The Bimolecular Hydrogen–Deuterium Exchange Behavior of Protonated Alkyl Dipeptides in the Gas Phase*

Erez H. Gur, Leo J. de Koning, and Nico M. M. Nibbering
Institute of Mass Spectrometry, University of Amsterdam, Amsterdam, The Netherlands

As part of an ongoing characterization of the intrinsic chemical properties of peptides, thermal hydrogen–deuterium exchange has been studied for a series of fast-atom-bombardment–generated protonated alkyldipeptides and related model compounds in the reaction with D_2O, CH_3OD, and ND_3 in a Fourier transform ion cyclotron resonance mass spectrometer. Despite the very large basicity difference between the dipeptides and the D_2O and CH_3OD exchange reagents, efficient exchange of all active hydrogen atoms occurs. From the kinetic data it appears that exchange of the amino, amide, and hydroxyl hydrogens proceeds with different efficiencies, which implies that the proton in thermal protonated dipeptides is immobile. The selectivity of the exchange at the different basic sites is governed by the nature of both the dipeptide and the exchange reagent. The results indicate that reversible proton transfer in the reaction complexes, which effectuates the deuterium incorporation, is assisted by formation of multiple hydrogen bonds between the reagents. Exchange is considered to proceed via the intermediacy of different competing intermediate complexes, each of which specifically leads to deuterium incorporation at different basic sites. The relative stabilization of the competing intermediate complexes can be related to the relative efficiencies of deuterium incorporation at different basic sites in the dipeptide. For all protonated dipeptides studied, the exchange in the reaction with ND_3 proceeds with unit efficiency, whereas all active hydrogen atoms are exchanged equally efficiently. Evidently specific multiple hydrogen bond formations are far less important in the reversible proton transfers with the relatively basic ammonia, which allows effective randomization of all active hydrogen atoms in the reaction complexes. *(J Am Soc Mass Spectrom 1995, 6, 466–477)*

Mass spectrometry has become a well established method for structural analysis of peptides [1, 2]. In general, these analyses are based on the extensively studied characteristic unimolecular decomposition behavior of the cationized species. The present level of sophistication of mass spectrometric instrumentation also has led to a growing interest to probe the bimolecular chemical behavior of these and related species. In particular, hydrogen–deuterium exchange in the gas-phase reactions of the protonated species with deuterated solvent molecules can provide insight into, for instance, the number of active hydrogens, site of protonation, and mobility of the proton over the different functional groups in the molecules [3, 4].

The site of protonation of peptides appears to be a continuing theme of debate. It is commonly proposed that backbone fragmentation of peptides is initiated by protonation at the nitrogen atom of the amide group [1, 5]. Heterogeneous populations of the peptides, which are protonated at the nitrogen atoms of the various basic amide groups, can account for the backbone fragmentation behavior [1, 6–8]. However, it is pointed out that this fragmentation behavior does not necessarily reflect the lowest energy structures of protonated peptides [9]. Accordingly, amides are generally considered to be favorably protonated at the oxygen rather than at the nitrogen atom [9–12]. Stabilization of the protonated peptides by intramolecular hydrogen bond formation is becoming a well accepted concept [9, 10, 13, 14]. For peptides larger than six amino acid building blocks it was suggested that protonation at one of the similarly basic amide oxygen atoms is stabilized by hydrogen bridging to other basic sites. Peptides smaller than four amino acid building blocks are considered to be favorably protonated at the terminal amino group with stabilizing hydrogen bridging to the amide oxygen atoms [10]. This is supported by semiempirical [9] and ab initio [15] calculations of $GlyGlyH^+$, which in addition show that structures with different hydrogen bond stabilizations can be quite close in energy. Consequently, dynamic mobility of the proton over different basic sites in protonated peptides seems energetically feasible [9, 16].

The present study is concerned with this proton mobility. The approach in this work, independently set up and parallel to that recently reported by Lebrilla

Address reprint requests to Nico M. M. Nibbering, Institute of Mass Spectrometry, University of Amsterdam, Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands.
*Presented at the 13th IMSC, Budapest, Hungary, 29 August–2 September 1994.

1044-0305/95/$9.50
SSDI 1044-0305(95)00189-K

Received September 27, 1994
Revised December 23, 1994
Accepted January 4,1995

and co-workers [17], is to monitor proton mobility via study of the kinetics of hydrogen–deuterium exchange in the reactions of a series of protonated alkyldipeptides with deuterated solvent molecules with varying proton affinity under the low pressure conditions in a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer [18, 19]. The results have been compared with the corresponding hydrogen–deuterium exchange behavior of a number of protonated model molecules that contain different structural features of the dipeptides.

Experimental

Experiments were performed with a Bruker Spectrospin CMS 47X Fourier transform ion cyclotron resonance mass spectrometer (Bruker-Franzen Analytik GMBH, Bremen, Germany) equipped with a 4.7-T magnet and an external ion source. Detailed descriptions of the instrument and general operating procedure for ion manipulation and detection have been provided previously [20–22].

Reactant ions were generated by fast-atom bombardment [23] (FAB) in the external ion source by using a 10-keV Xe atom beam from a Capillatron DIP Gun from Phrasor Scientific, Inc. (Duarte, CA). Solid samples were dissolved until saturation in a glycerol matrix containing 0.1-vol % trifluoroacetic acid. 1,4-diaminobutane and ethylamine were added as 10 and 2% of the matrix, respectively.

Standard H/D exchange reactions were initiated by FAB generation of $[M + H]^+$ ions that were gated to the FT-ICR cell for 50 ms. Subsequently the trapped $[M + H]^+$ ions were mass selected [22] from the ion mixture and effectively thermalized in an atmosphere of argon that was admitted to the FT-ICR cell through a pulsed valve for 120 ms up to a maximum pressure of approximately 10^{-5} mbar. Reaction of the selected thermalized $[M + H]^+$ ions with the H/D reagent gases was reproducibly monitored as a function of time up to a reaction time of 960 s. The reagent gases were introduced to the cell via a leak valve up to a pressure that ranged from 10^{-8} to 10^{-7} mbar (ionization gauge reading) with a background pressure of about 10^{-9} mbar. Similarly, reactions of nonthermalized $[M + H]^+$ ions were monitored by omission of the pulsed valve addition of argon.

A number of exchange reactions with D_2O were performed in the presence of pyridine and 2-fluoropyridine, at partial pressures of 2.1×10^{-7} and 1.4×10^{-7} mbar (ionization gauge reading), respectively.

To study the effect of thermal excitation on the behavior of the d_1-GlyGlyH$^+$ ions, protonated GlyGly first was thermalized by pulsed valve argon addition and reacted with D_2O for only 3 s. Then the resultant d_1-GlyGlyH$^+$ H/D exchange product ions carefully were selected [22] and subsequently excited [24] in three separate experiments to a translational energy of 0, 2.6, and 10.4 eV, respectively. Thereafter the excited ions were rethermalized by a second pulse of argon gas and the exchange reaction with D_2O as a function of reaction time continued to be monitored under thermal conditions.

Materials

N-Ethylglycylamide was synthesized in the reaction between glycine methyl ester and ethylamine. All other chemicals employed were commercially available and used without further purification.

Determination of Reaction Rate Constants

Pseudo-first-order rate constants for the decay of $[M + H]^+$ ions in the H/D exchange reactions were determined by fitting the experimental kinetic data for the decay of $[M + H]^+$ ions to $[M + H]_t^+ = [M + H]_0^+ e^{-nkt}$, where t is the reaction time (seconds), k is the pseudo-first-order reaction rate constant (cubic centimeters per molecule per second), and n is the number of H/D exchange reagent molecules per cubic centimeter. In all cases the fit to the experimental data had a regression factor greater than 0.998.

The n values were calculated from the partial pressures of the deuterated reagent gases in the FT-ICR cell. These pressures were determined from the ionization gauge readings that were corrected for the sensitivity, R_X relative to N_2, of the ionization gauge manometer for the nondeuterated gas X, as determined by Bartmess and Georgiadis [25]. Next, the corrected ionization gauge readings were multiplied by a factor of 1.9, which corresponds to the pressure gradient between the cell and the ionization gauge manometer (von Koding, H.; Pinkse, F. A.; Nibbering, N. M. M., unpublished). This pressure gradient was determined experimentally for methane by a fit of the determined rate constant for the reaction between $CH_4^{+\cdot}$ and CH_4 to the literature value [26]. Finally, the n values for the exchange reagents in the FT-ICR cell, which were obtained from the partial pressures, were multiplied with the actual deuterium label content (percent) of the exchange reagents. The actual deuterium label contents of the D_2O, CH_3OD, and ND_3 exchange reagents were determined for a series of exchange reactions by recording the self-chemical ionization mass spectra of the reagent gases in the FT-ICR cell and are included in the footnotes of Tables 1, 3, and 4, respectively. The purpose of the corrections of n for incomplete deuterium labeling is to discard reactions with unlabeled reagent molecules that do not contribute to deuterium incorporation in the protonated peptides. Maximum errors in the resulting n values and consequently in the reaction rate constants are estimated to be 30%.

Collision rate constants for the studied reaction systems without deuterium labeling were calculated by using the AADO (Average Dipole Orientation Theory with Conservation of Angular Momentum) theory [27]. For these calculations, values for dipole moments [28],

polarizabilities [29], dipole locking constants [27], and rotational constants [28] were taken from the literature.

Results and Discussion

H/D Exchange in the Reaction with D_2O

For a series of protonated alkyldipeptides and related model compounds the incorporation of deuterium in the thermal reaction with D_2O was monitored under identical conditions as a function of time: As an illustration, the kinetic data for the H/D exchange in protonated GlyAla and AlaGly are plotted in Figure 1. Qualitatively, these results indicate that for the protonated GlyAla incorporation of each of the first three deuterium atoms proceeds with the same rate, whereas incorporation of the fourth and fifth deuterium is increasingly less efficient (see Figure 1a). Clearly, the exchange behavior of the isomeric protonated AlaGly (see Figure 1b) is different. The overall H/D exchange is considerably less efficient. However, for this compound incorporation of each of the first four deteurium atoms appears to proceed with comparable rates, whereas the fifth deuterium atom is incorporated dramatically more slowly.

The recognition of different efficiencies of H/D exchange at the amino, amide, and carboxylic acid functional groups implies that in the thermal protonated dipeptides no effective randomization of active hydrogen atoms over the three functional groups has occurred. For all studied reaction systems pseudo-first-order reaction rate constants k_{exp} have been determined for the decay of the protonated molecules MH^+. The results are listed in Table 1 together with the efficiencies for the decay of MH^+, which is represented by the ratio of the experimental reaction rate constant and the corresponding calculated AADO collision rate constant k_{exp}/k_{AADO}.

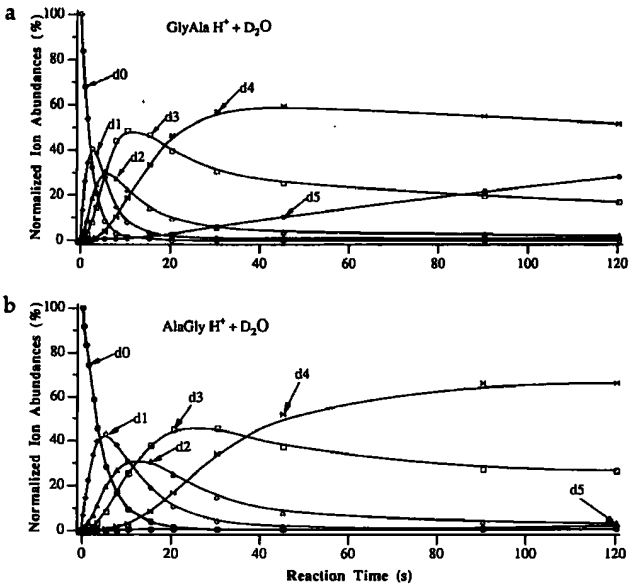


Figure 1. Deuterium incorporation in the reactions of D_2O with (a) protonated GlyAla and (b) protonated AlaGly as a function of reaction time at a D_2O pressure of 3.7×10^{-8} mbar. $d_0, \ldots, d_5$ represent the normalized abundances of the $d_0, \ldots, d_5$-GlyAlaH$^+$ and $d_0, \ldots, d_5$-AlaGlyH$^+$ ions, respectively.

To simplify the analysis of the kinetics of the consecutive deuterium atom incorporations, the macroscopic degree of deuterium incorporation was calculated from the raw data sets such as shown in Figure 1 by using eq. 1,

$$[\text{degree of D incorporation}]_t\ (\%) = \frac{100}{L} \sum_{n=0}^{n=N} \frac{n}{N} [(d_n)MH^+]_t \qquad (1)$$

where $[(d_n)MH^+]_t$ is the normalized abundance in percentage of the MH^+ ions with n deuterium atoms

Table 1. Hydrogen–deuterium exchange in the reaction between $[M + H]^+$ and D_2O[a]

M	$k_{exp} \times 10^{10}$[c] (cm^3 molecule^{-1} s^{-1})	k_{exp}/k_{AADO}	Relative rate of D incorporation[b] (%) $d_{1,2,3}$	d_4	d_5	d_6
GlyGly	6.3	0.23	100	50	10	
GlyAla	6.8	0.25	100	60	10	
GlyVal	6.0	0.22	100	85	10	
AlaGly	3.4	0.13	100	90	10	
AlaAla	3.7	0.14	100	95	15	
AlaVal	3.7	0.14	100	100	20	
ValGly	1.4	0.05	100	100	< 10	
ValVal	1.4	0.05	100	100	< 10	
GlyGlyGly	3.2	0.12	100	60	10	10
$EtNH_2$	—	—	—			
$H_2N(CH_2)_4NH_2$	0.26	0.01	100	100	100	
$H_2NCH_2C(O)NHEt$	5.6	0.20	100	5		
$H_2N(CH_2)_4COOH$	—	—	—	—		

[a] The pressure of the exchange reagent in the FT-ICR cell was determined to be 3.7×10^{-8} mbar. For the determination of k_{exp} this was corrected for the deuterium label content, which throughout the series was monitored to be 89 ± 1% (see Experimental section).
[b] Qualitative changes in the rate of D incorporation during the progression of H/D exchange as determined from the kinetic data in Figure 2 (see also text).
[c] Rate constant for the decay of the $[M + H]^+$ ions (see Experimental section).

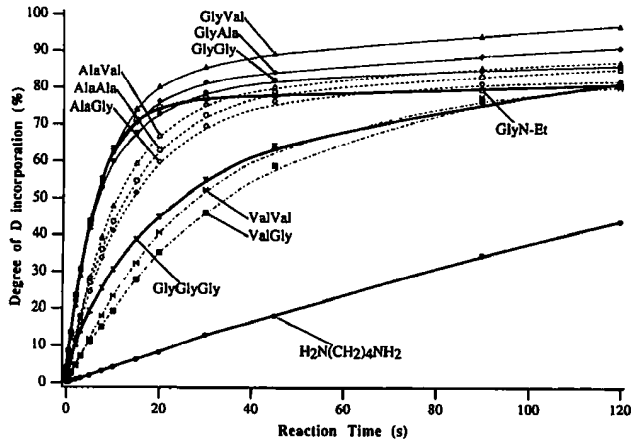


Figure 2. Macroscopic degree of D incorporation in the protonated di(tri)peptides and related model compounds in the reaction with D_2O as a function of reaction time at a D_2O pressure of 3.7×10^{-8} mbar.

incorporated at reaction time t, N is the number of active hydrogen atoms (five for the protonated dipeptides), and L is the D-label content of the exchange reagent (see Experimental section). The results of the data processing are shown in Figure 2.

If all the active hydrogen atoms were to exchange with equal efficiency, the macroscopic degree of D incorporation could be modeled with the quasi pseudo-first-order kinetics of eq. 2,

$$\text{degree of D incoropration} = 100 - 100e^{-rt} \quad (2)$$

where r represents an overall H/D exchange rate. However, as is already apparent from the raw kinetic data, the H/D exchange rate decreases as incorporation advances. A consequence of the decreasing exchange rate is that the data sets [except for putrescine, $H_2N(CH_4)_4NH_2$] in Figure 2 could not be fitted satisfactorily to eq 2.

By fitting segments of the data sets, the change in incorporation rate could be revealed qualitatively. For instance, for $GlyGlyH^+$ a perfect fit was obtained over the data range between 0 and about 60% of D incorporation (this fit is shown in Figure 4, which is discussed in more detail later on in this section). This result indicates that the exchange of the first three out of five active hydrogen atoms proceeds with the same rate. A fit over the data range from about 60 to 80% of D incorporation reveals that the exchange rate of the fourth hydrogen is only 50% of the initial exchange rate, whereas a fit over the data range from about 80 to 100% of D incorporation shows that the exchange rate of the fifth hydrogen is less than 10% of the initial exchange rate. The results of an analogous analysis of the data sets for each studied MH^+ are included in Table 1 in terms of $d_{1,2,3}$, d_4, d_5, and d_6, which represent the qualitative relative rates of H/D exchange of the first three hydrogen atoms and when applicable of the fourth, fifth, and sixth hydrogen atom, respectively.

As an aid to the rationalization of the results, selected proton affinities (PA) and gas-phase basicities at 300 K (GB_{300}) are listed in Table 2. Values in parentheses are based on assumptions (see the footnotes to Table 2) and therefore may suffer from relatively large inaccuracies. Nonetheless, it is obvious that D_2O is about 190–220 kJ mol^{-1} less basic than the di(tri)peptides studied, which ranged from GlyGly to ValVal. The large basicity differences imply that exchange of the active hydrogens via a simple reversible proton transfer in the reaction complex between D_2O and MH^+ is energetically hindered.

Apparently, proton transfer from MH^+ to D_2O becomes energetically accessible in the reaction complex because of formation of strongly stabilizing multiple hydrogen bonds between HD_2O^+ and the various basic sites in M. This concept is supported by the observation that protonated monofunctional ethylamine ($C_2H_5NH_2$) with a GB_{300} in the range of the studied dipeptides does not undergo H/D exchange, whereas protonated putrescine [$H_2N(CH_2)_4NH_2$] with two terminal amino groups is found to incorporate five deuterium atoms despite the much larger basicity difference with D_2O (see Table 2). If one of the amino groups in putrescine is replaced by a carboxylic group

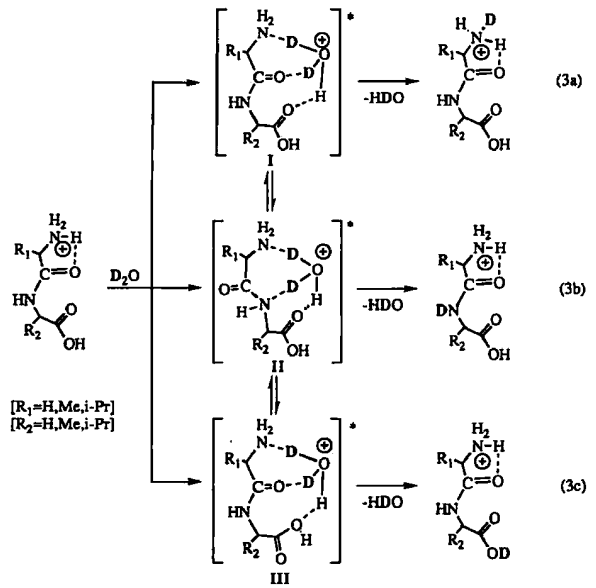
Table 2. Selected proton affinities (PA), entropies of protonation (ΔS_{base}), and gas-phase basicities (GB_{300})

	PA[a] (kJ mol^{-1})	ΔS_{base}[b] (J mol^{-1} K^{-1})	GB_{300}[c] (kJ mol^{-1})
$H_2O(D_2O)$	697	112	663
$C_6H_6(C_6D_6)$	759	85[d]	735
$CH_3OH(CH_3OD)$	761	109	728
CH_3COOH	796	115	762
$NH_3(ND_3)$	854	121	818
C_5H_4FN(2-fluoropyridine)	881	109	848
Gly	883[e]	118[f]	848
$CH_3CONHCH_3$	890[g]	107[g]	858
Ala	894[e]	109[f]	861
GlyGly	895[h]	(154)[i]	(849)
Val	903[e]	109[f]	861
GlyGlyGly	(908)[h]	(154)[i]	(862)
$C_2H_5NH_2$	908	118	873
GlyVal,ValGly,AlaVal[j]	(917)	(154)[i]	(871)
ValVal[j]	(928)	(154)[i]	(881)
$H_2N(CH_2)_4NH_2$(putrescine)	994	199	934

[a] PA values taken from ref 11, unless noted otherwise.
[b] ΔS values taken from Aue, D. H.; Bowers, M. T. In *Gas Phase Ion Chemistry*, Vol. 2; Bowers, M. T., Ed.; Academic Press: New York, 1979; pp 1-51.
[c] Calculated gas-phase basicity at 300 K.
[d] ΔS calculated from the data in Meot-Ner, M.; Sieck, L. W. *J. Am. Chem. Soc.* **1991**, *113*, 4448-4460.
[e] PA values for the amino acids taken from Li, X.; Harrison, A. G. *Org. Mass Spectrom.* **1993**, *28*, 366-371.
[f] ΔS for Gly and Ala taken from Meot-Ner, M.; Hunter, E. P.; Field, F. H. *J. Am. Chem. Soc.* **1979**, *101*, 686-689. ΔS for Val approximated with ΔS of Ala.
[g] Calculated from the data in Meot-Ner, M. *J. Am. Chem. Soc.* **1984**, *106*, 278-283.
[h] PA for GlyGly and GlyGlyGly taken from the data in ref 9 and adjusted to the scale of ref 11.
[i] ΔS taken as calculated in ref 9 for a dipeptide protonated at the amino group with hydrogen bonding to the amide oxygen atom.
[j] PA and GB_{300} are calculated from the available GB_{350} data in ref 13 and adjusted to the scale of ref 11.

to give 5-aminovaleric acid [$H_2N(CH_2)_4COOH$], the H/D exchange with D_2O again becomes too slow to be observed. Apparently, the initial proton transfer from protonated 5-aminovaleric acid to D_2O is hindered because the intermediate reaction complex between HD_2O^+ and 5-aminovaleric acid is insufficiently stabilized by multiple hydrogen bonds. This insufficient stabilization is considered to be because of the large basicity difference between the carboxylic and the amino groups as indicated by the difference in basicity of CH_3COOH and $C_2H_5NH_2$ of about 111 kJ mol^{-1} (see Table 2). Consistent with this assumption, protonated *N*-ethylglycylamide [$H_2NCH_2C(O)NHC_2H_5$] is found to undergo H/D exchange because stabilization of the intermediate reaction complex by multiple hydrogen bonds can be very efficient because of the relatively small basicity difference between the terminal amino and the amide group, as indicated by the difference in basicity of $C_2H_5NH_2$ and $CH_3C(O)NHCH_3$ of only 15 kJ mol^{-1} (see Table 2).

Clearly, the H/D exchange results of the protonated model compounds [$C_2H_5NH_2$, $H_2NCH_2C(O)NHC_2H_5$, and $H_2N(CH_2)_4COOH$] demonstrate that in the H/D exchange of the protonated dipeptides the amide group plays an essential role because it promotes the reversible proton transfer within the reaction complex with D_2O. However, the carboxylic group appears to have an insignificant effect on the efficiency of the exchange reaction. Based on the foregoing considerations the H/D exchange behavior of the protonated dipeptides can be rationalized with the mechanism summarized in eq. 3:



As a guideline in the discussion, the local basicities of the different basic sites in, for instance, GlyGly can be approximated roughly (see Table 2). The GB_{300} of the amino group is expected to be close to that of CH_3NH_2 at 861 kJ mol^{-1}. For the amide carbonyl oxygen atom the GB_{300} is anticipated to be close to that of $CH_3CONHCH_3$ at 858 kJ mol^{-1}. The GB_{300} of the amide nitrogen atom can be estimated to be about 60 kJ mol^{-1} lower at 798 kJ mol^{-1}, as indicated by ab initio calculations on $HCONH_2$ [12]. The GB_{300} for the carboxylic carbonyl oxygen atom can be compared with that of CH_3COOH at 762 kJ mol^{-1}, whereas the GB_{300} of the carboxylic hydroxyl oxygen atom can be estimated about 120 kJ mol^{-1} lower at 642 kJ mol^{-1}, as indicated by ab initio calculations on HCOOH [30].

Conceivably, the proton in thermal protonated dipeptides is strongly bonded to the basic amino group, whereas stabilization can occur through hydrogen bond formation with the almost equally basic amide carbonyl oxygen atom. This hydrogen bond stabilization appears to be canceled by the destabilizing inductive effect of the amide carbonyl as indicated by the basicity of GlyGly, which is approximated to be slightly smaller than that of the monofunctional $C_2H_5NH_2$ (see Table 2). A structure of protonated dipeptides where the proton is localized between the amino group and the amide carbonyl oxygen atom also has been advocated by other research groups [9, 13, 15].

Furthermore, the relatively low basicity of the amide nitrogen atom and the hydroxyl oxygen atom is expected to hamper the mobility of the proton over all functional groups under thermal conditions. Indeed, this immobility follows from the present results, which show different efficiencies of deuterium incorporation at different functional groups.

Computational studies [12, 30–32] have shown that, in general, stabilization via hydrogen bond formation to carbonyl oxygen atoms is extremely critical with respect to the geometry of the hydrogen bond. Therefore, the geometric restriction in the protonated dipeptides can be anticipated to have an unfavorable influence on the strength of the hydrogen bond. Consequently, despite the relatively low basicity, the incoming D_2O molecule can reorganize the complex to form a geometrically optimized hydrogen bond. This results in a charge delocalization toward the water molecule that enables multiple hydrogen bond formations to the amino and the two carbonyl oxygen atoms in the dipeptide. The flexibility of the reaction complex allows geometric optimization of the hydrogen bonds to maximize stabilization of a complex **I** in eq. 3a between HD_2O^+ and the dipeptide.

The energetic accessibility of complex **I** seems to be supported by several ab initio studies on the complexation of various protonated carbonyl-containing molecules with water [12, 30–32]. From these studies it appears that at certain bond lengthening and relative angular orientation of the bases to the hydrogen bond, the equilibrium position of the proton shifts toward the less basic moiety. This seemingly anomalous effect was traced to the differences in intrinsic flexibility, lone-pair distribution, and, most importantly, dipole moments of the two bases. For formamide [12], formic

acid [30], and ammonia [33] it was calculated that enlargement of the internuclear distance resulted in two minimum energy structures, which corresponded to a shift in the equilibrium position of the proton from the more basic molecules to water. In all three cases the strength of the hydrogen bond from the bases to H_3O^+ appeared to be significantly stronger than the hydrogen bond from water to the protonated bases.

It thus seems that complex I can be an accessible intermediate in which randomization can occur between only three out of five active hydrogen atoms of the protonated dipeptide and the two deuterium atoms in D_2O that lead to deuterium incorporation (eq 3a). Consequently, at least three hydrogen atoms are expected to be exchanged with equal efficiency, which agrees with the experimental results. Because there is no significant mobility of the hydrogen atoms in the thermal protonated dipeptides, exchange of the amide hydrogen requires an intermediate complex that involves a hydrogen bond between HD_2O^+ and the amide nitrogen atom such as in complex **II** in eq. 3b. Similarly, exchange of the hydroxyl hydrogen requires an intermediate complex that involves a hydrogen bond between HD_2O^+ and the hydroxyl hydrogen atom such as in complex **III** in eq 3c. It thus appears that the relative rate of deuterium incorporation at the different functional groups is related to the relative stabilities of the corresponding intermediate complexes. Clearly, complex **I** seems to be stabilized best, which leads to the most efficient exchange of the three hydrogen atoms at the amino group. In complex **II** the hydrogen bond to the amide carbonyl oxygen atom is replaced by a hydrogen bond to the less basic (by about 60 kJ mol^{-1}) amide nitrogen atom, which leads to a reduced efficiency of exchange of the amide hydrogen atom. Similarly, in complex **III** the hydrogen bond to the carboxylic carbonyl oxygen atom is replaced by a hydrogen bond to the less basic (by about 120 kJ mol^{-1}) hydroxyl oxygen atom, which leads to a reduced efficiency of exchange of the hydroxyl hydrogen atom. Because of this large difference in basicity between the carboxylic carbonyl and hydroxyl oxygen atom it is tempting to conclude that exchange of the hydroxyl hydrogen is expected to be less efficient than the exchange of the amide hydrogen atom. However, the different geometric restrictions to the hydrogen bonds in complexes **II** and **III** make estimates of the relative stabilities of the two complexes hazardous. Note that the mechanism described in eq 3 must be regarded as a simplified model to explain the H/D exchange behavior because it is likely that the reaction profile accommodates many reactive multiple hydrogen bonded complexes, similar to those discussed in the preceding text, from which deuterium incorporation may occur.

If the results in Table 1 are examined more closely, two trends become obvious. The first trend involves the efficiencies that can be associated with incorporation of the first three deuterium atoms at the amino group as indicated by k_{exp}/k_{AADO} in Table 1. In agreement with the proposed model summarized in eq 3, these efficiencies appear to correlate with the basicity of the *N*-terminal amino acid residues. The largest efficiencies are found for the dipeptides with the least basic Gly terminal amino acids, GlyGly, GlyAla, and GlyVal,which are all close to 0.25 (see Table 1). Clearly, formation of intermediate complex I is promoted if the difference between the basicity of D_2O and the *N*-terminal amino acid residue becomes smaller. A similar efficiency of 0.20 is found for model compound *N*-ethylglycylamide [$H_2NCH_2C(O)NHC_2H_5$]. This result again shows that stabilization via hydrogen bond formation to the carboxylic group in the intermediate complex **I** is relatively insignificant. For the tripeptide GlyGlyGly the efficiency is found to be significantly lower at 0.12. This may imply that in protonated GlyGlyGly the proton is more efficiently hydrogen bonded to the more remote amide carbonyl oxygen atom, as a consequence of which hydrogen bond formation to D_2O become less competitive.

Upon increasing the difference between the basicity of D_2O and the *N*-terminal amino acid residue the determined efficiencies are reduced. For AlaGly, AlaAla, and AlaVal the efficiencies all become about 0.14, whereas for ValGly and ValVal a further reduction to 0.05 is found (see Table 1).

The second trend involves the efficiencies that can be associated with incorporation of the fourth deuterium atom as indicated by d_4 in Table 1. In the series of protonated GlyGly, GlyAla, and GlyVal, the rates associated with incorporation of the fourth deuterium relative to the rate of incorporation of the first three deuterium atoms are reduced to 50, 60, and 85%, respectively. Evidently, enhancement of the basicity of the *C*-terminal amino acid residue relative to the *N*-terminal amino acid residue enhances the strength of the applicable hydrogen bonds in either intermediate complex **II** or **III**, which promotes incorporation of the fourth deuterium atom. A similar trend in the relative rate of incorporation of the fourth deuterium atom is detected in the series of protonated AlaGly, AlaAla, and AlaVal, although the reduction relative to the rate of incorporation of the first three deuterium atoms is less substantial. This tendency is continued in the series of protonated ValGly and ValVal, where no difference in the rates of incorporation of the first three and the fourth deuterium atoms could be detected (see Table 1). Interestingly, the relative rate of incorporation of the fourth deuterium in N-ethylglycylamide [$H_2NCH_2C(O)NHC_2H_5$] is found to be dramatically reduced to about 5%. Evidently, the carboxylic group strongly promotes incorporation of the fourth deuterium.

Finally, the results for the protonated tripeptide GlyGlyGly show that incorporation of the fourth deuterium proceeds with about 60% of the efficiency of the incorporation of the first three deuterium atoms, whereas incorporation of both the fifth and sixth deu-

terium atoms appears to proceed with a relative efficiency of about 10%. This deuterium incorporation pattern may misleadingly suggest that the hydroxyl hydrogen in the tripeptide exchanges more efficiently than the two amide hydrogen atoms.

To examine the effect of the thermalization of the protonated di(tri)peptides on the exchange rate, the decays of nonthermalized protonated di(tri)peptides in the reactions with D_2O have been compared with the decays of the corresponding reactions after thermalization in an argon bath gas (see Experimental section). From the results it appears that initially the reactions of both the somewhat suprathermal protonated GlyGly and GlyGlyGly, injected into the FT-ICR cell from the FAB ion source, are extremely slow. However, one or two nonreactive collisions with the D_2O reagent molecules lead to effective thermalization, as indicated by the enhancement of the reaction rate up to a value found for the reactions of the prethermalized protonated molecules under identical D_2O pressure conditions. This result reveals a large negative temperature effect, in agreement with an anticipated large activation entropy for the formation of multiply hydrogen bonded intermediate complexes.

The mobility of the proton in thermal protonated dipeptides has been investigated by comparison of the kinetic data of the successive deuterium atom incorporation in the reaction with D_2O at different D_2O pressures. If the mobility of the proton between collisions with D_2O were to lead to some redistribution of deuterium and hydrogen between the amino and the other functional groups, then it would be expected that at a lower pressure (collision frequency) and given a longer time in which intramolecular deuterium–hydrogen redistribution can occur, the incorporation of the fourth and fifth (and sixth for GlyGlyGly) atoms would become relatively more efficient. However, no significant change in the relative efficiencies of incorporation of the deuterium atoms has been found with variation of the D_2O pressure. From this and the applied D_2O pressures it follows that the unimolecular rate constant for the redistribution of the different active hydrogen atoms in thermal protonated di(tri)peptides is considerably smaller than 1 s^{-1}.

Next, the mobility of the proton in suprathermal protonated dipeptides was investigated. To this end, initially formed d_1-GlyGlyH$^+$ ions in the reaction of protonated GlyGly with D_2O were selected from the ion mixture and subsequently excited in three separate experiments to a translational energy of 0, 2.6, and 10.4 eV, respectively (see Experimental section). The selected translationally excited d_1-GlyGlyH$^+$ ions were allowed to collide with argon to convert the 0-, 0.6-, and 2.4-eV center-of-mass collision energy to internal energy, after which the d_1-GlyGlyH$^+$ ions were rethermalized by successive collisions with argon. Finally, the progress of the H/D exchange reaction of the rethermalized d_1-GlyGlyH$^+$ ions with D_2O was monitored.

The kinetic data for the decay of the d_1-GlyGlyH$^+$ ions, temporarily heated by 0, 0.6, and 2.4 eV, respectively, and the corresponding successive formations of d_2-GlyGlyH$^+$, d_3-GlyGlyH$^+$, and d_4-GlyGlyH$^+$ ions are plotted in Figure 3. The results show that temporarily heating the d_1-GlyGlyH$^+$ ions does not affect the decay of these ions. This is to be expected because the decay of these ions is predominantly related to deuterium incorporation at the amino group. However, it is clearly shown that the efficiency of formation of the d_4-GlyGlyH$^+$ ions relative to formation of the d_2-GlyGlyH$^+$ and d_3-GlyGlyH$^+$ ions is enhanced upon increasing the energy with which the d_4-GlyGlyH$^+$ ions are temporarily heated. Consequently, it follows that under suprathermal conditions intramolecular exchange can occur between the hydrogen atoms from the amino to the other functional groups in the protonated dipeptide.

Furthermore, the mobility of the proton in protonated dipeptides was examined during interaction with a nonprotic base of which the basicity approaches that of the dipeptides. For this purpose, the H/D exchange reactions of protonated GlyGly, GlyVal, and ValGly were monitored in the presence of 2-fluoropyridine with a basicity close to that of GlyGly (see Table 2). The kinetic data obtained for the reactions in the presence and in the absence of 2-fluoropyridine were analyzed as previously discussed, where the macroscopic degree of deuterium incorporation is calculated as a function of the reaction time by using eq 1. The results in Figure 4 show that for the exchange reaction of protonated GlyGly in the absence of 2-fluoropyridine only segments of the data can be fitted satisfactorily to eq. 2, from which it follows that relative to the incorporation rate of the first three deuterium atoms, the rate of incorporation of the fourth and fifth deuterium atoms is reduced to about 50 and 10%, respectively, as already shown (see Table 1). Strikingly, the complete

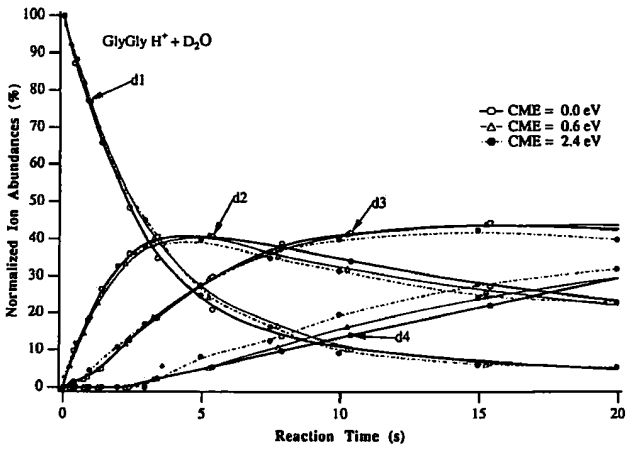


Figure 3. Deuterium incorporation in initially formed d_1-GlyGlyH$^+$ ions in the reaction with D_2O as a function of reaction time at a D_2O pressure of 3.7×10^{-8} mbar after temporary collisional heating with 0-, 0.6-, and 2.4-eV center-of-mass collision energy (CME) in an argon bath gas. $d_1, \ldots, d_4$ represent the normalized abundances of the $d_1, \ldots, d_4$-GlyGlyH$^+$ ions.

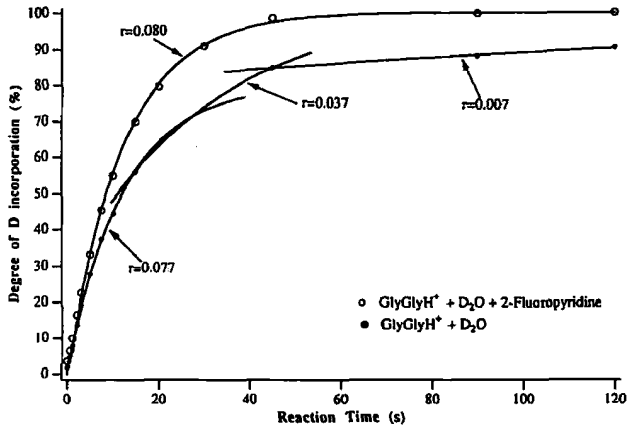
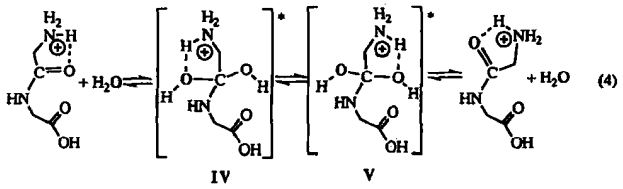


Figure 4. Macroscopic degree of D incorporation in protonated GlyGly in the reaction with D_2O as a function of reaction time at a D_2O pressure of 1.9×10^{-8} mbar in the absence and in the presence of 2-fluoropyridine at a partial pressure of about 5×10^{-8} mbar. The rate of deuterium incorporation is indicated by r, which is obtained from a fit of segments of the data sets to eq. 2.

data set for the exchange reaction of protonated GlyGly in the presence of 2-fluoropyridine can be fitted very successfully to eq. 2 (see Figure 4), which shows that incorporations of all five deuterium atoms proceed with equal efficiencies. Similar results were obtained for protonated GlyVal and ValGly. Evidently, each incorporation of a deuterium atom by D_2O, for which the rate is limited by that of the exchange of the amino hydrogen atoms, is followed by an efficient randomization of all active hydrogen atoms in the protonated dipeptide as a result of nonselective reversible proton transfer to the basic nonprotic 2-fluoropyridine.

Although the mechanism summarized in eq. 3 seems to rationalize very satisfactorily the hydrogen exchange behavior in the reaction with the relatively low basic water molecule, an alternative mechanism can be proposed, where the water molecule initially induces a proton catalyzed nucleophilic addition to the amide carbonyl group, which leads to the intermediate **IV** shown in eq. 4. Facile randomization of the three amino and the two individual hydroxyl hydrogen atoms in the corresponding intermediate complexes **IV** and **V** followed by the loss of a water molecule, can account for the incorporation of deuterium atoms in the reaction with D_2O.



(4)

Obviously the exchange mechanism described in eq. 4 does not suffer from the relatively low basicity of the water molecule. Furthermore, it is compatible with the results obtained for the model compounds, which show that the amide group plays a critical role in the exchange, contrary to the carboxylic group (see preceding text). Moreover, it seems to be in agreement with the observed trend that the initial efficiency of the exchange is reduced if the basicity of the *N*-terminal amino acid residue is increased; we assume that this leads to a stronger bond between the proton and the amino group, which thereby reduces the polarization of the amide carbonyl bond. If the mechanism described in eq 4 is applicable, reaction with $H_2{}^{18}O$ should result in the incorporation of ^{18}O via the equilibrium between the corresponding intermediates **IV** and **V**. However, because no incorporation of ^{18}O has been found in the reaction between $H_2{}^{18}O$ and protonated GlyGly, it appears that reversible nucleophilic addition of water to the amide carbonyl bond has been disproved.

H/D Exchange in the Reaction with CH_3OD

The kinetic data obtained from the thermal reactions with CH_3OD were analyzed in the same way as those obtained from the reaction with D_2O (see preceding text). The results of these analyses are presented in Table 3 and Figure 5.

The results for the reactions with D_2O show that the efficiency of the decay of the protonated molecules k_{exp}/k_{AADO} is enhanced if the basicity of the *N*-terminal amino acid residue becomes lower, whereas the relative efficiency that is associated with the incorporation of the fourth deuterium atom d_4 is enhanced if the basicity of the *C*-terminal amino acid residue becomes larger (see Table 3). In general, the decays of the protonated molecules are significantly more efficient. This can be attributed to the higher basicity of methanol relative to water, which consequently closes the basicity gap between the di(tri)peptides by about 65 kJ mol^{-1} (see Table 2). Nonetheless, this leaves a gap of at least 130 kJ mol^{-1}. Obviously, reversible proton transfer from the protonated di(tri)peptides to methanol still requires very efficient multiple hydrogen bond stabilization of the intermediate complexes between the di(tri)peptides and protonated methanol. Protonated methanol can form two relatively efficiently stabilizing hydrogen bonds, rather than three as in the case of protonated water. Nonetheless, the efficiency for the incorporation of the first three deuterium atoms is significantly higher in the reaction with CH_3OD than in the reaction with D_2O. Again this shows that for the hydrogen exchange at the amino group, hydrogen bond formation to the carboxylic group in the corresponding intermediate complex is insignificant. Consequently, incorporations of the first three deuterium atoms in the protonated model compound *N*-ethylglycylamide $[H_2NCH_2C(O)NHC_2H_5]$ and the protonated GlyGly, GlyAla, and GlyVal are found to be equally efficient (see Table 3). However, stabilization of the intermediate complex via hydrogen

Table 3. Hydrogen–deuterium exchange in the reaction between $[M + H]^+$ and MeOD[a]

M	$k_{exp} \times 10^{10}$[c] (cm^3 $molecule^{-1}$ s^{-1})	k_{exp}/k_{AADO}	Relative rate of D incorporation[b] (%)			
			$d_{1,2,3}$	d_4	d_5	d_6
GlyGly	12.7	0.64	100	35	15	
GlyAla	13.7	0.70	100	40	15	
GlyVal	12.3	0.64	100	50	20	
AlaGly	11.1	0.57	100	35	10	
AlaAla	13.0	0.67	100	40	10	
AlaVal	11.9	0.62	100	70	10	
ValGly	5.5	0.29	100	40	10	
ValVal	8.6	0.45	100	60	05	
GlyGlyGly	6.7	0.35	100	70	15	15
$EtNH_2$	—	—	—			
$H_2N(CH_2)_4NH_2$	1.1	0.05	100	100	100	
$H_2NCH_2C(O)NHEt$	13.7	0.62	100	2		
$H_2N(CH_2)_4COOH$	—	—	—	—		

[a] The pressure of the exchange reagent in the FT-ICR cell was determined to be 3.7×10^{-8} mbar. For the determination of k_{exp} this was corrected for the deuterium label content, which throughout the series was monitored to be 72 ± 4% (see Experimental section).
[b] Qualitative changes in the rate of D incorporation during the progression of H/D exchange as determined from the kinetic data in Figure 5 (see also text).
[c] Rate constant for the decay of the $[M + H]^+$ ions (see Experimental section).

bond formation to the amide carbonyl oxygen atom is crucial, as follows from the observation that the protonated model compounds ethylamine ($C_2H_5NH_2$) and 5-aminovaleric acid [$H_2N(CH_2)_4COOH$] are unreactive toward CH_3OD.

Strikingly, the relative efficiencies, which can be associated with the incorporation of the fourth deuterium atom d_4, are significantly lower than in the reaction with D_2O. Most prominently this is illustrated in the reaction of CH_3OD with protonated AlaVal, ValGly, and ValVal, where a considerable decrease in the efficiency for the incorporation of the fourth deuterium atom is found relative to the incorporation of the first three deuterium atoms, which is in sharp contrast to the corresponding results obtained for the reaction with D_2O (see Tables 1 and 3). Evidently, the intermediate complexes that lead to incorporation of the fourth deuterium atom are relatively better stabilized by three hydrogen bonds with protonated water than by two hydrogen bonds with protonated methanol. For the least efficient incorporation of the fifth deuterium atom this effect seems to be insignificant as follows from the relative rates of the successive deuterium incorporations (see Tables 1 and 3).

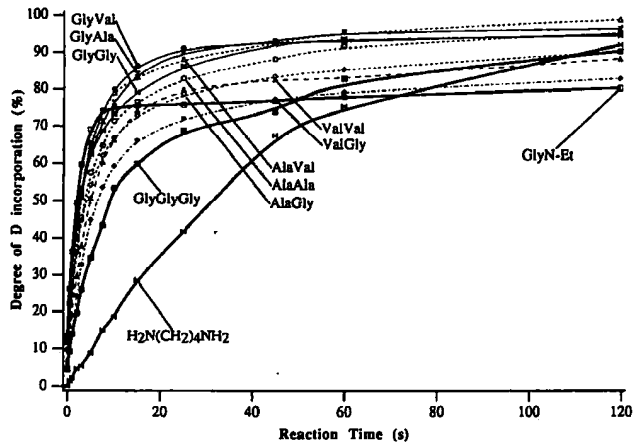


Figure 5. Macroscopic degree of D incorporation in the protonated di(tri)peptides and related model compounds in the reaction with CH_3OD as a function of reaction time at a CH_3OD pressure of 3.7×10^{-8} mbar.

For the reaction of the protonated tripeptide GlyGlyGly the overall efficiency for deuterium incorporation is found to be about three times greater in the reaction with CH_3OD than in the reaction with D_2O. Nevertheless, the relative rates of the successive deuterium atom incorporations appear to be quite similar.

For the reactions with D_2O, the effect of thermalization on the decay of the protonated di(tri)peptides was examined in the reactions with CH_3OD. Again it appears that the nonthermalized protonated peptides are less reactive than the thermalized protonated peptides. Qualitatively, the associated negative temperature effect is smaller than in the reactions with D_2O, in agreement with the expectation that entropy unfavorable multiple hydrogen bond formations in the intermediate complexes are more important in the reactions with D_2O.

Also in the reactions with CH_3OD no pressure dependence was found with respect to the relative efficiencies of the successive incorporation of the deuterium atoms, which implies that no redistribution of active hydrogen atoms between functional groups is detected in thermal protonated di(tri)peptides.

H/D Exchange in the Reaction with ND_3

The kinetic data obtained from the thermal reactions with ND_3 were analyzed in the same way as those

obtained from the reaction with D_2O and CH_3OD (see preceding text). The results of these analyses are presented in Table 4 and Figure 6. Within experimental errors the decays of all the protonated dipeptides indicate unit efficiencies ($k_{exp}/k_{AADO} \approx 1$; see Table 4). The corresponding unsegmented data sets in Figure 6 could be fitted very satisfactorily to the single exponential function of eq 2, which clearly shows that successive exchanges of all active hydrogen atoms are equally efficient as indicated by $d_{1,2,3}$, d_4, and d_5 in Table 4.

The use of ND_3 closes the gap between the basicity of the dipeptides and the exchange reagent to about 30 kJ mol^{-1}. Consequently, reversible proton transfer has become energetically accessible and does not necessarily require specific multiple hydrogen-bonded stabilized intermediate complexes. Hence, randomization within the reaction complex of the three ammonia and the five active hydrogen atoms from the protonated dipeptides leads to random incorporation of deuterium atoms.

In agreement, all protonated model compounds readily exchange all active hydrogens for deuterium atoms in the reaction with ND_3. These model compounds include the monofunctional ethylamine ($C_2H_5NH_2$) and 5-aminovaleric acid [$H_2N(CH_2)_4COOH$] despite the large difference between the basicity of the amino and carboxylic groups. This result implies that in the H/D exchange reaction of the protonated dipeptides with ND_3 the amide group has lost its leading role. However, it is found that the amide hydrogen atom in protonated *N*-ethylglycylamide [$H_2NCH_2C(O)NHC_2H_5$] still exchanges exceptionally slowly; no obvious explanation is available.

Compared to the dipeptides, the efficiency of the exchange reaction for protonated GlyGlyGly appears to be somewhat lower. This may be because of the assumed stronger hydrogen bond in the protonated GlyGlyGly, which may be responsible for a higher activation energy for the reversible proton transfer. Furthermore, one of the six active hydrogen atoms in protonated GlyGlyGly appears to exchange significantly less efficiently (see Table 4), and for this exceptional behavior there is also no apparent explanation.

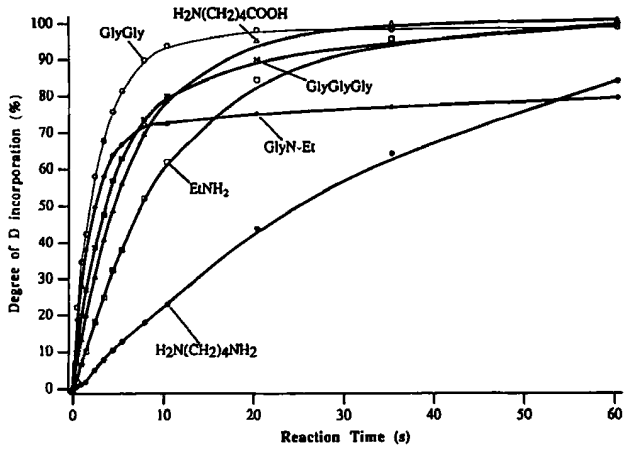


Figure 6. Macroscopic degree of D incorporation in the protonated GlyGly and related model compounds in the reaction with ND_3 as a function of reaction time at a ND_3 pressure of 1.9×10^{-8} mbar. The corresponding data obtained for the reactions of the remaining studied protonated dipeptides are identical to those of GlyGly.

The effect of thermalization on the decay of the protonated di(tri)peptides in the reactions with ND_3 is found to be relatively small. The exchange reactions are initially slightly slowed down if the thermalization event is omitted. The indicated small negative temperature effect is compatible with the conclusion that reversible proton transfer, which effectuates deuterium incorporation, does not necessarily have to be assisted

Table 4. Hydrogen–deuterium exchange in the reaction between $[M + H]^+$ and ND_3 [a]

M	$k_{exp} \times 10^{10}$ [c] (cm^3 $molecule^{-1}$ s^{-1})	k_{exp}/k_{AADO}	Relative rate of incorporation [b] (%)			
			$d_{1,2,3}$	d_4	d_5	d_6
GlyGly	26.0	1.08	100	100	100	
GlyAla	26.3	1.09	100	100	100	
GlyVal	23.8	1.01	100	100	100	
AlaGly	26.5	1.10	100	100	100	
AlaAla	26.7	1.10	100	100	100	
AlaVal	27.2	1.13	100	100	100	
ValGly	25.2	1.05	100	100	100	
ValVal	25.4	1.05	100	100	100	
GlyGlyGly	21.2	0.88	100	100	100	25
$EtNH_2$	7.0	0.27	100			
$H_2N(CH_2)_4NH_2$	3.4	0.14	100	100	100	
$H_2NCH_2C(O)NHEt$	25.0	1.04	100	5		
$H_2N(CH_2)_4COOH$	13.8	0.58	100	80		

[a] The pressure of the exchange reagent in the FT-ICR cell was determined to be 1.9×10^{-8} mbar. For the determination of k_{exp} this was corrected for the deuterium label content, which throughout the series was monitored to be 85 ± 3% (see Experimental section).

[b] Qualitative changes in the rate of D incorporation during the progression of H/D exchange as determined from the kinetic data in Figure 6 (see also text).

[c] Rate constant for the decay of the $[M + H]^+$ ions (see Experimental section).

by multiple hydrogen bond formations between the reactants.

As expected for the ND_3 exchange reagent, no pressure dependence has been found with respect to the efficiencies of deuterium incorporations in the protonated di(tri) peptides.

H/D Exchange in the Reaction with C_6D_6

Only protonated GlyGly, GlyVal, and GlyGlyGly were subjected to H/D exchange in the reaction with C_6D_6. The efficiencies of the exchanges were found to be extremely low. Under conditions equivalent to those applied for the reactions with D_2O, CH_3OD, and ND_3, only a few percent of the ions appear to incorporate one deuterium atom, whereas the protonated model compounds were found to be unreactive toward C_6D_6. Because the basicity of benzene is very close to that of methanol (see Table 2), the poor performance of C_6D_6 can be attributed to the inability of protonated benzene to form efficiently stabilizing multiple hydrogen bonds in the intermediate reaction complexes [34].

The H/D exchange behavior in the reactions with D_2O and CH_3OD is considered to reflect the relative stabilities of the competing intermediate complexes that lead to selective deuterium incorporations at the different functional groups. From the trends in the results it has been considered that stabilization of the competing intermediate complexes occurs via formation of specific multiple hydrogen bonds whose strengths are governed by the local basicities of the different basic sites in the dipeptides. However, if geometric optimization of the hydrogen bonds in the competing intermediate complexes is critical, the effects of the alkyl substituents on the H/D exchange behavior may be related not only to the individual enhancements of the local basicities at different basic sites, but also to the geometric distortion in the intermediate complexes due to steric hindrance. The effect of a possible steric hindrance can be examined by study of the H/D exchange behavior of protonated diastereomeric dipeptides with the assumption that the basicities of the different basic sites in the diastereomers are identical. A study of the H/D exchange behavior of protonated diastereomers and the effect of chiral exchange reagents [*R*(+) and *S*(−) butan-2-ol] is in progress.

In addition, a study is in progress where the incorporated deuterium atoms are traced within the protonated dipeptides through collision-induced dissociation of mass-selected protonated dipeptides at different stages of the deuterium incorporation reaction.

Conclusions

The proton in the thermal protonated alkyldipeptides is found to be immobile. Efficient H/D exchange of all active hydrogens in the protonated dipeptides via reversible proton transfers in the reactions with relatively low basic D_2O and CH_3OD is considered to be assisted by specific multiple hydrogen bond formations in the intermediate reaction complexes in which the amide group plays a crucial role. The relative stabilization of competing intermediate reaction complexes has been related to the observed relative efficiencies of deuterium incorporation at the amino, amide, and carboxylic groups.

Unit H/D exchange efficiency and effective randomization of all active hydrogen atoms in the reaction complexes in the reactions with ND_3 indicate that specific multiple hydrogen bond formations are far less important in the reversible proton transfers with the relatively basic ammonia.

References

1. Vath, J. E.; Biemann, K. *Int. J. Mass Spectrom. Ion Processes* **1990**, *100*, 287–299.
2. Desiderio, D. M., Ed. *Mass Spectrometry of Peptides*; CRC Press: Boca Raton, FL, 1991.
3. Winger, B. E.; Light-Wahl, K. J.; Rockwood, A. L.; Smith, R. D. *J. Am. Chem. Soc.* **1992**, *114*, 5897–5898.
4. Cheng, X.; Fenselau, C. *Int. J. Mass Spectrom. Ion Processes* **1992**, *122*, 109–119.
5. Orlando, R.; Wu, Z.; Fenselau, C.; Cotter, R. J. *Int. J. Mass Spectrom. Ion Processes* **1991**, *111*, 27–40.
6. Renner, D.; Spiteller, G. *Biomed. Environ. Mass Spectrom.* **1988**, *15*, 75–77.
7. Grese, R. P.; Cerny, R. L.; Gross, M. L. *J. Am. Chem. Soc.* **1989**, *111*, 2835–2842.
8. Leary, J. A.; Zhou, Z.; Ogden, S. A.; Williams, T. D. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 473–480.
9. Wu, J.; Lebrilla, C. B. *J. Am. Chem. Soc.* **1993**, *115*, 3270–3275.
10. Yeh, R. W.; Grimley, J. M.; Bursey, M. M. *Biol. Mass Spectrom.* **1991**, *20*, 443–450.
11. Lias, S. G.; Liebman, J. F.; Levin, R. D. *J. Phys. Chem. Ref. Data* **1984**, *13*.
12. Scheiner, S.; Wang, L. *J. Am. Chem. Soc.* **1993**, *115*, 1958–1963.
13. Gorman, G. S.; Amster, I. J. *J. Am. Chem. Soc.* **1993**, *115*, 5729–5735.
14. Wu, Z.; Fenselau, C. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 863–866.
15. Zhang, K.; Zimmerman, D. M.; Chung-Phillips, A.; Cassady, C. J. *J. Am. Chem. Soc.* **1993**,*115*, 10812–10822.
16. Burlet, O.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 461–469.
17. Gard, E.; Green, M. K.; Bregar, J.; Lebrilla, C. B. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 623–631.
18. Freiser, B. S. In *Techniques for the Study of Ion Molecule Reactions*, Vol. 20; Farrar, J. M.; Saunders, W. H., Jr., Eds.; Wiley: New York, 1988; pp 61–118.
19. Marshall, A. G.; Schweikhard, L. *Int. J. Mass Spectrom. Ion Processes* **1992**, *118/119*, 37–70.
20. de Koning, L. J.; Fokkens, R. H.; Pinkse, F. A.; Nibbering, N. M. M. *Int. J. Mass Spectrom. Ion Processes* **1987**, *77*, 95–105.
21. Heck, A. J. R.; Drewello, T.; de Koning, L. J.; Nibbering, N. M. M. *Int. J. Mass Spectrom. Ion Processes* **1990**, *100*, 611–624.
22. Heck, A. J. R.; de Koning, L. J.; Pinkse, F. A.; Nibbering, N. M. M. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 406–414.

23. Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *J. Chem. Soc., Chem. Commun.* **1981**, 325–327.
24. Heck, A. J. R.; de Koning, L. J.; Nibbering, N. M. M. *J. Phys. Chem.* **1992**, *96*, 8870–8876.
25. Barmess, J. E.; Georgiadis, R. M. *Vacuum* **1983**, *33*, 149–166.
26. Bowers, M. T.; Neilson, P. V.; Kemper, P. R.; Wren, A. G. *Int. J. Mass Spectrom. Ion Phys.* **1977**, *25*, 103–116.
27. Su, T.; Su, E. C. F.; Bowers, M. T. *J. Chem. Phys.* **1978**, *69*, 2243–2250.
28. *Landolt–Börnstein Numerical Data and Functional Relationships in Science and Technology New Series, Group II: Atomic and Molecular Physics, Molecular Constants*, (a) Vol. 6; Hellwege, K.-H.; Hellewege, A. M., Eds.; 1974; (b) Vols. 14a and 14b; Hellwege, K.-H.; Hellewege, A. M., Eds.; 1982; (c) Vols. 19a and 19c; Hüttner, W., Ed.; 1990; Springer-Verlag, Berlin.
29. Miller, K. J. *J. Am. Chem. Soc.* **1990**, *112*, 8533–8542.
30. Hillenbrand, E. A.; Scheiner, S. *J. Am. Chem. Soc.* **1986**, *108*, 7178–7186.
31. Scheiner, S.; Hillenbrand, E. A. *J. Phys. Chem.* **1985**, *89*, 3053–3060.
32. Cybulski, S. M.; Scheiner, S. *J. Am. Chem. Soc.* **1989**, *111*, 23–31.
33. Scheiner, S. *J. Chem. Phys.* **1982**, *77*, 4039–4050.
34. Lau, Y. K.; Nishizawa, K.; Tse, A.; Brown, R. S.; Kebarle, P. *J. Am. Chem. Soc.* **1981**, *103*, 6291–6295.