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FOCUS: BIO-ION CHEMISTRY: INTERACTIONS OF BIOLOGICAL IONS WITH IONS, MOLECULES, SURFACES, ELECTRONS, AND LIGHT: RESEARCH ARTICLE

Enhanced Reactivity in Nucleophilic Acyl Substitution Ion/Ion Reactions Using Triazole-Ester Reagents

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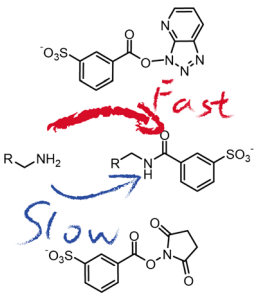

Abstract. The acyl substitution reactions between 1-hydroxy-7-aza-benzotriazole (HOAt)/1-hydroxy-benzotriazole (HOBt) ester reagents and nucleophilic side chains on peptides have been demonstrated in the gas phase via ion/ion reactions. The HOAt/HOBt ester reagents were synthesized in solution and ionized via negative nano-electrospray ionization. The anionic reagents were then reacted with doubly protonated model peptides containing amines, guanidines, and imidazoles in the gas phase. The complexes formed in the reaction cell were further probed with ion trap collision induced dissociation (CID) yielding either a covalently modified analyte ion or a proton transfer product ion. The covalent reaction yield of HOAt/HOBt ester reagents was demonstrated to be higher than the yield with *N*-hydroxysuccinimide (NHS) ester reagents over a range of equivalent conditions. Density functional theory (DFT) calculations were performed with a primary amine model system for both triazole-ester and NHS-ester reactants, which indicated a lower transition state barrier for the former reagent, consistent with experiments. The work herein demonstrates that the triazole-ester reagents are more reactive, and therefore less selective, than the analogous NHS-ester reagent. As a consequence, the triazole-ester reagents are the first to show efficient reactivity with unprotonated histidine residues in the gas phase. For all nucleophilic sites and all reagents, covalent reactions are favored under long time, low amplitude activation conditions. This work presents a novel class of reagents capable of gas-phase conjugation to nucleophilic sites in analyte ions via ion/ion chemistry.

Keywords: Ion/ion reactions, HOAt/HOBt ester, Nucleophilic attack

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Introduction

Tandem mass spectrometry is a well-established approach for obtaining structural information from ions derived from biomolecules. The structural information derived from a molecule of interest is highly related to the gas-phase ion type that is subjected to activation [1]. Gas-phase ion/ion reactions have proven to be particularly useful for converting ions from one type to another. Examples include altering charge states and polarities with proton transfer reactions [2–5], converting multiply protonated species to radical hypervalent species via electron transfer reactions [6–8], and removing or replacing metal ions [9, 10]. In addition to the small charged particle transfer ion/ion reactions just mentioned, ion/ion reactions that involve selective covalent chemistry have been developed, thereby enabling the chemical modification of analyte ions in the gas phase. Examples of gas-phase covalent modification reactions include Schiff base chemistry between amines and aldehydes [11], Click chemistry between azides and alkenes [12], and oxidation chemistry between periodate and various reductive groups [13]. A reaction that is particularly useful for protein and peptide modification is the reaction between *N*-hydroxysuccinimide (NHS) esters and various nucleophiles commonly present in polypeptides such as neutral amines [14], neutral guanidines [15], and carboxylates [16]. The reaction involves the covalent attachment of an acyl group to the nucleophile via the displacement of an NHS leaving group, resulting in the formation of an amide on the N-terminal amine or lysine side chain [14], an *N*-acyl guanidine on arginine side chains [15],

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or an anhydride on the C-terminus or side chain carboxylates [16].

The gas-phase ion/ion reactions that result in new covalent bond formation have been noted to proceed through a long-lived complex derived from the condensation of the oppositely charged reactant ions. Such complexes are usually sufficiently long-lived that they become stabilized by collisions in the relatively high pressure environment of an electrodynamic ion trap (e.g., 1–10 mTorr). Subsequent ion activation of the complex leads to observation of the products of the covalent reaction. However, charge transfer without covalent bond formation, usually in the form of proton transfer, is a competing reaction pathway in the break-up of an ion/ion complex. Simple charge transfer therefore constitutes a "side-reaction" when the selective formation of a covalent bond is the desired objective. The observed efficiency of the covalent reaction is governed by the energies and entropies of the barriers on the potential energy surface (PES) for the competing reactions (i.e., proton transfer versus covalent reaction) and by the activation conditions [17]. While NHS esters have proven to be relatively efficient reagents for covalent bond formation, proton transfer is often highly competitive with covalent reaction. It is, therefore, of interest to explore the use of reagents that may be comprised of better leaving groups in order to increase the fraction of complexes that dissociate to yield the covalent reaction products. Triazoles, such as 1-hydroxy-benzotriazole (HOBt) and 1-hydroxy-7-aza-benzotriazole (HOAt), are a group of activating reagents for carboxylic acid functional groups and were originally used in solid-phase peptide synthesis [18]. They are also commonly used as carboxylate activators [19] to facilitate amide bond formation between amines and carboxylates. Here, we describe a novel gas-phase ion/ion reaction between HOAt/HOBt ester reagents and nucleophiles. Similar to NHS ester reagents, HOAt/HOBt ester reagents react by covalently attaching an acyl group to certain nucleophiles with the loss of neutral HOAt/HOBt molecules (i.e., leaving groups). Density functional theory (DFT) calculations on model systems that have been conducted to compare reaction barrier heights for HOAt- and NHS-based reagents suggest that the former can be expected to react faster than the latter. As described herein, HOAt/HOBt ester reagents have also been demonstrated experimentally to be more reactive than NHS ester reagents, which leads to greater reaction efficiencies as well as reactivity with weaker nucleophiles, such as histidine side-chains.

Experimental

Materials

N-hydroxysulfosuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) were purchased from Thermo Fisher Scientific (Rockford, IL, USA). 1-Hydroxybenzotriazole hydrate (HOBt·xH_2O), acetic anhydride, and sodium 3-sulfobenzoate were purchased from Sigma Aldrich (St. Louis, MO, USA). 1-Hydroxy-7-azabenzotriazole (HOAt) was purchased from ABBLIS Chemicals LLC (Houston, TX, USA). The peptides KGAGGKGAGGKL, RGAGGRGAGGRL, and Ac-HGAGGHGAGGHL-OMe were custom synthesized by NeoBioLab (Cambridge, MA, USA). Methanol, dimethylformamide (DMF), and glacial acetic acid were purchased from Mallinckrodt (St. Louis, MO, USA). Water (HPLC grade), and acetonitrile were purchased from Fisher Scientific (Hampton, NH, USA).

Reagents sulfo-benzoyl-HOAt, sulfo-benzoyl-HOBt, and sulfo-benzoyl-NHS were prepared by combining 10 uL of 100 mM EDC, 100 mM sodium 3-sulfobenzoate, and 100 mM HOAt/HOBt/NHS in DMF. The solution was then diluted 100× with acetonitrile for nano electrospray ionization (nESI). Water is avoided as the reagents hydrolyze within a few hours at room temperature.

The N-terminus of the peptide RGAGGRGAGGRL was protected by combining the peptide with excessive acetic anhydride in pH 8 aqueous ammonium bicarbonate buffer. The solution was incubated for 2 h at room temperature. The reaction was quenched by the addition of glacial acetic acid to drop the pH to 2–3. All peptide solutions for positive nano-electrospray (nESI) were prepared in a 50/50 (vol/vol) solution of methanol/water at a concentration of ~1 mM.

Mass Spectrometry

Experiments were performed using a QTRAP 4000 hybrid triple quadrupole/linear ion trap and a TripleTOF 5600 System (SCIEX, Concord, ON, Canada). Both instruments were modified for ion/ion reactions; modifications were similar to those published previously [20, 21]. Anions and cations were sequentially injected into the instruments via alternately pulsed nESI [22], isolated in transit through Q1, and transferred into the Q2/q2 reaction cell. The ions were mutually stored in the reaction cell for a defined period of time ranging from 10 to 500 ms. In the QTRAP 4000, reaction products were transferred to Q3, where they were further probed via resonance collisional induced dissociation (CID) and mass-analyzed using mass-selective axial ejection (MSAE) [23]. In the TripleTOF 5600, the reaction products were probed directly in Q2 by either resonance CID or dipolar direct current (DDC) CID [24, 25] and then mass-analyzed using the orthogonal TOF.

Rate Measurements

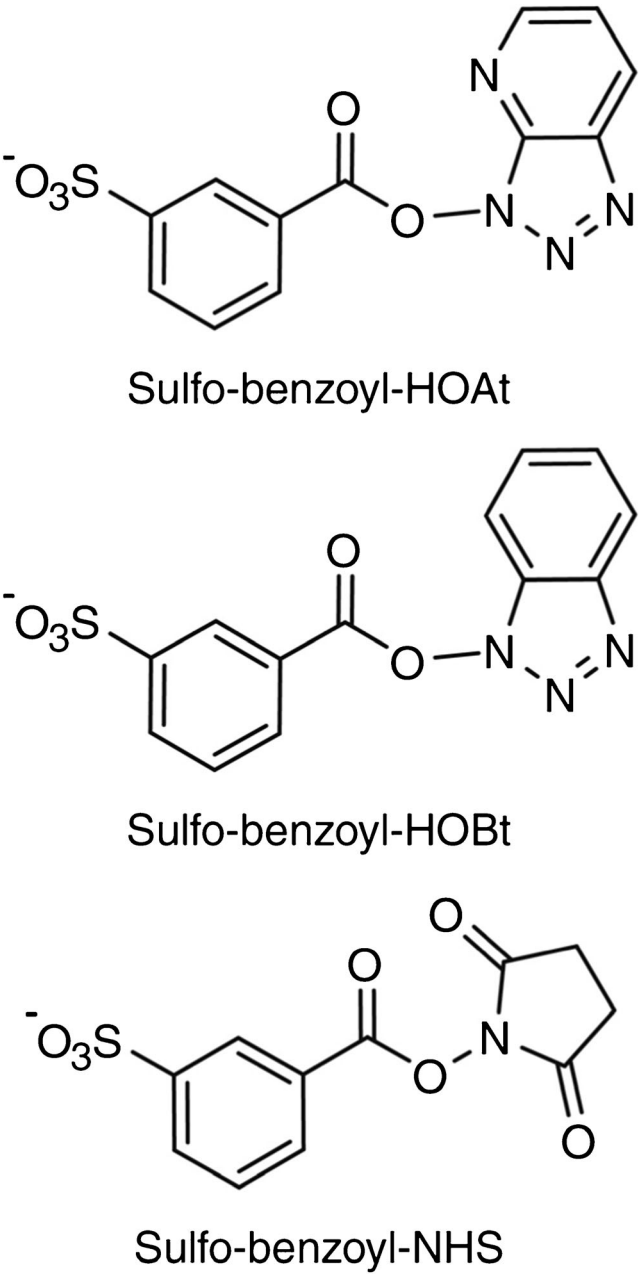
The ion/ion reaction rate measured via DDC kinetic experiments has been described previously [17]. Briefly, the dissociation of the precursor ion follows pseudo-first-order reaction kinetics. Therefore, dissociation rates can be determined from product ion spectra acquired as a function of activation time at various DDC amplitudes. Multiple pathways may exist in the dissociation process and compete with each other. The rate of appearance of each product can be calculated based on the partitioning between the two product peaks in the product spectrum and the overall dissociation rate.

DFT Calculations

DFT calculations were used to characterize reaction pathways. Optimizations and zero-point corrected energies (ZPE) were calculated using the Gaussian 09 package [26] at the unrestricted B3LYP/6-311G++(d,p) level of theory. Since the model molecules are small, all structures were directly optimized in Gaussian 09. Transition states were searched using the QST3 option and confirmed with intrinsic reaction coordinate (IRC) calculations [27]. All stationary points have been subjected to frequency calculations and identified as local minima (zero imaginary frequencies) or transition states (one imaginary frequency). See Supplementary Material for the coordinates of all of the calculated structures.

Results and Discussion

Lysine, arginine, and histidine are common basic residues in proteins. They are comprised of the amine, guanidine, and imidazole nucleophilic groups, respectively. Amines and guanidines have been shown to be highly reactive with NHS ester-based reagents [14, 15], while minimal imidazole reactivity has been observed. This work compares the use of HOAt-ester and HOBt-ester reagents with an analogous NHS ester reagent to determine if enhanced reactivity can be expected with these reagents in the gas phase. The structures of the HOAt/HOBt-based reagents and the analogous NHS-ester reagent used in this work are shown in Scheme 1. A sulfonate group is included on the benzoyl moiety of each reagent to facilitate the formation of a long-lived ion/ion complex via a strong electrostatic interaction with a protonated site on the analyte ion. The gas-phase reactions are expected to proceed similarly for all three reagents in that they all form complexes with multiply protonated peptide cations via the electrostatic interaction just mentioned. The nucleophilic groups on the peptides (e.g., primary amines), initiate an attack on the carbonyl carbon of the sulfobenzoyl group resulting in the formation of covalent bonds, followed by the loss of neutral HOAt/HOBt/NHS molecules. The final cationic product is the same in all cases with the only difference being the leaving group and the associated PES. Here, model peptides with specific residues are reacted with both NHS ester-based reagents and HOAt/HOBt ester-based reagents to compare reactivities.



Scheme 1. Structures of the reagents used in this work

For a given analyte ion in reactions with reagents such as those shown in Scheme 1, the likelihood for the formation of a long-lived ion/ion complex is very similar because the reagents are of similar physical size and they share the same anionic functional group (i.e., a sulfonate). The key factor determining the relative reactivities of different reagents for generating a covalent bond with the analyte is the competition between simple proton transfer, for which no covalent reaction occurs, and covalent reaction upon activation of the ion/ion complex (see Figure 1). For a given analyte ion, the barrier to proton transfer is expected to be very similar for each reagent because it is largely determined by the strength of the electrostatic interaction between the sulfonate of the reagent and a protonated site on the analyte ion. The main difference between the reagents is expected to arise from the barrier leading to covalent reaction. Given the relatively weak interaction of the leaving group with the ionic product of the covalent reaction, it is expected that the rate-determining step for the observation of the covalent reaction product will be passage over the transition state for the reaction. Therefore, the relative propensities for proton transfer versus covalent reaction in comparing different reagents should be most sensitive to differences in the transition states for covalent reaction. Given the very similar mechanisms expected to be involved (i.e., nucleophilic attack on the carbonyl carbon of the ester), similar transition state entropies can be expected. The main difference is, therefore, expected to be manifest in the energies of the transition states. Under these

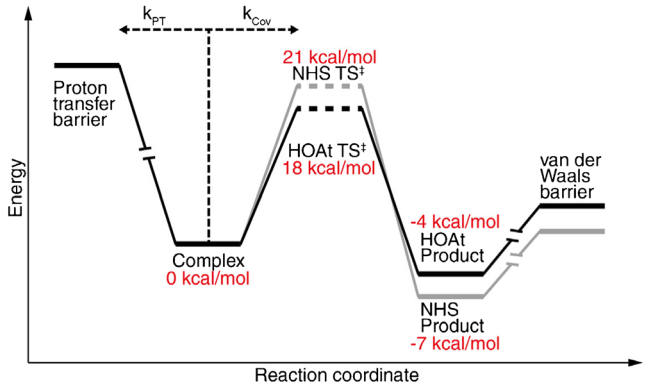


Figure 1. The reaction coordinate displaying the energies of reaction barriers and products of various pathways. The energies are calculated relative to the electrostatic ion/ion complex. The pathway involving HOAt ester reagent is plotted in black, whereas that of the NHS ester reagent is plotted in gray

circumstances, even in the absence of a competing proton transfer process, it is possible to evaluate the reactivities of the various reagents via measurement of the ion/ion complex dissociation rates under a fixed set of conditions (see below).

DFT calculations using the Gaussian 09 package were performed on simple model systems to compare the energy requirements associated with the covalent reactions of HOAt ester and NHS ester reagents with primary amines. Here, neutral methyl amine was used to represent the analyte and a neutral acetyl HOAt/NHS ester was used to represent the reagent. The energy of the electrostatic complex was assigned 0 kcal/mol. The two reaction pathways are indicated on either side of the complex in Figure 1, although no modeling of the proton transfer process was performed, as this barrier is expected to be similar for all reagents with a given analyte ion. For reference, the proton transfer barrier between a doubly protonated amine system to a sulfonate has been reported previously as 28 kcal/mol [17]. In the covalent modification pathway, to the right of the complex, the electrostatic complex overcomes a transition state barrier to yield the product complex, then breaks relatively weak van der Waals interactions between the modified peptide and the neutral HOAt/NHS leaving group to yield the covalently modified product. The transition state barrier of the HOAt ester reagent was determined to be 18 kcal/mol whereas that of the NHS ester pathway requires 21 kcal/mol of energy. Interestingly, while the calculations indicate that the NHS ester products are favored thermodynamically by 3 kcal/mol, they also suggest that the HOAt ester products should be expected to be favored on kinetic grounds. As CID conducted in tandem mass spectrometry is under kinetic control, this calculation suggests that HOAt esters should be more reactive than NHS ester reagents. Calculations were also conducted using benzoyl HOAt ester and sulfo-benzoyl HOAt ester reagents resulting in differences in transition state energies of ≤0.9 kcal/mol and product complex energies of ≤1.1 kcal/mol. All calculations are consistent with higher reactivity for HOAt esters. Results of the calculations are provided in SI Table 1 as well as listings of the coordinates for calculated structures.

We recently published an approach to characterizing the competition between proton transfer and covalent reaction based on the measurement of CID kinetics of relevant ion/ion complexes [17]. It is based on measuring the overall dissociation rate, k_{diss}, as well as the rates for proton transfer, k_{PT}, and covalent bond formation, k_{Cov}, where $k_{diss} = k_{PT} + k_{Cov}$, as a function of ion trap collisional activation conditions. For each ion activation amplitude, CID spectra are collected as a function of time while monitoring the rate of loss of the precursor ion as well as the rates of appearance of the proton transfer and covalent reaction (i.e., the ion generated by loss of the leaving group) products. By attaching the strongly interacting sulfonate group to the portion of the reagent that remains bonded to the analyte ion, we generate the condition in which the rate-determining step for observation of the loss of the leaving group is the barrier for covalent reaction (i.e., the '**case 1**' condition described in our earlier work [17]). The following sections relate our findings with respect to dissociation rates of ion/ion complexes comprised of peptide ions with the three different reagents, as a probe of relative reactivity. We employ DDC as the activation method for our detailed kinetics measurements because it is a broad-band activation approach and is, therefore, much less tuning-intensive relative to resonance ion acceleration based on the use of a supplementary AC signal. However, based on the finite time required to reach the full DC voltage in our DDC experiments, rates greater than roughly 30 s^{-1} could not be measured reliably because most, if not all, of the precursor ions were depleted before the final DC level was reached. It is straightforward to achieve higher dissociation rates using resonance AC CID. For this reason, a few spectra obtained using AC CID at relatively high dissociation rates are included here to illustrate the partitioning between proton transfer and covalent reaction under such conditions.

Amine Reactivity

The deprotonated sulfo-benzoyl-HOAt and sulfo-benzoyl-NHS reagents were each reacted with the doubly protonated peptide KGAGGKGAGGKL. In this experiment, all strong nucleophilic sites on the peptide are primary amines (i.e., the N-terminus or lysine ε-amino group). Hence, this peptide serves as a model to test relative reactivities of the reagents with primary amines. After isolation, the complexes were subjected to DDC CID at various voltages and times in order to generate series of rate measurements for each reagent with the model peptide. The net dissociation rate, k_{diss}, and the rates for the two dissociation pathways, k_{PT} for proton transfer and k_{Cov} for loss of the leaving group, are plotted versus the DDC voltage in Figure 2. Solid markers represent data obtained for the HOAt reagent, whereas the hollowed markers represent the data obtained for the NHS reagent. Two main observations can be made from this data set. First, the rate of the proton transfer pathway, represented by the red lines for each reagent, are very close to zero for all DDC voltages, indicating that the yield of the covalent reaction is almost 100% for both the HOAt ester reagent and the NHS ester reagent on the DDC CID time scale,

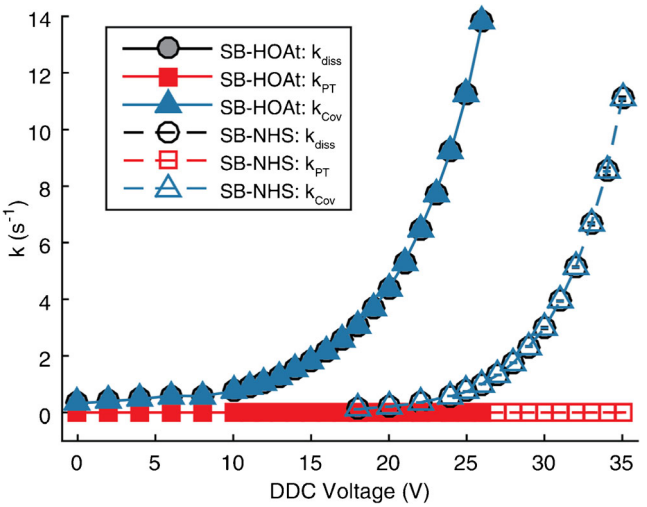


Figure 2. Dissociation rates of ion/ion complexes involving the doubly protonated peptide KGAGGKGAGGKL with the deprotonated sulfo-benzoyl-HOAt or deprotonated sulfo-benzoyl-NHS. The complex dissociation rate, k_{diss} (black), proton transfer (PT) reaction rate, k_{PT} (red), and covalent (Cov.) modification reaction rate, k_{Cov} (blue), are plotted versus DDC voltage. The solid markers represent the sulfo-benzoyl-HOAt group, whereas the hollowed markers represent the sulfo-benzoyl-NHS group. The error bar represents one standard deviation of the fitting parameters

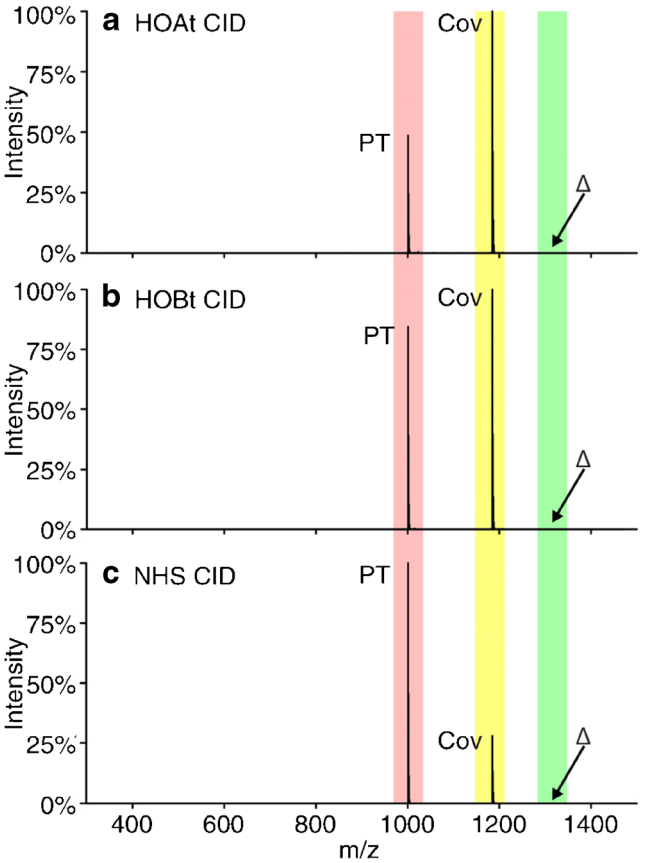


Figure 3. Single frequency AC ion trap CID of the ion/ion complexes derived from the attachment of doubly protonated KGAGGKGAGGKL to **(a)** the sulfo-benzoyl-HOAt anion, **(b)** the sulfo-benzoyl-HOBt anion, and **(c)** the sulfo-benzoyl-NHS anion. The *m/z* regions of the proton transfer products are shaded red, the *m/z* regions of the covalent products are shaded yellow, and the *m/z* regions of the ion/ion complexes are shaded green. Under these conditions, the entire precursor complex is dissociated

which is 30 to 5000 ms in this experiment. Secondly, the rate of the covalent reaction is significantly faster with the HOAt reagent compared with the NHS ester reagent at each DDC amplitude, which indicates that the transition state barrier for the HOAt ester reagent is lower than that of the NHS ester reagent. We noted that even at 0 V DDC, a non-zero complex dissociation rate that goes through the covalent pathway was observed for the HOAt ester reagent. This shows that the reaction between the peptide and HOAt ester reagent proceeds at an observable rate under the near-thermal trapping conditions used in this work. All this evidence indicates that HOAt ester reagents are more reactive than NHS ester reagents when exposed to primary amines.

Previous work has indicated that the transition state for the proton transfer channel is 'looser' than that for covalent reaction using NHS-ester reagents [17]. Therefore, at higher dissociation rates, proton transfer can become more competitive. Due to the relatively slow rise-time associated with the DDC experiments in our instrument, it is not possible to obtain reliable DDC data at dissociation rates significantly higher than those shown in Figure 2. However, single frequency AC CID at relatively high amplitudes and short times can be effected to demonstrate product ion partitioning at dissociation rates higher than those of Figure 2. Figure 3 compares single-frequency ion trap CID product ion spectra of the complexes generated from doubly protonated KGAGGKGAGGKL with singly deprotonated sulfo-benzoyl-HOAt (Figure 3a), sulfo-benzoyl-HOBt (Figure 3b), and sulfo-benzoyl-NHS (Figure 3c) obtained using an amplitude of 150 mV at a common pseudo-potential well depth, which results in dissociation rates in excess of 10^3 s^{-1}. The activation time (100 ms) was sufficiently long in each case to completely deplete the precursor ion population. At the higher dissociation rates, proton transfer is competitive in all three cases with the relative abundances of the proton transfer and covalent reaction products being indicative of relative reactivities. The HOAt and HOBt reagents are both clearly more reactive than the NHS reagent with the HOAt reagent being the most reactive of the three.

Guanidine Reactivity

The peptide Ac-RGAGGRGAGGRL was used to compare the relative reactivities of the reagents with guanidine, the arginine side chain functionality. Here the N-terminus was protected to rule out the possible participation of N-terminal amine reactivity. DDC dissociation kinetics were determined for the ion/ion complexes derived from the doubly protonated peptide with the sulfo-benzoyl-HOAt anion and with the sulfo-benzoyl-NHS anion (see Figure 4). In analogy with the data for the lysine containing model peptide (KGAGGKGAGGKL), covalent

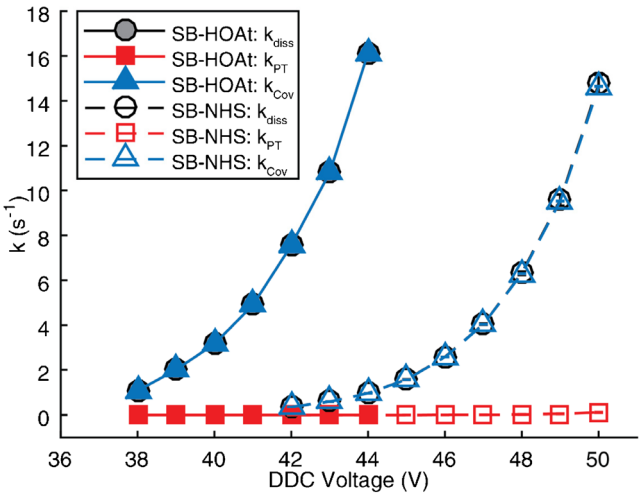


Figure 4. Dissociation rates of ion/ion complexes involving the doubly protonated peptide Ac-RGAGGRGAGGRL with the deprotonated sulfo-benzoyl-HOAt or deprotonated sulfo-benzoyl-NHS. The complex dissociation rate, k_{diss} (black), proton transfer (PT) reaction rate, k_{PT} (red), and covalent (Cov.) modification reaction rate, k_{Cov} (blue), are plotted versus DDC voltage. The solid markers represent the sulfo-benzoyl-HOAt group, whereas the hollowed markers represent the sulfo-benzoyl-NHS group. The error bars represent one standard deviation of the fitting parameters

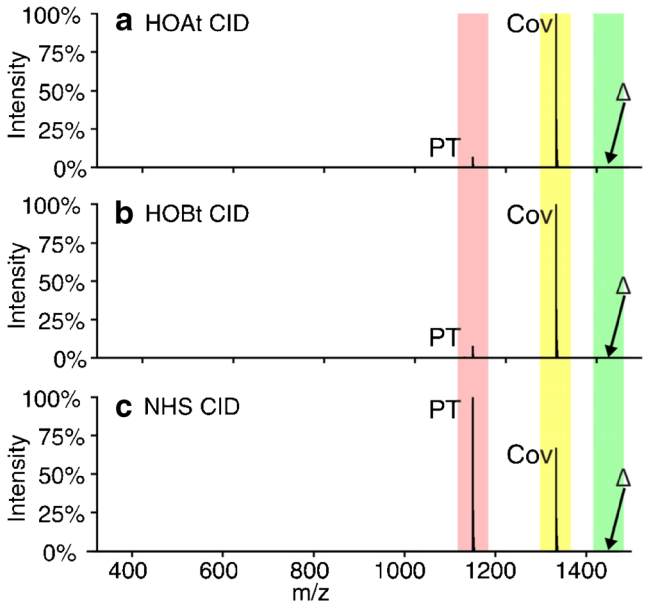


Figure 5. Single frequency AC ion trap CID of the ion/ion complexes derived from the attachment of doubly protonated Ac-RGAGGRGAGGRL to **(a)** the sulfo-benzoyl-HOAt anion, **(b)** the sulfo-benzoyl-HOBt anion, and **(c)** the sulfo-benzoyl-NHS anion. The *m/z* regions of the proton transfer products are shaded red, the *m/z* regions of the covalent products are shaded yellow, and the *m/z* regions of the ion/ion complexes are shaded green. Under these conditions, the entire precursor complex is dissociated

bond formation dominates over the entire DDC voltage range used in this work and the absolute rates are greater for the sulfo-benzoyl-HOAt reagent than for the sulfo-benzoyl-NHS reagent at all DDC voltages.

The DDC conditions used to generate the kinetic data of Figure 4 gave rise to very little proton transfer. However, as with the cases involving the lysine model peptide (see Figure 3), dissociation of the arginine model peptide complexes at higher rates using single frequency ion trap CID gave rise to some proton transfer, as shown in Figure 5. Data are shown for complexes involving doubly protonated Ac-RGAGGRGAGGRL with the sulfo-benzoyl-HOAt anion, the sulfo-benzoyl-HOBt anion, and the sulfo-benzoyl-NHS anion using an amplitude of 250 mV for 100 ms. In all three cases, the complex ion population was completely depleted well before 100 ms with k_{diss} values in excess of 10^3 s^{-1}. For the reagents comprised of the triazole ester reagents, relatively little proton transfer was noted even under these higher rate conditions, whereas proton transfer led to the most abundant product ion in the case of the NHS ester. We note that the barrier to proton transfer is likely to be significantly higher for the complexes comprised of Ac-RGAGGRGAGGRL than for those comprised of KGAGGKGAGGKL due to a stronger electrostatic interaction between protonated arginine with sulfonate versus that for protonated lysine with sulfonate [17]. For this reason, the ratios noted in Figures 3 and 5 cannot be compared directly to draw conclusions regarding the relative reactivities of a given reagent with lysine or arginine. Rather, these results are restricted to the comparison of the reagents in reaction with a given peptide. As with the lysine case, the triazole-ester reagents are clearly more reactive with the guanidine group than is the NHS-ester reagent.

Imidazole Reactivity

The third nucleophilic site examined in this work is the imidazole side chain of histidine. The peptide Ac-HGAGGHGAGGHL-OMe was used as the model analyte. Here both the N-terminus and the C-terminus were protected to avoid reactions at the amine group and the carboxylic acid group. Carboxylic acids are relatively weak nucleophiles and are generally considered unreactive with NHS ester reagents in the presence of lysine or arginine residues. To avoid ambiguity, the C-terminus was protected in this case because the nucleophilicity of histidine residues is also relatively low. Figure 6 summarizes the dissociation rate data for the complexes derived from doubly protonated Ac-HGAGGHGAGGHL-OMe and the reagents derived from sulfo-benzoyl-HOAt or deprotonated sulfo-benzoyl-NHS as a function of DDC voltage. In this case, significant fractions of both proton transfer and covalent bond formation were noted over the DDC voltage range examined. The inserts to the right of the plots summarize the relative product partitioning between the two channels as a function of DDC voltage. In the case of the sulfo-benzoyl-HOAt reagent, covalent bond formation was strongly dominant at the lowest DDC voltages, which corresponds to the lowest dissociation rates, whereas proton transfer was observed to dominate at the highest DDC voltages (i.e., highest DDC rates).

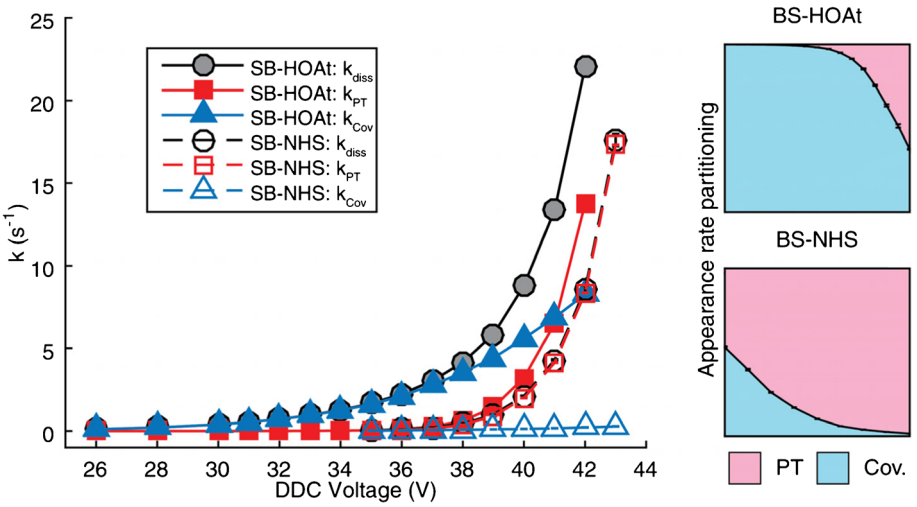


Figure 6. Dissociation rates of ion/ion complexes involving the doubly protonated peptide Ac-HGAGGHGAGGHL-OMe with the deprotonated sulfo-benzoyl-HOAt or deprotonated sulfo-benzoyl-NHS. The complex dissociation rate, k_{diss} (black), proton transfer (PT) reaction rate, k_{PT} (red), and covalent (Cov.) modification reaction rate, k_{Cov} (blue), are plotted versus DDC voltage. The solid markers represent the sulfo-benzoyl-HOAt group, whereas the hollowed markers represent the sulfo-benzoyl-NHS group. The error bars represent one standard deviation of the fitting parameters. The fractional partitioning between the proton transfer pathway and the covalent pathway as a function of DDC voltage is summarized in the inserts at the right. The cyan area shows the covalent yield, whereas the pink area shows the proton transfer yield

In the case of the sulfo-benzoyl-NHS reagent, covalent reaction was observed at a measureable extent only at the lowest DDC voltages. Proton transfer dominated at all DDC voltages and was the only process observed at the highest DDC voltages. Under single frequency ion trap CID conditions similar to those used to generate the data of Figures 3 and 5, proton transfer was the only process observed for all three reagents (data not shown). These data are consistent with past observations that histidine shows little or no reactivity with NHS-ester reagents. Indeed, only under very slow activation conditions is any evidence for reactivity noted. On the other hand, these results show that slow activation conditions can lead to efficient covalent modification of histidine residues using the more reactive triazole ester reagents. However, covalent reaction is not competitive with proton transfer under more energetic activation conditions.

Conclusions

This work demonstrates gas-phase nucleophilic ion/ion reactions between triazole-ester reagents and various common neutral nucleophiles. Similar to other gas-phase ion/ion reactions that lead to selective covalent bond formation, this nucleophilic attack proceeds via a long-lived complex facilitated by the strong electrostatic interaction between anchoring groups on the analyte and the reagent (i.e., a sulfonate group on the reagent and a protonated site on the analyte). All of the experimental results in this study are consistent with triazole-ester reagents being more reactive than the analogous NHS-ester reagent, which is also consistent with a lower transition state barrier obtained via DFT calculations with a model primary amine. The triazole-ester reagents are the first to show efficient gas-phase reactivity with an imidazole, which makes these reagents less 'selective' than analogous NHS-ester reagents. In all cases, regardless of reagent or nucleophile, activation conditions can play a major role in the observed reaction efficiency. The main competing reaction, proton transfer, is increasingly favored as the activation conditions become more energetic. These nucleophilic displacement reactions are, therefore, most efficient using conditions that give rise to relatively low dissociation rates (e.g., low amplitudes and long times in ion trap CID).

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