Chemical Papers (2022) 76:879–888
https://doi.org/10.1007/s11696-021-01906-2

ORIGINAL PAPER

Chemical decarboxylation kinetics and identification of amino acid standards by benchtop NMR spectroscopy

Angelika Czajkowska[1] · Defne Ilayda Dayi[1] · Helga Weinschrott[1] · Hans-Peter Deigner[1,2,3] · Magnus S. Schmidt[1,4]

Received: 5 August 2021 / Accepted: 24 September 2021 / Published online: 8 October 2021

Abstract

In this paper, we examined the competence of amino acids as standards for instrumental biochemical analysis. The chosen amino acids were first dissolved in various aquatic solutions and then measured in a benchtop NMR spectrometer, which is not a common choice in such analytical investigations. Analysis by mass spectrometry was used in addition. As part of these investigations, we examined and determined the stability of the amino acids ornithine, glutamic acid, alanine, glycine, proline, pyroglutamic acid, phenylalanine and trans-4-hydroxy-D-proline under critical basic and acidic pH conditions and under various other conditions. We observed that not all solutions of the amino acid standards remain stable under the given conditions and a chemical transformation takes place. Given our findings by mass spectroscopy, additional kinetic measurements were carried out with the benchtop NMR spectrometer. We discovered that pyroglutamic acid becomes unstable under basic conditions and decarboxylates to pyrrolidone.

Keywords Amino acids · NMR spectroscopy · Chemical transformation · ^{1}H NMR · Kinetics · Decarboxylation

Introduction

Amino acids describe the vast, complex and diverse group of molecules that include both an amino and a carboxyl group (Lu and Freeland 2016). They are the building blocks of proteins and can be considered the most important structures in the creation and proper function of life itself. The mystery of how amino acids were created in the primitive and extreme conditions of early earth moves scientist to this day (Miller 1953). It was discovered that not all prebiotically synthesized amino acids ended up in the standard alphabet and that not all structures of the standard alphabet could have been prebiotically synthesized. The standard alphabet consists of 20 amino acids abbreviated into a three letter or one letter code, which is intended to reduce the size of the data files needed to describe the sequence of the amino acids within a protein. Some amino acids, like proline, do not even meet the minimal requirements of an amino acid, because of its cyclic structure. The carbon here binds back to the 'backbone' nitrogen, generating a secondary amino group instead of a primary amine (Lu and Freeland 2016). Therefore, the analysis of those structures, free amino acids and proteins plays an important role in today's chemistry and biochemistry as well as in various other fields. Most suitable

✉ Magnus S. Schmidt
magnus.schmidt@hs-furtwangen.de

Angelika Czajkowska
angelika.czajkowska@hs-furtwangen.de

Defne Ilayda Dayi
defne.ilayda.dayi@hs-furtwangen.de

Helga Weinschrott
Helga.Weinschrott@hs-furtwangen.de

Hans-Peter Deigner
Hans-Peter.Deigner@hs-furtwangen.de

1 Institute of Precision Medicine, Medical and Life Sciences Faculty, Furtwangen University, Jakob-Kienzle-Str. 17, 78054 VS-Schwenningen, Germany

2 Fraunhofer Institute IZI Leipzig, EXIM Department, Schillingallee 68, 18057 Rostock, Germany

3 Faculty of Science, Associated Member of Tuebingen University, Auf der Morgenstelle 8, 72076 Tübingen, Germany

4 Organic and Bioorganic Chemistry Labs, Furtwangen University, Jakob-Kienzle-Str. 17, 78054 VS-Schwenningen, Germany

for such structure and functional group analysis is NMR spectroscopy.

Chemical shifts in nuclear magnetic resonance (NMR) measure the locally induced magnetic field on a nucleus that is sensitive to the chemical environment (Friebolin 2011). They can be used as fingerprints for detailing the chemical composition of condensed matter. The analysis of chemical shifts plays an increasingly important role in structural peptide and protein analyses (Wishart et al. 1995). A systematic study of the NMR chemical shifts of free amino acids is, therefore, highly desirable and could lay the ground for studies of protein shifts in complex biological environments. The position of the resonance is the first step in the NMR-based structure determination of proteins. Because of limiting protein concentrations or their low stabilities, a new strategy centered around the evaluating of the NMR spectra of individual amino acids has been established (Bellstedt et al. 2013). It was found that, when calculating the NMR shifts, the effect of the environment on the chemical shifts of these amino acids corresponds to the influence of the intermolecular hydrogen bonds or electrical dipole field effects (Yoon et al. 2004). It was also found that in aqueous solutions the pH influences the phenomenon of chemical exchange. The chemical shifts are sensible toward specific intermolecular effects between the labile protons of amino acids and the ones of water (Khallouk et al. 2005).

The NMR spectroscopy with the benchtop device can be used for such analytic work and offers many advantages. Among other things, it has low acquisition and operating costs, is easy to use, has a high spectral resolution, no cryogens needed, is easy to use via Windows PC and is mobile and compact. Following these advantages, the device also allows smaller organizations the entry into this analysis method and could be used as a fully automated analyzer. However, negative aspects, such as the spectral width, must also be taken into account. The lower field strength of the systems also often requires the use of model-based approaches for spectra evaluation (Tables 1, 2).

This motivated the present work on the behavior and stability of amino acids and amino acid standards in benchtop NMR spectroscopy. We discovered that such solutions are potentially unstable under various conditions (Airaudo et al. 1987), and therefore, we have conducted certain investigations. By using an internal standard, the concentrations of amino acid standard solutions which in solid state are difficult to weigh were determined by quantitative NMR spectroscopy (Yamazaki et al. 2017). For these experiments, we choose six naturally occurring L-amino acids and the non-proteinogenic ornithine and

Table 1 Initial weighted masses (m) and concentration (c) of the amino acids in buffer, water, deuterium chloride (DCl) and sodium hydroxide (NaOD)

Amino acids	m in water [mg]	c in water [mmol/L]	m in buffer [mg]	c in buffer [mmol/L]	m in DCl [mg]	c in DCl [mmol/L]	m in NaOD [mg]	c in NaOD [mmol/L]
Ornithine	128.8	76.4	63.8	75.7	25.3	75	25.4	75.3
Glutamic acid	111.1	75.5	58.7	79.8	22.3	75.8	22.9	77.8
Alanine	68.4	76.8	34.5	77.4	14.4	80.8	14	78.6
Glycine	57.7	76.9	30.2	80.5	12.1	80.6	12.5	83.3
Proline	87.3	75.8	49.2	85.5	18.2	79	18.2	79
Pyroglutamic acid	98.4	76.2	49	75.9	19.8	76.7	20.2	78.2
Phenylalanine	125.2	75.8	62.8	76	24.9	75.4	25.4	76.9
Trans-4-hydroxy-D-proline	75.6	72.1	25.5	64.8	19.8	75.5	20.5	78.2

Table 2 Measured pH value of the amino acids in water

Amino acid	1.pH	2.pH	3.pH	Arithmetic mean	SD	Variance
Alanine	6.10	6.12	6.00	6.07	$5.25 * 10^{-2}$	$2.76 * 10^{-3}$
Phenylalanine	5.36	5.43	5.33	5.37	$4.19 * 10^{-2}$	$1.76 * 10^{-3}$
Glycine	5.63	5.76	5.92	5.77	$1.19 * 10^{-1}$	$1.41 * 10^{-2}$
Glutamic acid	3.09	3.09	3.09	3.09	0	0
Proline	5.97	5.83	5.95	5.92	$6.18 * 10^{-2}$	$3.82 * 10^{-3}$
Ornithine	5.32	5.31	5.24	5.29	$3.56 * 10^{-2}$	$1.27 * 10^{-3}$
Pyroglutamic acid	2.09	2.13	2.14	2.12	$2.16 * 10^{-2}$	$4.67 * 10^{-4}$
Trans-4-Hydroxy-D-Prolin	4.33	4.39	4.40	4.37	$3.09 * 10^{-2}$	$9.56 * 10^{-4}$

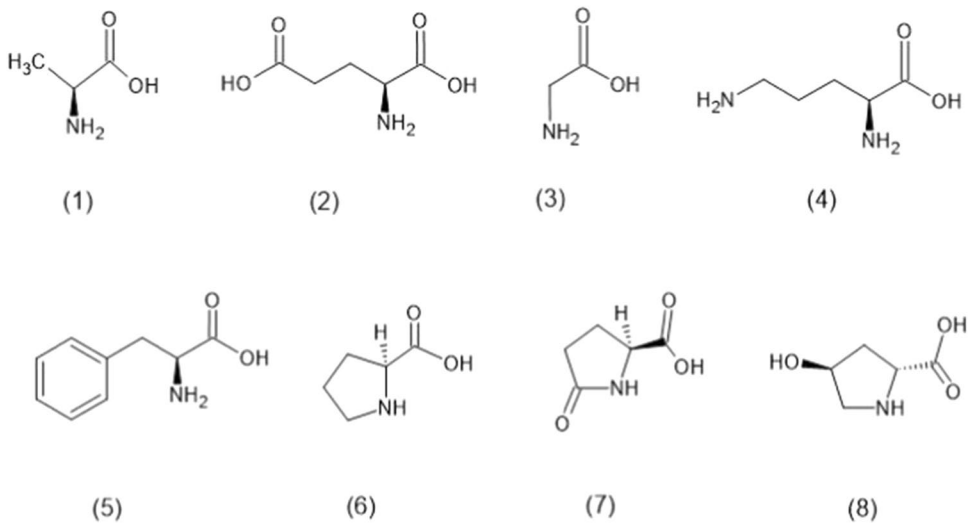

Fig. 1 Structural formulas of the amino acids (1) alanine, (2) glutamic acid, (3) glycine, (4) ornithine, (5) phenylalanine, (6) proline, (7) pyroglutamic acid, (8) trans-4-hydroxy-D-proline

Table 3 Measured pH value of the amino acids buffer solutions

Amino acid	1.pH	2.pH	3.pH	Arithmetic mean	SD	Variance
Alanine	8.07	8.08	8.08	8.08	$4.71 * 10^{-3}$	$2.22 * 10^{-5}$
Phenylalanine	7.88	7.89	7.93	7.90	$2.16 * 10^{-2}$	$4.67 * 10^{-4}$
Glycine	7.83	7.83	7.83	7.83	$8.88 * 10^{-16}$	$7.89 * 10^{-31}$
Glutamic acid	4.53	4.56	4.57	4.55	$1.70 * 10^{-2}$	$2.89 * 10^{-4}$
Proline	8.04	8.04	8.04	8.04	0	0
Ornithine	7.44	7.44	7.45	7.44	$4.71 * 10^{-3}$	$2.22 * 10^{-5}$
Pyroglutamic acid	3.64	3.67	3.66	3.66	$1.25 * 10^{-2}$	$1.56 * 10^{-4}$
Trans-4-Hydroxy-D-Prolin	7.88	7.90	7.91	7.90	$1.25 * 10^{-2}$	$1.56 * 10^{-4}$

pyroglutamic acid. To remove any strong solvent signal in the 1 H-NMR spectra, the samples were measured with water suppression. Complementary mass spectroscopy is carried out for the solutions, since this analytical method is very useful for amino acids (Johnson 2011). In mass spectrometric experiments, we observed unexpected degradation processes and decided to investigate them further. In this paper, we present the investigation of amino acids and amino acid standards regarding their stability in extreme conditions.

Material and methods

Chemicals and reagents were purchased from Carl Roth and were used without further purification. The amino acids examined and used in this study were alanine, glutamic acid, glycine, ornithine, phenylalanine, proline, pyroglutamic acid, trans-4-hydroxy-D-proline.

The structural formulas of the amino acids are shown below (Fig. 1; Tables 3, 4).

Table 4 Time and the respective temperature of the oil bath while the glutamic acid is heated in 4 M DCl solution

Time [Min]	Temperature [°C]
0	85
8	79
20	80
30	80

No experiments on human participants or animals were carried out during the implementation of these experiments, nor were any human or animal rights violated (Tables 5, 6).

NMR sample preparation

For sample preparation, the amino acids were first weighed (a Sartorius precision balance with the MC1 AC 210 P) and then transferred into 15 ml falcon for Sect. 2.1.1 or 2-ml Eppendorf tubes for Sects. 2.1.2 to 2.1.4. These amino acids were then dissolved in different solutions.

Table 5 Five different NaOD solutions with the respective weight of the pyroglutamic acid and the concentrations of TMSP-d_4 and pyroglutamic acid, which were prepared for the kinetics measurements

NaOD solutions [M]	Concentration of TMSP-d_4 [mM]	Initial weight of pyroglutamic acid [mg]	Concentration of pyroglutamic acid [mM]
0.08	50	10.8	84
0.16	50	10.7	83
0.5	25	10.4	81
1	50	10.7	83
4	50	10.7	83

Table 6 Calculated concentrations of the buffer solutions are compared with the concentrations determined from the NMR measurements

Amino acids	Calculated Concentrations [mmol/L]	Determined concentrations [mmol/L]
Pyroglutamic acid	80.0	75.9
Glutamic acid	89.7	79.8
Glycine	90.0	80.5
Ornithine	98.0	75.7
Phenylalanine	85.3	76.0
Proline	90.1	85.5
Trans-4-hydroxy-D-proline	80.9	64.8
Alanine	79.0	77.4

Sample preparation with water and buffer

The first set of amino acids was dissolved in 10 ml H_2O by shaking and the second set in 5 ml 50 mM ammonium bicarbonate buffer (NH_4HCO_3). An exception was the solution with trans-4-hydroxy-D-proline which was dissolved in 8 ml H_2O and 3 ml ammonium bicarbonate buffer, due to material shortage of this amino acid. However, the concentration of all amino acids in the solutions was about 0.075 M. The buffer solution was prepared in deuterium oxide (D_2O) with 0.4005 g NH_4HCO_3 and 89.4 mg 3-(trimethylsilyl)-2,2,3,3-tetradeuteropropionic acid sodium salt (TMSP-d_4). Before starting the NMR measurement, the pH of every water and buffer sample was determined with the potentiometric titrator 916 Ti-Touch which is used for routine analysis.

Sample preparation with NaOD

The amino acids were dissolved in 2 ml of a 4 M NaOD solution by shaking. From each sample, 0.6 ml was transferred to standard NMR tube and measured in the benchtop NMR spectrometer (Tables 7, 8).

Table 7 Calculated concentrations of the DCl solutions are compared with the concentrations determined from the NMR measurements

Amino acids	Calculated concentrations [mmol/L]	Determined concentrations [mmol/L]
Pyroglutamic acid	88.4	76.7
Glutamic acid	97.3	75.8
Glycine	90.0	80.6
Ornithine	97.4	75.0
Phenylalanine	89.8	75.4
Proline	85.3	79.0
Trans-4-hydroxy-D-proline	88.6	75.5
Alanine	81.9	80.8

Table 8 Reaction rate constants of the kinetics measurements

	4 M NaOD	1 M NaOD	0.5 M NaOD
Reaction rate constant from ln(c) straight	0.0016 s^{-1}	0.0002 s^{-1}	$7*10^{-5}$ s^{-1}
Reaction rate constant from ln(c0/c) straight	0.0016 s^{-1}	0.0002 s^{-1}	$7*10^{-5}$ s^{-1}

Sample preparation with DCl

The amino acids were dissolved in 2 ml of 4 M DCl solution containing 21.8 mg TMSP-d_4. From each sample, 0.6 ml was transferred to a standard NMR tube and measured in the benchtop NMR spectrometer.

Additionally, 0.6 ml of the glutamic acid sample was filled into a sealed 5-mm standard NMR tube and heated for 30 min at 80 °C in an oil bath before the measurement. During the heating time, the temperature was controlled four times, to make sure that it stays constant.

Sample preparation for kinetics

Five similar TMSP-d_4 containing D_2O and NaOD solutions were prepared for the kinetics measurement.

For a 4 M NaOD solution in D_2O, 45.2 mg of TMSP-d_4 was dissolved in 5.25 ml. For the next three solutions, 1 M, 0.08 M and 0.16 M, 43.1 mg of TMSP-d_4 were dissolved in 5 ml. The 0.5 M NaOD and TMSP-d_4 solution was made by mixing the 1 M solution with pure D2O in 1:1 ratio.

For each kinetics measurement, pyroglutamic acid was dissolved in 1 ml of each of the basic solutions. 0.6 ml of these samples was transferred to a standard NMR tube and used for the reaction monitoring measurement in the NMR spectrometer.

General procedure for NMR measurements

After the preparations, about 0.6 ml of these samples was transferred into sealed 5-mm standard NMR tubes. The ^{1}H NMR spectra were then recorded on a Magritek Spinsolve 60 Carbon Benchtop NMR spectrometer. The Chemical shifts are reported in ppm relative to solvent signal (HDO: δH = 4.79 ppm). The spectra were recorded at a temperature of 25 °C with the 1D Proton standard protocol using 16 scans with a 90-degree excitation pulse (7 microseconds) covering a spectral range from 46 to −37 ppm (Magritek Spinsolve standard NMR conditions). 32 k data points are acquired with an acquisition time of 6.5 s and a repetition time (recycle delay) of 15 s. In advance to every long-time experiment (like kinetic measurements) and at the start of each working day, a so-called powershim has been performed within Spinsolve, where all available shims get optimized. The final scan records a spectrum and analyzes the linewidths at 50% and 0.55% of peak maximum, the Signal-to-Noise ratio (SNR), peak amplitude (signal) and the mean noise level. The settings of the 1D Presat protocol, which suppresses the large solvent peaks of water, exactly match the settings of the standard protocol. The frequency search was set to automatic, the SAT power was −60 dB, the SAT period was 3 s, and 2 dummy scans were carried out. Data processing has been performed with MestReNova version 14.1.0 including zeroth and first-order phase correction and baseline correction with a Bernstein polynomial fit of third order.

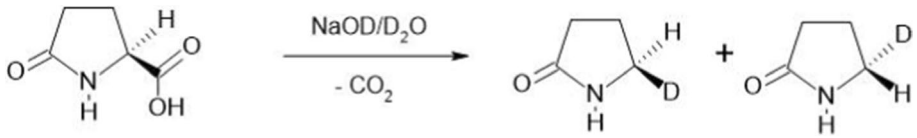

Fig. 2 Decarboxylation of pyroglutamic acid

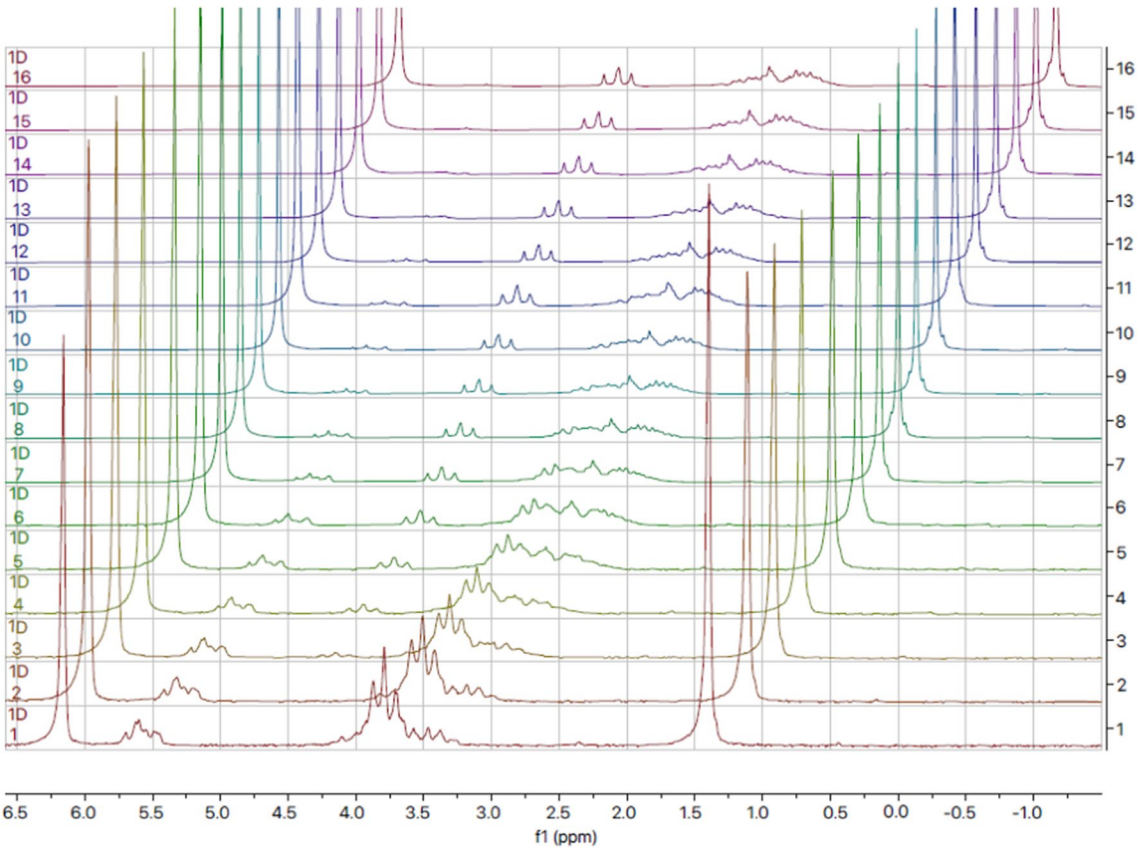

Fig. 3 Stacked graph shows the NMR spectrum of the 1 M pyroglutamic acid kinetics measurement from the beginning of the decarboxylation (spectrum 1) to the end of the decarboxylation (spectrum 16). It can be observed that the peaks by 5.60 ppm decrease over time till they completely disappear. Also new peaks by 4.50 ppm appear and increase in size over time

Mass spectroscopy

Electrospray ionization mass spectra (ESI) were performed on Sciex API QTRAP Mass Spectrometer (AB Sciex LLC, Framingham, MA, USA). The mass spectrometer was operated in the positive ion mode with an electrospray voltage of 5000 V at 200 °C, curtain gas at 25 psi, collision gas at 6 psi, nebulizing gas at 25 psi and auxiliary gas at 25 psi. All quadrupoles were working at unit resolution.

To carry out the Q1 mass spectra, all 4 M DCl and 4 M NaOD samples were diluted with MS-grade methanol.

Results and discussion

NMR spectroscopy and the stability of the amino acids

If we look at the stability of the amino acids, we observed that seven of the eight amino acids tested were stable at given experimental conditions. The first H-NMR spectra of the simple water solution were poor to evaluate because the water signal was wide and covered the signals of the amino acids. The amino acid buffer solutions then were measured with water suppression. No signal and peaks of the amino acid were covered or distorted by the buffer. However, many amino acids show peaks near the 4.79 ppm mark of the wide water signal, which hindered the quantification of the integrals. All the amino acids also remained stable in the buffer solution for a week.

The spectra of the NaOD solution were easy to evaluate. The water peak was almost absent and shifted to around 5.30 ppm in most samples. The reason for the shift of the water peak in different samples is their pH-dependency. Some peaks of the samples also shifted or changed their distance to one another, for example, the peaks of trans-4-hydroxy-D-proline have split up considerably. After a week, the samples were measured a second time and it was discovered that the spectra of amino acid (7) had changed its appearance, indicating a chemical transformation. All the other amino acids remained stable in the basic solution.

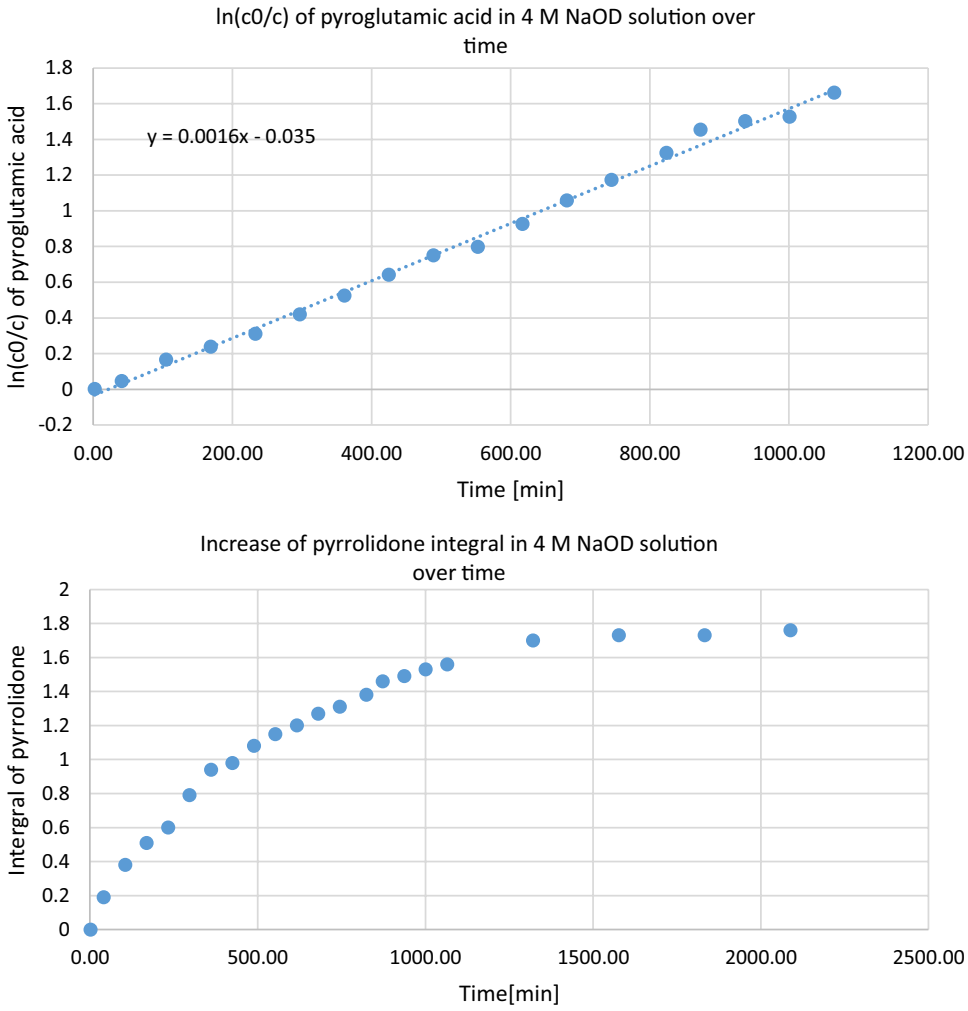

Fig. 4 Kinetics of the 4 M NaOD solutions: consumption of pyroglutamic acid shown with ln(c0/c) and formation of pyrrolidone over time

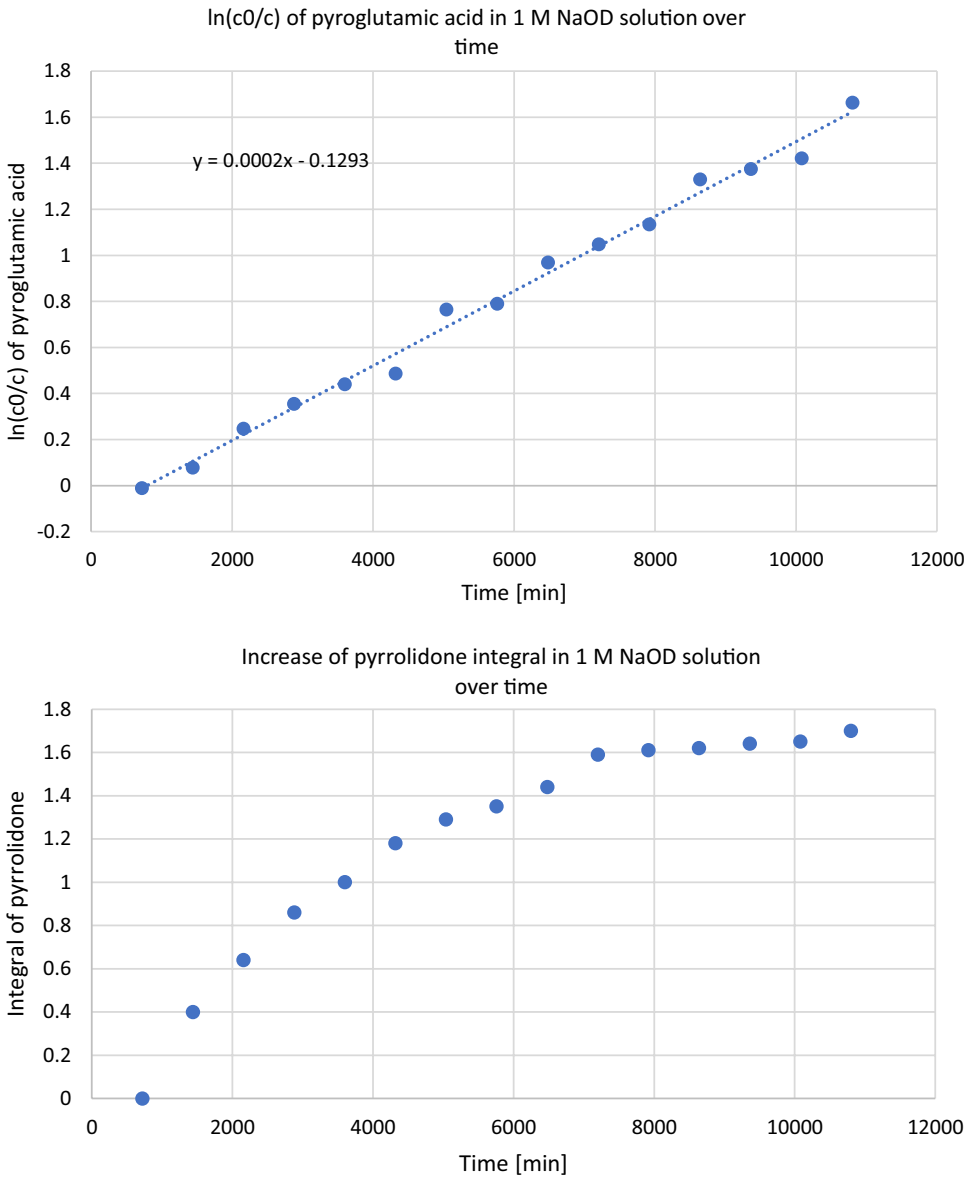

Fig. 5 Kinetics of the 1 M NaOD solutions: consumption of pyroglutamic acid shown with ln(c0/c) and formation of pyrrolidone over time

The spectra of the DCl solution were very similar to the measurements carried out in the NaOD solution. The water peak shifted again, but this time to around 6.00 ppm. Some of the amino acid peaks also shifted compared to the buffer spectra. As a second measurement after a week showed, all of the eight amino acids remained stable in acidic solution.

In order to calculate the concentration of the amino acids from the measured and processed spectra, we used the formula shown below. From the buffer solutions and DCl solutions runs, four spectra of sample were used to determinate the arithmetic mean of all the specific integrals of each amino acid. The mean value of the integrals is then divided by the number of protons and multiplied by the concentration of the standard.

$$c\left[\frac{\text{mol}}{L}\right] = \frac{\bar{x}(\text{Integrals})}{\text{Proton number}} * c(\text{Standard})\left[\frac{\text{mol}}{L}\right]$$

As it can be seen from the following tables, the concentrations determined by the NMR measurement tend to have higher values than the calculated concentrations. When determining the concentrations using the NMR measurements, we have potential sources of error which could cause the results to deviate from the concentrations calculated from the gravimetric sample preparation. These potential sources of error include phase correction and baseline correction, which were carried out when evaluating the NMR spectra. Further sources of error could be overlapping peaks and the choice of the

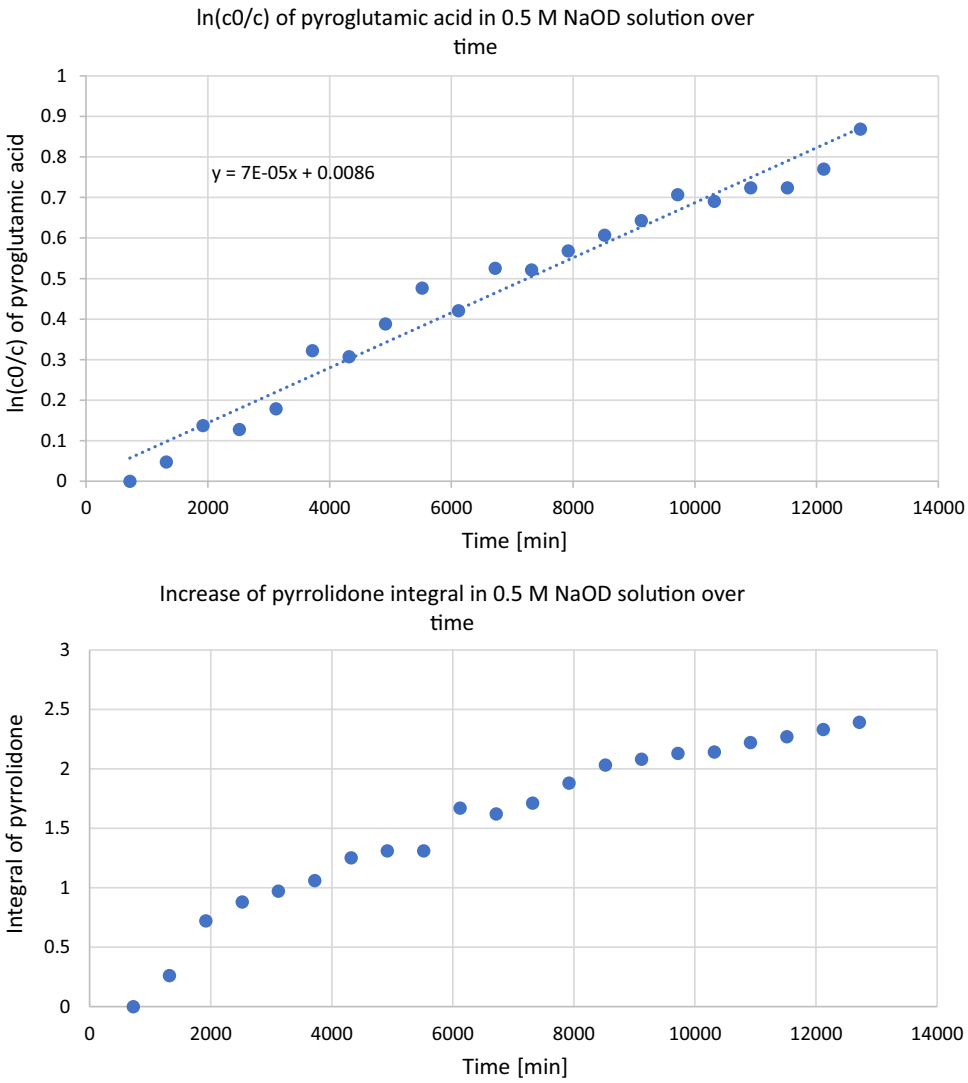

Fig. 6 Kinetics of the 0.5 M NaOD solutions: consumption of pyroglutamic acid shown with ln(c0/c) and formation of pyrrolidone over time

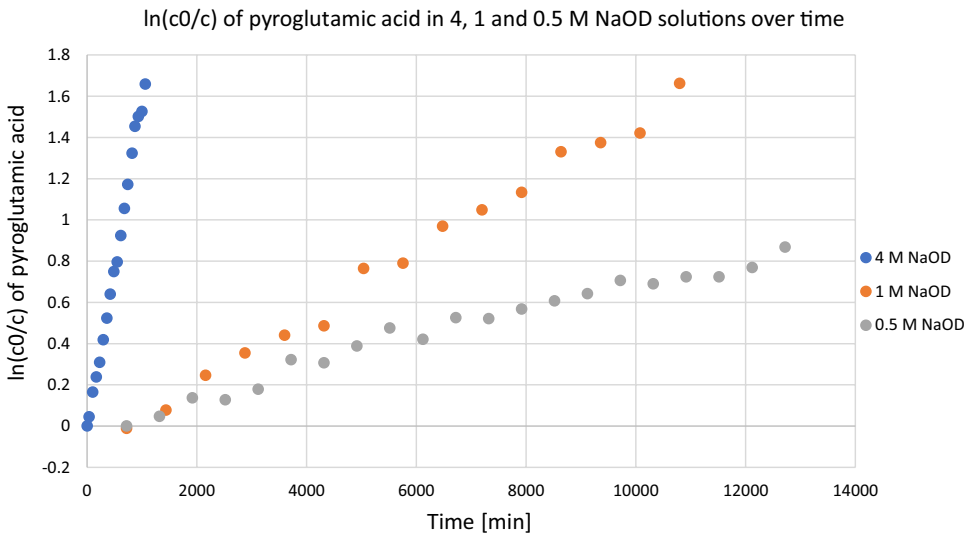

Fig. 7 Kinetics over time for the 4 M, 1 M and 0.5 M NaOD solutions

bounds of integration. Those potential sources of error can influence the integrals, which in turn influence the determined concentrations.

Stability of glutamic acid in 4 M DCl solution

For future investigation of the stability, the glutamic acid with 4 M DCl solution was heated for 30 Minutes at 80 °C. The NMR spectrum result has shown no changes. So, it can be concluded that no change in the structure of the glutamic acid was caused by brief heating. In addition, the glutamic acid sample was also stable for a week in the refrigerator and for several hours at room temperature.

The kinetics of pyroglutamic acid

With the information from mass spectroscopy and additional research (Dose 1957; Derek et al. 1984; Claes et al. 2019; Steffen et al. 1991; Snider and Wolfenden 2000; Thanassi and Aminomalonic acid. 1970), we came to the conclusion that the pyroglutamic acid, after a few days in NaOD solution, is decarboxylating. Here the reaction equation that fits our hypothesis is shown in deuterated water (Fig. 2).

The 4 M kinetic measurement was carried out at 30-min intervals and ran for four days, but the reaction ended on the second day.

The 1 M kinetic measurement was carried out at 30-min intervals and ran over a full eight days (Fig. 3).

The 0.5 M kinetics measurement was carried out at two-hour intervals and ran for eleven days, after which the reaction was not fully completed. Even after multiple days of run time (>8 days), the kinetics measurements with the 0.08 M and 0.16 M NaOD solutions did not show any signs of a chemical transformation.

The overall process of the decarboxylation is a one molecular disintegration reaction. In this case, we assume the observed reaction is of (pseudo) first order, because the rate of reaction linearly depends only on the concentration of the pyroglutamate itself. This would align with the overall formation of pyrrolidone over time illustrated in the following graphs (Figs. 4, 5, 6, 7):

Mass spectroscopy

Since we observed changes in the NMR spectra of the pyroglutamic acid, we carried out mass spectroscopy for all amino acids in NaOD and DCl after the kinetic measurements. The results of mass spectroscopy support the above observation that decarboxylation or chemical transformation occurs in the case of pyroglutamic acid. Another supporting fact is that the masses of the other amino acid samples in NaOD and DCl showed no change in their mass in the mass spectra.

Conclusion

Almost every amino acid that was used in the experiment was stable at the critical pH conditions, with the exception of pyroglutamic acid. This shows that the amino acids have great potential as standards in the field of biochemical analysis particularly in NMR spectroscopy. They stay stable over time, in very acidic and very basic conditions, at high temperatures and have good recognizable peaks.

Keeping in mind that benchtop NMR spectroscopy cannot be the method of choice for everyday quantification and identification of amino acids due to more suitable methods such as HPLC–MS or GC–MS, the method can still be of great use for solving more complex analytical tasks. It is also a good option for quick measurements because it can be varied out easily.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11696-021-01906-2.

Aknowledgements We like to thank the ReAching program of the faculty MLS for support.

Funding Open Access funding enabled and organized by Projekt DEAL.

Declarations

Conflicts of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript. All authors declare that they have no conflict of interest.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Code availability not applicable.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Airaudo CB, Gayte-Sorbier A, Armand P (1987) Stability of glutamine and pyroglutamic acid under model system conditions: influence of physical and technological factors. J Food Sci 52(6):1750–1752. https://doi.org/10.1111/j.1365-2621.1987.tb05926.x

Bellstedt P, Seiboth T, Häfner S, Kutscha H, Ramachandran R, Görlach M (2013) Resonance assignment for a particularly challenging protein based on systematic unlabeling of amino acids to complement incomplete NMR data sets. J Biomol NMR 57(1):65–72. https://doi.org/10.1007/s10858-013-9768-0

Claes L, Janssen M, De Vos DE (2019) Organocatalytic decarboxylation of amino acids as a route to bio-based amines and amides. ChemCatChem 11(17):4297–4306. https://doi.org/10.1002/cctc.201900800

Derek HRB, Herve T, Potier P, Thierry J (1984) Reductive radical decarboxylation of amino-acids and peptides. J Chem Soc Chem, Commun 19:1298–1299

Dose K (1957) Catalytic decarboxylation of α-amino-acids. Nature 179(4562):734–735. https://doi.org/10.1038/179734b0

Friebolin H (2011) Basic one- and two-dimensional NMR spectroscopy, completely revised and enlarged, 5th edn. Wiley, Hoboken

Johnson DW (2011) Free amino acid quantification by LC-MS/MS using derivatization generated isotope-labelled standards. J Chromatogr B Anal Technol Biomed Life Sci 879(17–18):1345–1352. https://doi.org/10.1016/j.jchromb.2010.12.010

Khallouk M, Rutledge DN, Silva AMS, Delgadillo I (2005) Study of the behaviour of amino acids in aqueous solution by time-domain NMR and high-resolution NMR. Magn Reson Chem MRC 43(4):309–315. https://doi.org/10.1002/mrc.1538

Lu Y, Freeland S (2016) On the evolution of the standard amino-acid alphabet. Genome Biol 7:102

Miller SL (1953) A production of amino acids under possible primitive earth conditions. Sci (n Y) 117(3046):528–529. https://doi.org/10.1126/science.117.3046.528

Snider MJ, Wolfenden R (2000) The rate of spontaneous decarboxylation of amino acids. J Am Chem Soc 122(46):11507–11508. https://doi.org/10.1021/ja002851c

Steffen LK, Glass, RS, Sabahi M, Wilson GS, Schoeneich, C, Mahling S, Asmus KD (1991) Hydroxyl radical induced decarboxylation of amino acids. Decarboxylation vs bond formation in radical intermediates. J Am Chem Soc 113(6), 2141–2145

Thanassi JW (1970) Aminomalonic acid. Spontaneous decarboxylation and reaction with 5-deoxypyridoxal. Biochemistry 9(3):525–532. https://doi.org/10.1021/bi00805a011

Wishart DS, Bigam CG, Holm A, Hodges RS, Sykes BD (1995) 1H, 13C and 15N random coil NMR chemical shifts of the common amino acids. I. Investigations of nearest-neighbor effects. J Biomol NMR 5(1):67–81. https://doi.org/10.1007/BF00227471

Yamazaki T, Eyama S, Takatsu A (2017) Concentration measurement of amino acid. Anal Sci 33:369–373. https://doi.org/10.2116/analsci.33.369

Yoon Y-G, Pfrommer BG, Louie SG, Canning A (2004) NMR chemical shifts in amino acids: effects of environments in the condensed phase. Solid State Commun 131(1):15–19. https://doi.org/10.1016/j.ssc.2004.04.023