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Water molecule assisted proton mobility in gaseous protonated GlyPro and ProGly

C.G. Sinnige, L.J. de Koning, N.M.M. Nibbering*

Institute of Mass Spectrometry, University of Amsterdam, Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands

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Abstract

The thermal hydrogen/deuterium exchange behaviors of $GlyProH^+$, $ProGlyH^+$, and $ValGlyH^+$ in the reactions with D_2O have been studied in a Fourier transform ion cyclotron resonance mass spectrometer. The analyzed behaviors are consistent with our earlier conclusions that more basic N-terminal amino acids lower the overall efficiency of the hydrogen atom exchange in protonated alkyl dipeptides. It appears that the mobility of a proton over the basic sites in peptides can be restricted drastically by N-terminal proline, relative to peptides with other N-terminal alkyl amino acids. (Int J Mass Spectrom 195/196 (2000) 115–119) © 2000 Elsevier Science B.V.

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1. Introduction

While most of the mass spectrometric research of peptides and proteins focuses on identification and characterization, a number of research groups have studied the bimolecular gas-phase chemistry of small protonated peptides in hydrogen/deuterium exchange with deuterated solvent molecules [1–12] and in coupling reactions with substrate molecules [12,13]. In particular, the studied hydrogen/deuterium exchange behavior can give insight into the heterogeneity of protonation over the different amide bonds in a peptide. This heterogeneous protonation is considered to specifically drive the backbone fragmentations of protonated peptides, which are commonly analyzed in terms of an amino acid sequence [14,15]. Furthermore, a fundamental study of the hydrogen/deuterium exchange behavior of small protonated peptides can help rationalize the gas-phase exchange behavior of larger peptides and proteins in terms of specific folding under mass spectrometric conditions.

In one of our earlier papers [4] on this subject we have reported that the selectivity of the exchange of the labile hydrogen atoms at the different basic sites of protonated alkyl dipeptides is governed by the nature of both the dipeptide and the exchange reagent molecule. With the relatively low basic water molecule, exchange at the different basic sites is achieved by mobilizing the proton over the different basic sites via multiple hydrogen bonding. This results in a very specific consistent exchange behavior, where more basic N-terminal amino acids lower the overall efficiency of the hydrogen atom exchange, while more

* Corresponding author. E-mail: nibberin@ims.chem.uva.nl

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basic C-terminal amino acids enhance the efficiency of exchange of the amide and acid hydrogen atoms, relative to the exchange of the amino hydrogen atoms, in the protonated alkyl dipeptides.

Mobilization of the proton over the different basic sites in the alkyl dipeptide is energetically more feasible when assisted by the more basic ammonia molecule. For this exchange reagent, multiple hydrogen bonding is less important, as a consequence the efficiencies of the exchanges of the hydrogen atoms at the different basic sites are found to be comparable and independent of the nature of the studied alkyl dipeptides.

Commonly, it appears that backbone fragmentation of protonated peptides shows a preference for cleavage of peptide bonds adjacent to proline building blocks. This can be rationalized by assuming a heterogeneous distribution of the proton over the peptide bonds, which favors the peptide bonds, involving the highly basic proline building block. To explore the effect of proton mobility and distribution in protonated peptides involving a proline building block, we have prolonged our studies of the hydrogen/deuterium exchange of protonated alkyl dipeptides by investigating the exchange behavior of protonated GlyPro and ProGly in the reaction with D_2O.

2. Experimental

Experiments were performed with a Bruker Apex II 47e Fourier transform ion cyclotron resonance (FTICR) mass spectrometer (Bruker Daltonics, Billerica, MA, USA), equipped with 4.7 T superconducting magnet, an external ion source, a 6-cm-diameter cylindrical infinity cell™ [16] with a quadrupolar excitation/axialization facility, a pulsed valve inlet and two liquid inlet lines with leak valves for the introduction of compounds in the FTICR cell. Background pressure was better than 1×10^{-9} mbar.

Reactant ions were generated by fast-atom bombardment (FAB) in the external source by using a 10 keV Xe atom beam from a Capillatron DIP Gun from Phrasor Scientific, Inc. (Duarte, CA, USA). Solid samples were dissolved until saturation in a glycerol matrix containing 1% trifluoroacetic acid.

The H/D exchange reactions were initiated by FAB generation of $[M + H]^+$ ions that were gated to the FTICR cell for 80 ms. The trapped ions were effectively thermalized in an atmosphere of argon that was admitted to the FTICR cell through a pulsed valve for 150 ms up to a maximum pressure of 10^{-5} mbar. After a pump-down/thermalization period of 2s, the trapped $[M + H]^+$ ions were carefully mass selected from the ion mixture using the correlated harmonic excitation fields (CHEF) procedure [17]. Subsequently, the reactant ions were allowed to react with D_2O that was introduced to the cell via a leak valve up to a stationary pressure of 5.2×10^{-8} mbar. Finally, the exchange reaction progress was reproducibly monitored up to a reaction time of 420 s.

In order to maximize and stabilize the deuterium label content of the exchange reagent, the cell vacuum system was flushed several times with D_2O. The actual label content was determined before and after a series of exchange reactions, from the completed H/D exchange reactions with protonated glycerol and the proton-bound dimer of glycerol, using the measured H/D isotope distributions after correction for ^{13}C contributions. From these isotope distributions the label content was calculated according to

$$L(\text{label content}) = \frac{\sum_{n=0}^{n=N}(I_n n)}{N} \times 100\% \qquad (1)$$

where I_n is the relative abundance of the ion, in the isotopic cluster, that has exchanged n hydrogen atoms for deuterium atoms, and N is the maximum number of exchangeable hydrogen atoms ($N = 4$ for protonated glycerol and $N = 7$ for the proton-bound dimer of glycerol). Both from the exchange of protonated glycerol and from the exchange of the proton-bound dimer of glycerol the actual label content of D_2O was determined to be $88.8 \pm 0.3\%$ for all exchange reactions with the protonated alkyl dipeptides.

All chemicals employed were commercially available and were used without further purification.

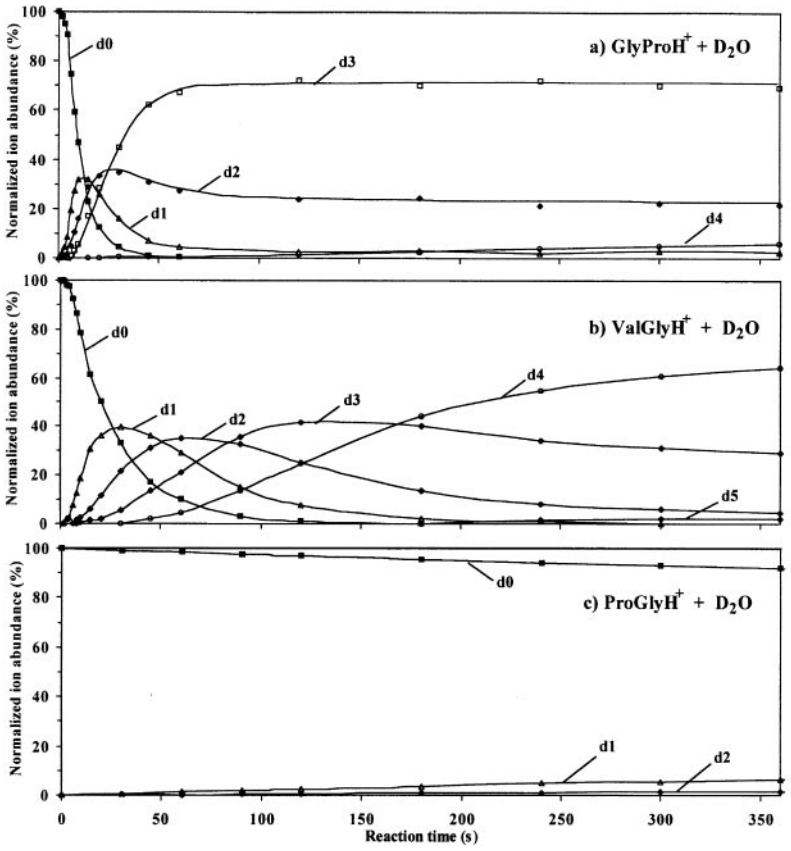

Fig. 1. Deuterium incorporation in the reactions of D_2O with (a) protonated GlyPro, (b) protonated ValGly, and (c) protonated ProGly, as a function of the reaction time, at a D_2O pressure of 5.2×10^{-8} mbar. d0, $\cdots$, d5 represent the normalized abundances of the protonated dipeptides with 1, $\cdots$, 5 deuterium atoms incorporated.

3. Results and discussion

Fig. 1(a), (b), and (c) show the progress of deuterium incorporation in the reactions between D_2O and $GlyProH^+$, $ValGlyH^+$, and $ProGlyH^+$, respectively, recorded under identical experimental conditions. The H/D exchange of $ValGlyH^+$ has been included as a reference reaction, enabling the comparison of the exchange behavior of $GlyProH^+$ and $ProGlyH^+$ with the exchange behavior of the previously studied alkyl dipeptides [4,9].

For $GlyProH^+$, the exchange of the first three hydrogen atoms by deuterium atoms proceeds relatively fast, whereas the exchange of the fourth hydrogen atom is significantly slower [see Fig. 1(a)]. Note that $GlyProH^+$ and $ProGlyH^+$, have only four exchangeable hydrogen atoms, in contrast to the previously studied protonated alkyl dipeptides [4,9], that have five exchangeable hydrogen atoms. As in our previous studies [4,9], the analysis of the consecutive deuterium incorporation can be simplified, correcting for the incomplete labeling of the D_2O exchange reagent, by calculating the macroscopic degree of deuterium incorporation by using

$$[\text{degree of } D \text{ incorporation}]_t(\%) = \frac{100}{L} \sum_{n=0}^{n=N} \frac{n}{N} [(d_n)\mathrm{MH}^+]_t \quad (2)$$

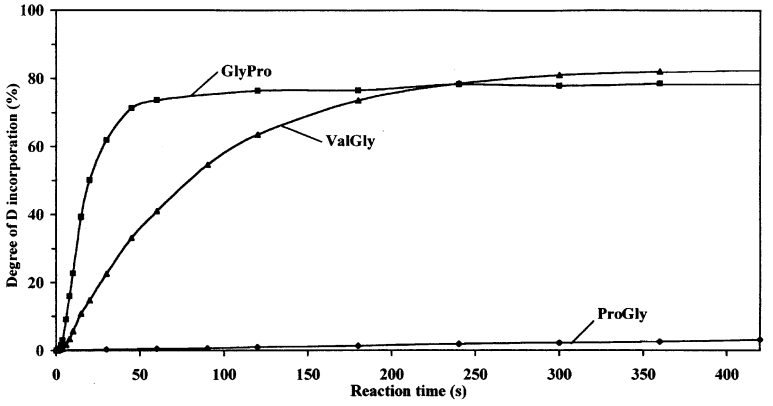

Fig. 2. Macroscopic degree of D incorporation in protonated GlyPro, ValGly, and ProGly in the reactions with D_2O as a function of the reaction time at a D_2O pressure of 5.2×10^{-8} mbar.

where $[(d_n)MH^+]_t$ is the normalized abundance in percentage of the MH^+ ions with n deuterium atoms incorporated at reaction time t, N is the maximum number of exchangeable hydrogen atoms ($N = 4$ for $GlyProH^+$ and $ProGlyH^+$ and $N = 5$ for $ValGlyH^+$), and L is the label content of D_2O (88.8%, see sec. 2).

For $GlyProH^+$, the results of the calculations, shown in Fig. 2, indicate that up to 75% deuterium incorporation is relatively fast, whereas from 75% to 100% completion the deuterium incorporation becomes extremely slow. Clearly, the three amino hydrogens are exchanging considerably faster than the carboxylate hydrogen, implying that mobility of the proton in the reaction complex from the amino group to the carboxylate group is very inefficient.

For $ValGlyH^+$, the exchange of the first three hydrogen atoms by deuterium atoms proceeds slower, relative to $GlyProH^+$, while the exchange of the fourth hydrogen atom proceeds with a rate comparable to the rate of the exchange of the first three hydrogen atoms. The exchange of the fifth hydrogen atom is significantly slower [see Fig. 1(b)]. The macroscopic deuterium incorporation shown in Fig. 2 indicates that for up to 80% deuterium incorporation the exchange rate is constant, after which the exchange slows down considerably. Apparently, mobility of the proton in the exchange complex from the terminal amino group to the amide group is relatively efficient, whereas the mobility of the proton toward the carboxylate group is very inefficient, in agreement with the results from one of our previous studies [4] on related systems.

For $ProGlyH^+$, incorporation of deuterium has become remarkably inefficient in the reaction with D_2O [see Figs. 1(c) and 2]. From this, it may be concluded that the proton becomes very strongly bonded to the amine nitrogen atom in peptides with N-terminal proline, which may considerably hamper the mobility of the proton over the other basic sites in the peptide.

4. Conclusion

The above discussed hydrogen/deuterium exchange behaviors of $GlyProH^+$ and $ProGlyH^+$ in the reactions with D_2O are consistent with our earlier conclusions that more basic N-terminal amino acids lower the overall efficiency of the hydrogen atom exchange in protonated alkyl dipeptides. It appears that the mobility of the proton over the basic sites in peptides can drastically be restricted by N-terminal proline (with a proton affinity, PA, of 920.5 kJ mol^{-1} [18]), relative to peptides with other N-terminal alkyl amino acids, such as glycine (PA = 886.5 kJ mol^{-1} [18]), alanine (PA = 901.6 kJ mol^{-1}) [18]), and valine (PA = 910.6 kJ mol^{-1} [18]).

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