



An electrospray ionization-flow tube study of H/D exchange in the protonated serine dimer and protonated serine dipeptide

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Dedicated to Professor Robert C. Dunbar on the occasion of his 60th birthday.

Abstract

Serine is known to undergo a chiroselective self-directed oligomerization to form a singly-protonated octamer whose structure is made up of four dimer units. This has led to interest in the protonated dimer. An electrospray ionization-fast flow technique has been employed to study the hydrogen/deuterium (H/D) exchange reactions of the protonated dimer of serine and of the protonated dipeptide of serine with ND_3 and CH_3OD , respectively. Ammonia- d_3 reacts with the dimer with an overall rate constant of $(6.7 \pm 0.4) \times 10^{-10} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$. On a time scale of $\sim 12 \text{ ms}$, the protonated dimer was observed to undergo very efficient consecutive exchange with ND_3 of all of its nine labile hydrogens at ND_3 concentrations of $\leq 1.2 \times 10^{12} \text{ molecules mL}^{-1}$. Reaction with CH_3OD is less efficient in view of the lower proton affinity of the latter. In addition, the dipeptide was observed to exchange its seven labile hydrogens with ND_3 somewhat less efficiently than the dimer. The results are interpreted as indicating a rather open structure for the protonated dimer allowing easy complexation by ND_3 and involving very labile protons. This conclusion is discussed in the light of the known computed structures of the protonated dimer [Anal. Chem. 73 (2001) 3646]. H/D exchange of the dimer demonstrates a single structure—the ion–molecule form. Serine is not basic enough to stabilize a zwitterionic salt-bridge structure for the dimer.

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1. Introduction

Electrospray ionization–mass spectrometry (ESI–MS) has been known [1] to produce multiply-protonated peptides and proteins. The occurrence of ‘fractional charging’ by which cluster ions of amino acids are formed with more than one solute molecule

per charge was discovered as well [2]. Large multimers were observed in the ESI–MS of peptides [3].

The formation of non-covalent supramolecular assemblies plays a critical role in biological systems. The discovery of multimers led to a series of studies of the clustering of the natural amino acids [4,5], the formation of peptide aggregates [6] and the complexation and clustering of proteins [7] under ESI–MS.

A fascinating discovery has been [5] that serine undergoes a chiroselective self-directed oligomerization to form a singly-protonated octamer. Collision-induced

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dissociation that was performed led to the suggestion that the protonated octamer is composed of four hydrogen-bonded dimers, stabilized by further extensive hydrogen bonding. Density functional calculations supported this model. Several other groups have been involved in the experimental and theoretical study of the octamer of serine and larger serine clusters, including mass spectra and ion mobility studies, modeling of octamers comprising zwitterionic amino acid units [8] and discussions of the relevance of the data to the origin of life [9]. Several alternative structures of the protonated octamer have been considered [8,9].

We have studied recently [10] the H/D exchange reactions of protonated serine with ND_3 and CH_3OD . The present study is devoted to the dimer in view of its important role in the formation of the chiroselective octamer of serine. We are employing a unique combination of electrospray (ES) ionization with a fast flow apparatus. This setup has opened the way for the study of ion–molecule reactions and H/D exchange of protonated biomolecules under thermal high-pressure conditions [11–16].

Serine is an amino acid with a hydroxy-methyl side chain, $\text{H}_2\text{N}-\text{C}(\text{H})(\text{CH}_2\text{OH})-\text{COOH}$. Protonated serine has five labile hydrogens [10]. The protonated dimer of serine, $(\text{H}_2\text{N}-\text{C}(\text{H})(\text{CH}_2\text{OH})-\text{COOH})_2\text{H}^+$ has nine labile hydrogens (underlined): four at the two amine groups of the N-termini, two at the two car-

boxyl groups of the C-termini, two at the hydroxyls of the hydroxy-methyl side chains and, in addition, a single hydrogen due to the extra proton. Our aim has been to search for the H/D exchange of these labile hydrogens and to determine rate constants. Several experiments were also run on the protonated serine dipeptide $\text{SerSer}\cdot\text{H}^+$, $(\text{H}_2\text{N}-\text{C}(\text{H})(\text{CH}_2\text{OH})-\text{CO}-\text{NH}-\text{C}(\text{H})(\text{CH}_2\text{OH})-\text{COOH})\text{H}^+$ for comparison with the serine dimer data. The protonated dipeptide has seven labile hydrogens (underlined).

2. Experimental

The ES ionization-fast flow apparatus is shown schematically in Fig. 1. It consists of a SIFT apparatus that we constructed several years ago and modified to work with an ES source connected directly to the flow tube. The apparatus is made of a flow reactor that is 123 cm in length with an inner diameter (i.d.) of 74 mm. A neutral reagent is introduced into the flow tube through either one of two ring inlets. Tylan mass flow controllers define the flow rate of the neutral reactant into the flow tube. The quadrupole mass analyzer (652601 ABB EXTREL) is housed in a differentially pumped chamber that is separated from the flow tube by a nose cone (NC) skimmer with a 1.0 mm sampling orifice. A small NC voltage is used for focusing ions into the analysis quadrupole. Helium buffer gas

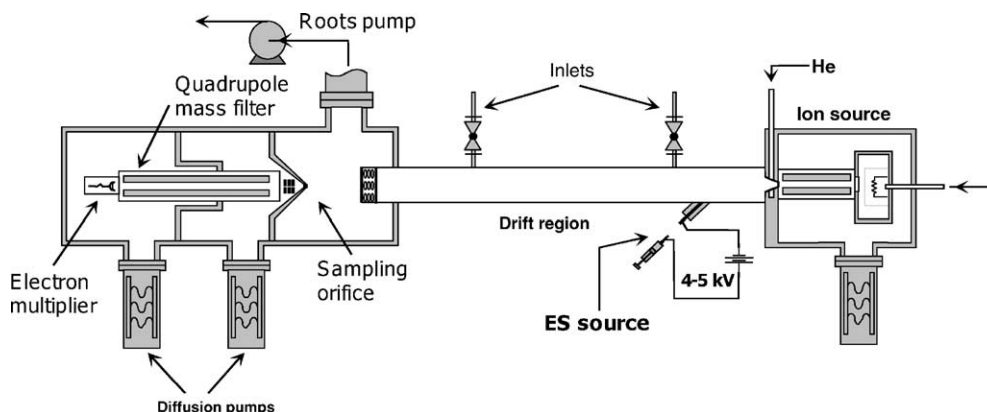


Fig. 1. Schematic drawing of the experimental setup combining electrospray (ES) ionization with a fast flow technique.

enters the flow tube at the upstream end near an electron impact ion source through another Tylan flow controller. It is pumped through the tube by a Roots blower.

The ES ion source was designed as follows. A capillary tube serves as the interface between the ES and the helium flow reactor. Stainless-steel tubes 15 cm in length and 0.05 cm i.d. are employed. The entire assembly is inserted into the flow tube at a distance of ~ 96 cm from the sampling orifice, 135° to the

direction of the helium flow, through an 'O'-ring type vacuum fitting. A capillary tube of 0.05 cm i.d. introduces an air leak into the flow tube with a pressure of 0.07 Torr and a flow rate of 1.3 L/min (STP); these numbers have to be added to the helium pressure and helium flow rate when calculating rate constants. The experiments to be described were carried out at total flow velocities of ~ 6000 – 7000 cm s^{-1} leading to typical flow tube pressures of ~ 0.1 Torr and reaction times of several millisecond.

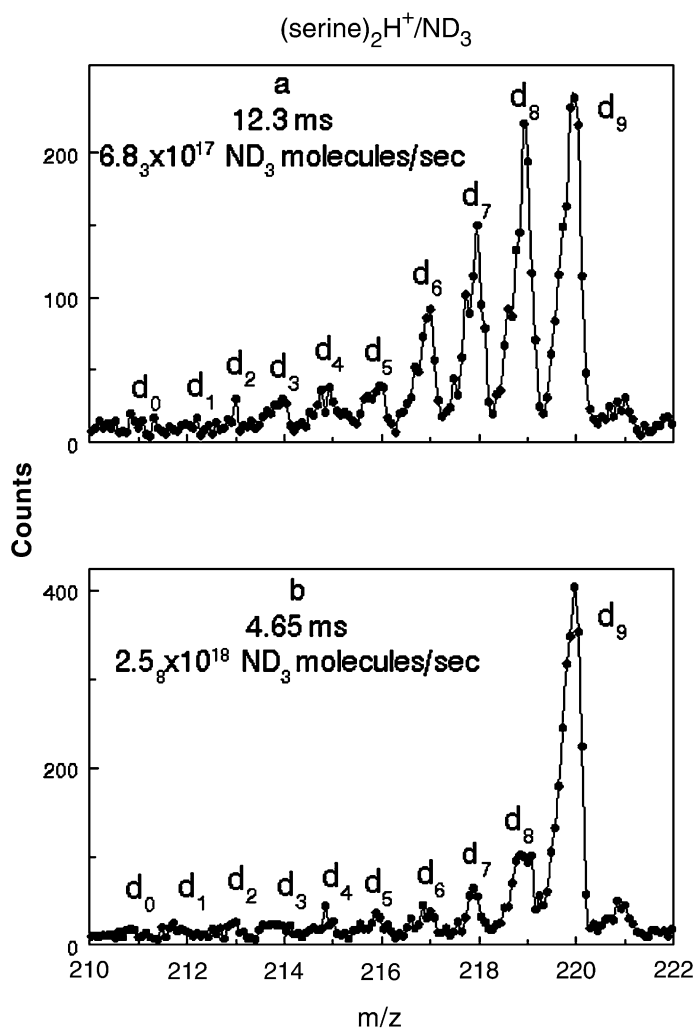


Fig. 2. Mass spectra obtained by the detector quad for the protonated dimer of serine following H/D exchange with ND_3 at the indicated reaction times and ND_3 flow rates. The spectra are uncorrected for ^{13}C or ^{15}N isotopic contributions.

Ions are electrosprayed ~ 10 mm through ambient air into the grounded capillary tube from a stainless-steel syringe needle biased at 5 kV DC. Dilute solutions of serine in a polar solvent are delivered to the ES needle at flow rates of $3\text{--}10 \mu\text{L min}^{-1}$ from a 5 mL syringe mounted on a model 100 KD scientific syringe pump. The temperature of the capillary tube as well as of the flow tube does not exceed $\sim 29^\circ\text{C}$.

The L-serine used in the experiments was purchased from Sigma–Aldrich (St. Louis, MO), with a stated minimum purity of 99%. ND_3 and CH_3OD were also from Sigma–Aldrich, with a stated isotopic purity that exceeds 99 at.% D. Stock solutions of water–methanol–acetic acid (50:50:0.1) and water–acetonitrile–acetic acid (50:50:1) were prepared for this series of experiments.

The low-resolution ESI mass spectra of water–methanol solutions (0.1 mM) of L-serine at a NC voltage of 20 V were reported earlier [10]. They demonstrate the monomeric ion $[\text{M} + \text{H}]^+$ at m/z 106 and a dimeric species $[2\text{M} + \text{H}]^+$ at m/z 211. The experimental conditions were optimized in order to allow running with confidence measurements of ion–molecule reactions on the dimer. This necessitated a minimum concentration of 2 mM serine.

3. Results and discussion

The protonated dimer of L-serine, $(\text{L-serine})_2\text{H}^+$ was produced by ES ionization and was injected into the flow tube. We reacted this ion in the flow tube with either ND_3 or CH_3OD and monitored the incorporation of deuterium as a function of flow rate of the deuterating reagent at a constant reaction time. All of the experiments were carried out under thermal, room temperature conditions.

One set of results is represented in the form of mass spectra in Fig. 2. A flow rate of 6.8×10^{17} molecules s^{-1} corresponding to a concentration of 2.4×10^{12} molecules mL^{-1} of ND_3 at an overall pressure of 0.11 Torr and a reaction time of 12.3 ms (Fig. 2a) is sufficient to exchange up to nine labile hydrogens. Raising the flow rate to

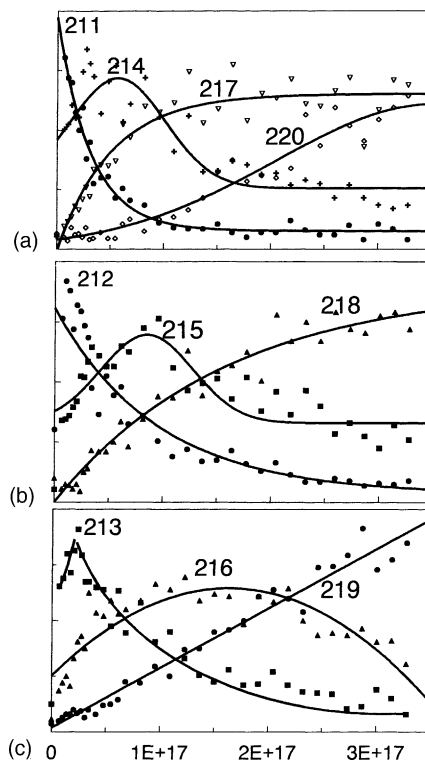


Fig. 3. Relative abundance vs. neutral ND_3 flow rate (in molecules s^{-1}) for the various indicated cations in the reaction of the protonated dimer of L-serine with ND_3 . The relative abundances given by the symbols are the raw experimental data for $M = 211$, $M + 1 = 212$, etc. The curves are not simulated fits but serve to lead the eye.

2.6×10^{18} molecules s^{-1} , corresponding to a ND_3 concentration of 9.5×10^{12} molecules mL^{-1} , enabled the nearly complete conversion of the protonated dimer to the d_9 isotopomer, already after 4.65 ms (Fig. 2b).

Fig. 3 represents the data for H/D exchange of the protonated dimer as a function of flow rate of ND_3 at a constant reaction time of 12.3 ms. The isotopic multiplet of $(\text{serine})_2\text{H}^+$ is observed to shift to higher masses (Figs. 2 and 3) upon introduction of ND_3 into the flow tube. The measured peaks are made up of contributions from the undeuterated dimer and its various deuterated isotopomers. The elemental formula of the protonated serine dimer is $\text{C}_6\text{H}_{15}\text{N}_2\text{O}_6$ (m/z 211). The undeuterated ion and its deuterated isotopomers each contribute a multiplet of isotopic peaks due to

the natural abundances of the various carbon, nitrogen, oxygen and hydrogen isotopes. The isotopic natural abundance distribution complicates the analysis of the deuterium labeling data. Successful interpretation of the experiments requires removal of the isotopic natural abundance distribution to reveal the underlying distribution of artificially introduced deuterium [17]. The goal can be achieved through isotopic deconvolution of the experimental data [14,15]. Experimental data can be isotopically deconvoluted and compared with simulated data to extract rate constants. Fig. 4 is a different presentation of the same data that were deconvoluted isotopically from ^{13}C , ^{15}N , etc. contributions. The highest ND_3 flow in these experiments was 3.5×10^{17} molecules s^{-1} (see Figs. 3 and 4) at a carrier gas velocity, $v = 6668.2$ cm s^{-1} . Since the flow tube diameter is 7.4 cm, this ND_3 flow corresponds to a maximum ND_3 concentration of 1.22×10^{12} molecules mL^{-1} . The results clearly indicate the consecutive nature of the H/D exchange process.

Optimum apparent and site-specific rate constants were deduced by simulated fits, based on solutions

of simultaneous first order differential equations for analogous data, for the protonated monomer of serine [10]. Attempts to apply the simulation program to the present data have so far been unsuccessful. We were able on the other hand to deduce the overall rate constant from the semi-log plot of the decay of the undeuterated reactant dimer ion, Fig. 5. The rate constant deduced for the reaction of the protonated dimer with ND_3 , $k = 6.7 \times 10^{-10}$ cm³ molecule⁻¹ s⁻¹, is just slightly higher than the one deduced previously [10], 6.1×10^{-10} cm³ molecule⁻¹ s⁻¹, for the reaction of the protonated monomer of serine with ND_3 .

The protonated dimer is also reactive with CH_3OD and undergoes H/D exchange. Whereas with ND_3 flows of 3×10^{17} molecules s^{-1} , the nine labile hydrogens are easily exchanged (Fig. 4) even higher flows of 5×10^{17} molecules s^{-1} of CH_3OD lead to only five exchanges and a very weak sixth exchange. This is as expected on the basis of the considerably lower proton affinity of CH_3OD (181.9 kcal mol^{-1}) compared to that of ND_3 (204 kcal mol^{-1}) [18] and the different proposed reaction mechanisms of the two

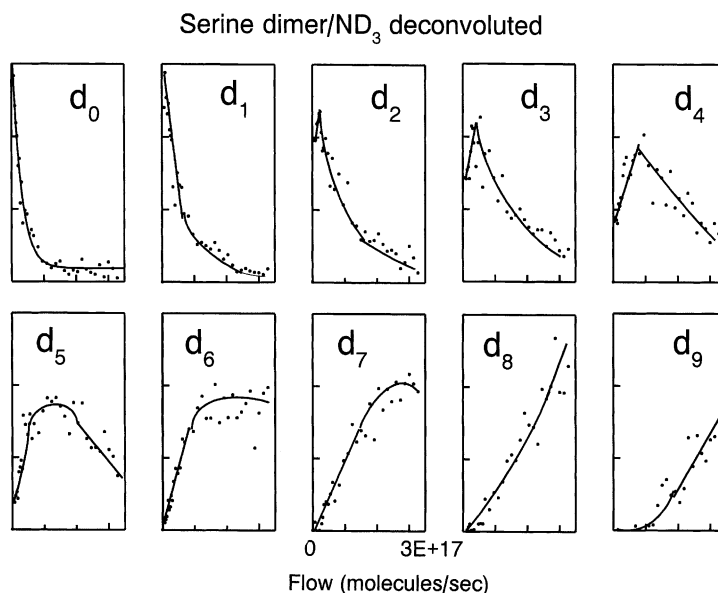


Fig. 4. Relative abundance vs. neutral ND_3 flow rate (in molecules s^{-1}) for the various indicated cations in the reaction of the protonated dimer of L-serine with ND_3 . The data of Fig. 3 were deconvoluted isotopically from ^{13}C , ^{15}N , etc. contributions. For zero ND_3 flow rate, the d_1 and d_2 peaks start at 0, but they rise very abruptly to finite values at the lowest ND_3 flow rate measured namely 6.83×10^{15} molecules s^{-1} .

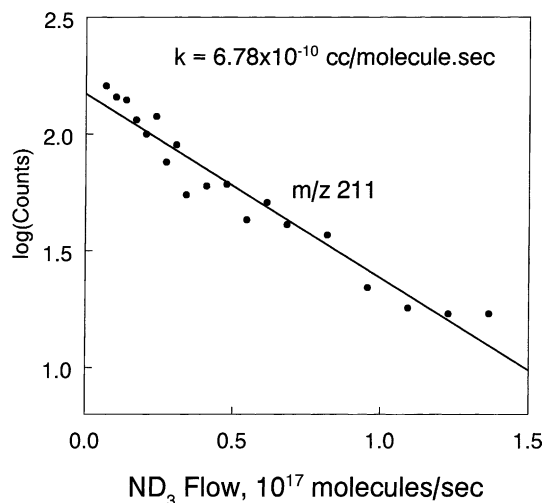


Fig. 5. Semi-logarithmic plot of the decay of primary ions as a function of the neutral flow rate for the reaction of the protonated dimer of L-serine with ND_3 .

deuterating reagents [10]. ND_3 is basic enough to gain a proton and be solvated by serine whereas CH_3OD is incapable of deprotonating the serine dimer and is postulated to exchange via a “relay mechanism,” in which the protonated dimer simultaneously gains a D while losing an H [19].

The protonated serine dipeptide, $\text{SerSer}\cdot\text{H}^+$ undergoes H/D exchange with ND_3 , exchanging its seven labile hydrogens. Six hydrogens are exchanged quite efficiently whereas the seventh is exchanged somewhat more slowly. For a similar reaction time and similar ND_3 flow rate, the protonated dimer exchanges the ninth labile hydrogen more efficiently.

Fig. 6 represents the data for H/D exchange of the protonated dipeptide as a function of flow rate of ND_3 , at a constant reaction time of 4.7 ms. The isotopic multiplet of $\text{SerSer}\cdot\text{H}^+$ is observed to shift to higher masses upon introduction of ND_3 into the flow tube. As before, the measured peaks are made up of contributions from the undeuterated dipeptide and its various deuterated isotopomers. The elemental formula of the protonated serine dipeptide is $\text{C}_6\text{H}_{13}\text{N}_2\text{O}_5$ (m/z 193). As was pointed out earlier for the dimer, successful interpretation of the experiments requires removal of the isotopic natural abundance distribution to reveal

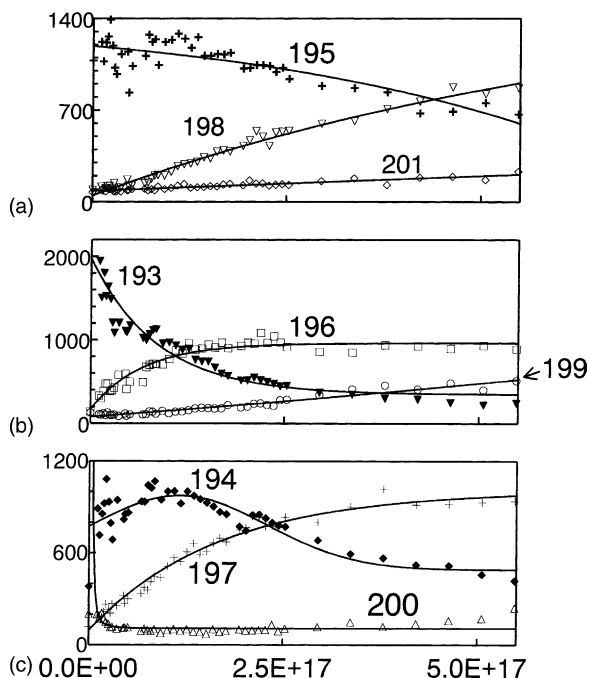


Fig. 6. Relative abundance vs. neutral ND_3 flow rate (in molecules s^{-1}) for the various indicated cations in the reaction of the protonated dipeptide of L-serine with ND_3 . The relative abundances given by the symbols are the raw experimental data for $M = 193$, $M + 1 = 194$, etc. The curves are not simulated fits but serve to lead the eye.

the underlying distribution of artificially-introduced deuterium. Fig. 7a is a different presentation of the same data as are presented in Fig. 6 that were deconvoluted isotopically. The results clearly indicate again the consecutive nature of the H/D exchange process. At considerable higher ND_3 flow rates, Fig. 7b, one observes the instantaneous drop of the reactant ion d_0 and the high abundances of d_6 and d_7 ions. The highest ND_3 flow in these experiments was 4.5×10^{18} molecules s^{-1} (see Fig. 7b) at a carrier gas velocity, $v = 6150.2$ cm s^{-1} . Since the flow tube diameter is 7.4 cm, this ND_3 flow corresponds to a maximum ND_3 concentration of 1.7×10^{13} molecules mL^{-1} . The reaction time in these latter experiments was 13.3 ms.

A comparison of the dimer/ ND_3 exchange data (Fig. 4) with the dipeptide/ ND_3 exchange data (Fig. 7a and b) is of interest. It demonstrates that considerably higher ND_3 flow rates and concentrations are required

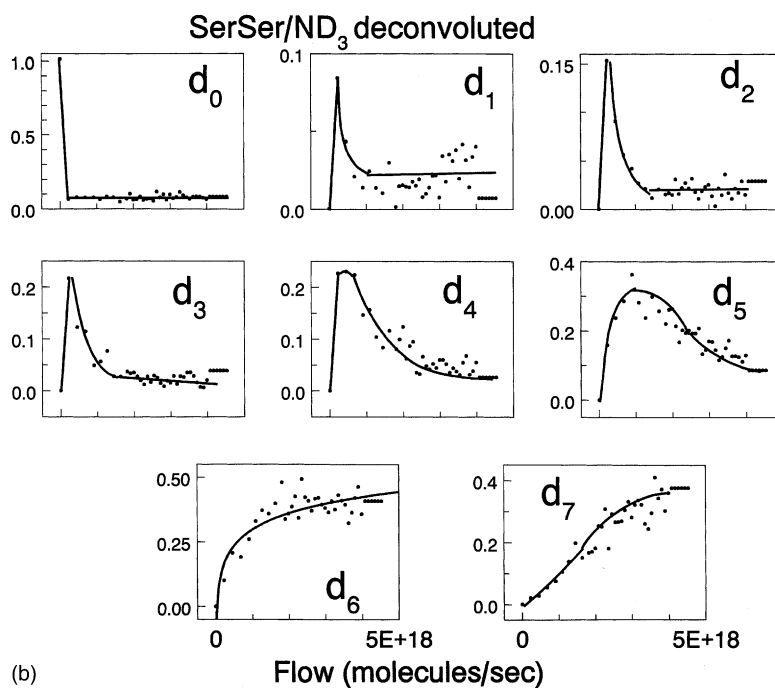
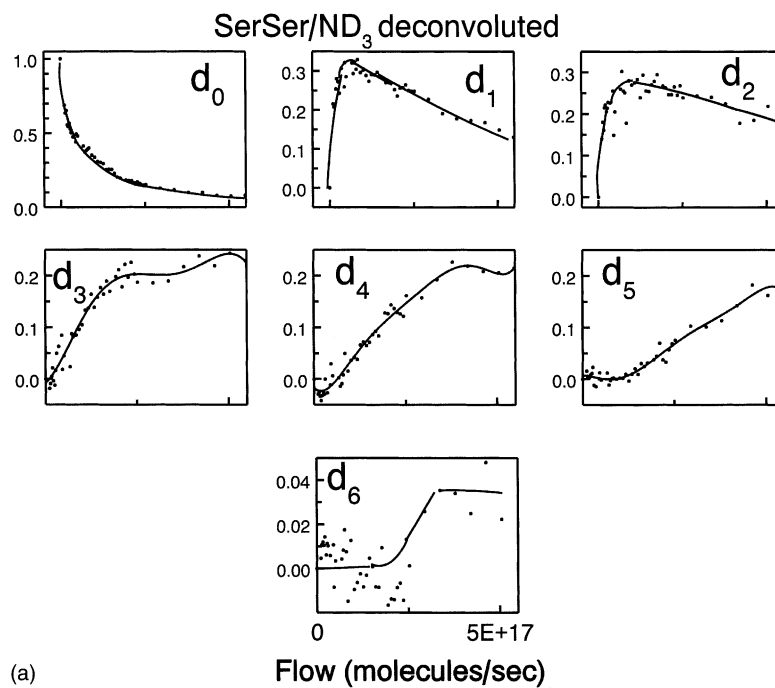


Fig. 7. Relative abundance vs. neutral ND₃ flow rate (in molecules s⁻¹) for the various indicated cations in the reaction of the protonated dipeptide of L-serine with ND₃. The raw data were deconvoluted isotopically from ¹³C, ¹⁵N, etc. contributions: (a) low flow rates; (b) high flow rates.

Table 1
Overall H/D exchange rate constants ($\text{cm}^3 \text{molecule}^{-1} \text{s}^{-1}$) for $(\text{serine})_2\text{H}^+$ and $\text{SerSer}\cdot\text{H}^+$ with ND_3 and CH_3OD , respectively

Ionic reactant	Deuterating reagent	Measured rate constant	Calculated rate constant
$(\text{Serine})_2\text{H}^+$	ND_3	$(6.7 \pm 0.4) \times 10^{-10}$	1.85×10^{-9}
	CH_3OD	$(1.5 \pm 0.3) \times 10^{-10}$	1.12×10^{-9}
$\text{SerSer}\cdot\text{H}^+$	ND_3	$(3.9 \pm 1) \times 10^{-10}$	1.86×10^{-9}
	CH_3OD	$(2.5 \pm 0.4) \times 10^{-10}$	1.13×10^{-9}

for a similar reaction time in order to observe similar degrees of exchange of the seven labile hydrogens of the dipeptide as are observed for the nine labile hydrogens of the dimer. This comparison indicates that the dipeptide has a tighter, more compact structure than the dimer structure and/or that the extra proton is less labile.

Overall disappearance rate constants are summarized in Table 1. The experimental values were obtained from semi-logarithmic decay plots similar to Fig. 5. Calculated values are the ion–polar molecule collision rate constants calculated according to the parameterized expression of Su and Chesnavich [20]. The disappearance rate constant measured for the fully protonated dimer with ND_3 is nearly equal to one half the calculated collision rate constant. H/D exchange takes place via the formation of an intermediate collision complex. Each collision complex can either break up leading back to non-deuterated reactant ions or to the deuterated product ions. The factor of one half between the experimental rate and the calculated collision rate is therefore the maximum expected on purely statistical grounds. This is another indication for the very efficient H/D exchange of the protonated dimer with ND_3 .

Evidence was presented recently for the existence in the gas phase of two isomers of the protonated arginine dimer [21]. The structures ascribed to them were the salt-bridge ion–zwitterion form and the simple protonated ion–molecule form, respectively. The present experiments do not display a bimodal decay for the protonated serine dimer. This is a strong indication that the protonated serine dimer exists in a major single isomeric structure—the ion–molecule form. Serine is not basic enough to stabilize a zwitterionic salt-bridge structure for the dimer [9].

4. Conclusion

H/D isotopic exchange is observed to be a very efficient reaction of the protonated serine dimer with ND_3 . This is an indication for a rather open structure for the protonated dimer. Calculations have shown that the most stable protonated dimer is the *N* atom protonated dimer **1** that has two COOH/COOH hydrogen bonds [5]. The two COOH/COOH hydrogen bonds of the dimer may prevent the exchange of the two carboxyl hydrogens by CH_3OD .

Previous studies by Beauchamp and coworkers [9] have indicated no clear preference for chirality by comparing predicted statistical distributions and observed distributions of the serine dimer. The open structure of the dimer demonstrated through our present H/D exchange data, is related to the observation of no preference for homochirality.

The open structure suggested here for the protonated serine dimer is in marked contrast with the compact structure of the octamer. The octamer has a rather tight multiply-hydrogen-bonded structure. One suggested structure of the protonated octamer has eight COOH/COOH bonds and eight NH_2/OH bonds and it forms a closed cage [5]. Alternative ones are a tightly packed structure made up of seven zwitterionic serine units and a single serine protonated at the amino terminus [8] or a cube in which all eight serines are bound via a central zwitterionic core and stabilized by hydrogen bonding between facing pairs of serines [9].

The strong preference for homochirality of the octamer is directly related to its tightly packed gas phase ‘cubic’ structure that is presumed to be formed from a homochiral solution phase precursor [9]. The octamer is expected to undergo very inefficient H/D exchange

even with ND₃. Experiments are underway to test this hypothesis.

Future studies involving simulations of the present data and extraction of site-specific rate constants will hopefully shed more light on the consecutive order of H/D exchange for different sites reacting at different rates.

Acknowledgements

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