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# Gas-phase H/D exchange of the protonated serine octamer cluster: “Ion ping pong” of populations A and B

Ulf Mazurek[a,*], Marianne Engeser[b], Chava Lifshitz[a]

[a] *Department of Physical Chemistry and The Farkas Center for Light-Induced Processes,*
*The Hebrew University of Jerusalem, Jerusalem 91904, Israel*
[b] *Kekulé-Institut für Organische Chemie und Biochemie, Rheinische Friedrich-Wilhelms-Universität Bonn,*
*Gerhard-Domagk-Str. 1, 53121 Bonn, Germany*

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Dedicated to the memory of Professor Chava Lifshitz, who untimely passed away while this investigation was in progress.

## Abstract

The stability of protonated serine octamer clusters was investigated by forward and reverse H/D exchange in the gas phase. The existence of two independent populations with different overall exchange rate constants and different stabilities toward collision-induced dissociation was confirmed.

*Keywords:* FTICR–MS; Serine octamer; Gas-phase chemistry; Hydrogen/deuterium exchange

## 1. Introduction

In this work, we re-address the structure and stability of the protonated serine octamer cluster, $[(\mathrm{ser})_8\mathrm{H}]^+$. Since its first observation [1,2] and even more since the discovery of its strong preference for homochiral formation [1,2] (which can be turned into a strong preference for *hetero*chiral formation of the decamer upon addition of a second proton to yield $[(\mathrm{ser})_{10}\mathrm{H}_2]^{2+}$ [3]), the structure of this cluster has been addressed with great interest and effort [4,5]. In brief, one isomer with a cross section of about 190 $\text{Å}^2$ has been observed in ion-mobility experiments [6–8] while hydrogen/deuterium exchange (HDX) experiments revealed the existence of two clusters (conveniently designated A and B [9] for the slower- and faster-exchanging population, respectively) as evident from a bimodal exchange kinetics [9–11]. However, a different setup of the HDX experiment revealed only one isomer [12]. An explanation for the apparent discrepancy is offered when assigning different stabilities to the two isomer populations. Thus, if isomer B is rather susceptible to collision-induced dissociation (CID), different experimental setups (varying in internal ion energies or pressure regimes) should yield substantially different results [9–11]. As an alternative, the question of potential conversion of one of the isomer populations into the other [12], initiated either by the mere fact of collisions or by specific interaction of the deuterating agent [13] with the serine octamer cluster, remained.

To investigate the potential interconversion of populations A and B, the cluster $[(\mathrm{ser})_8\mathrm{H}]^+$ was subjected to sequential forward and reverse H/D exchange in the gas phase. Interconversion of the two isomers should lead to a decline or disappearance of the population with lower stability. If in contrast the bimodal HDX kinetics reported for the forward exchange [9,10] is also observed during the reverse exchange, the interaction with the deuterating agent does *not* facilitate the populations’ interconversion, and the cluster’s structure is not altered in the course of H/D exchange.

## 2. Experimental

### 2.1. Chemicals and spectrometer

$\mathrm{CD_3OD}$ (Euriso-Top, 99.8% atom-D), $\mathrm{CH_3OH}$ (B.T. Baker, 99.8%) and L-serine (Aldrich, 99%, 97% e.e.) were used without further purification. All experiments were carried out in

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* Corresponding author. Tel.: +972 2 658 4834; fax: +972 2 561 8033.
*E-mail addresses:* ykv@chem.ch.huji.ac.il (U. Mazurek), Marianne.Engeser@uni-bonn.de (M. Engeser).

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a commercial Bruker APEX IV FT-ICR mass spectrometer equipped with a superconducting 7 T magnet and an Apollo electrospray ionization (ESI) source. A 1 mM solution of L-serine in $H_2O/CH_3OH$ (1:1, 2.5% $CH_3COOH$) was sprayed at a rate of 2–3 μL/min through a 70° off-axis, room-temperature stainless-steel capillary of 10 μm i.d. Ions were transferred in the first of three differential pump stages of the ion source with a nickel-coated glass capillary of 0.5 mm i.d. $N_2$ was used as spraying gas and drying gas, the temperature of the drying gas was set to 250 °C. The ions were accumulated in the instrument's hexapole for 0.5–1 s, introduced into the FT-ICR cell, subjected to H/D exchange, and detected in broadband mode (*m*/*z* range 100–1500, time-domain data size 512 kWords) by a standard excitation and detection sequence. The pressure in the ICR cell was measured with an uncalibrated Granville-Philips ion gauge and not corrected (background pressure: $p_{\text{cell}} = 3 \times 10^{-11}$ mbar).

### 2.2. *Forward and reverse H/D exchange*

*Forward H/D exchange* was achieved by opening a pulse valve connected to a 1 L reservoir containing gaseous $CD_3OD$ at a pressure of 8 mbar. The pulse valve opening time was 0.02 s throughout the experiments. The pulse valve opening and closing commands were placed inside a pulse valve operation loop of the spectrometer-controlling software. Different reaction periods were set by varying the number of loop executions in the range of 100–3000 corresponding to reaction times of 2–60 s. During this procedure, the deuterating agent's pressure in the ICR cell raised to $p_{\text{cell}} = 2.6 \pm 0.2 \times 10^{-8}$ mbar and, then, remained constant for the remaining exchange period. Completion of the pulse valve loop was followed by a 150 s pump-down delay which allowed the pressure to reach a value of $p_{\text{cell}} = 2.0 \pm 0.3 \times 10^{-10}$ mbar. The *sequential forward and*

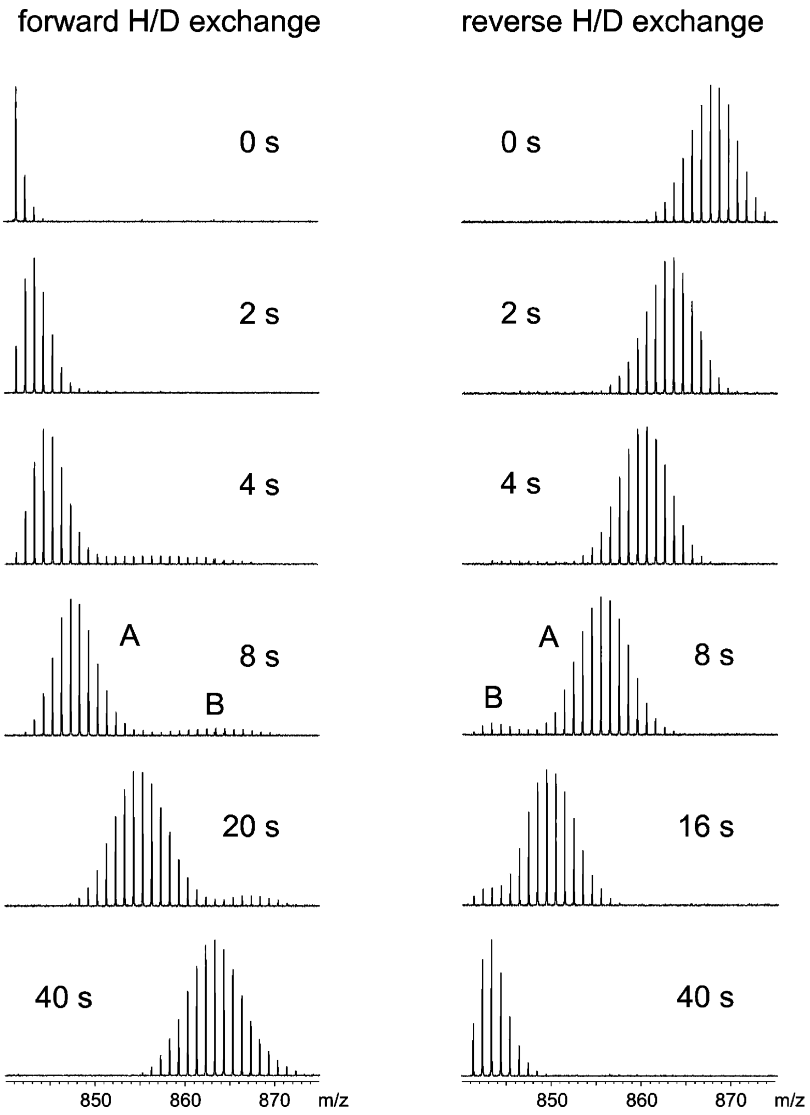

Fig. 1. Forward and reverse H/D exchange of $[(\text{ser})_8\text{H}]^+$ with $CD_3OD$ and $CH_3OH$, respectively. Left column: H/D exchange of $[(\text{ser})_8\text{H}]^+$ with $CD_3OD$ at a pressure of $p_{\text{cell}} = 2.6 \pm 0.2 \times 10^{-8}$ mbar; right column: H/D exchange of $[(\text{ser})_8\text{H}]^+$ with $CD_3OD$ at a pressure of $p_{\text{cell}} = 2.6 \pm 0.2 \times 10^{-8}$ mbar and a reaction period of 60 s, followed by reverse H/D exchange with $CH_3OH$ at a pressure of $p_{\text{cell}} = 7.2 \pm 0.3 \times 10^{-8}$ mbar. In both cases, the bimodal exchange kinetics is clearly visible. The reaction periods reported here are estimates based on the number of pulse valve openings (see Section 2).

*reverse H/D exchange* was achieved by opening the $CD_3OD$ pulse valve as described above (3000 repetitions of the pulse valve operation loop), followed by a 60 s pump-down delay to reach $p_{cell} = 3.6 \pm 0.1 \times 10^{-10}$ mbar and the subsequent opening of a second pulse valve connected to another 1 L reservoir containing gaseous $CH_3OH$ at a pressure of 8 mbar. The opening time of the second pulse valve again was 0.02 s, and a number of the respective pulse valve operation loop executions in the range of 100–2000 (2–40 s, $p_{cell} = 7.2 \pm 0.3 \times 10^{-8}$ mbar during the gas pulse) was followed by a 150 s pump-down delay, thus allowing $p_{cell}$ to reach a value of $2.3 \pm 0.3 \times 10^{-10}$ mbar. The reported data are the sums of 4 scans.

## 3. Results and discussion

In order to address the question of potential interconversion of the two serine octamer populations A and B (named according to Ref. [9]), we subjected the non-deuterated serine octamer cluster, $[(ser)_8H]^+$, to forward and subsequent reverse H/D exchange. During the forward exchange (Fig. 1, left column), the two different ion populations can be clearly distinguished on the basis of their different overall H/D exchange speed. At about 8 s exchange time, the two populations are fully separated. For longer reaction periods, population A catches up with its faster-exchanging counterpart B. After an exchange period of about 40 s, H/D exchange is nearly complete and the two populations are not distinguishable anymore. Clusters higher than the octamer or metaclusters were not observed in significant amounts, thus ruling out the formation of the second population by CID of larger clusters.

After H/D exchange was as complete as experimentally feasible (60 s), we subjected the ions to reverse H/D exchange (Fig. 1, right column). As it was for the forward exchange, two populations can be clearly distinguished, with the ions of faster-exchanging population B now appearing toward lower $m/z$ values, i.e., left to their A counterparts. Further, even the abundance ratio B/A is not reduced significantly. Accordingly, we conclude that there is no interconversion of the two ion populations A and B during HDX with methanol.

In order to avoid any possible excitation and destruction of an easily fragmenting population [10], a mass-selection step had been omitted in the experiments described above. To clarify the amount of unintended CID occurring during HDX, the experiment was repeated including a careful mass-selection of the octamer cluster prior to the H/D exchange (spectra not shown). Three decisive observations could be made. First, the interaction with methanol causes substantial fragmentation of the protonated octamer yielding protonated dimer and monomer as major ionic products during HDX. Second, the relative abundance of population B decreases substantially and even vanishes completely when a standard mass-selection routine is applied. Variation of the mass-selection parameters affects the relative amount of B tremendously. Despite considerable effort, we did not achieve to find a parameter combination for good octamer isolation without significant loss of population B. Third, the fragments' isotopic patterns indicate fragmentation of B rather than A. For example, after 12 s of HDX the dimer shows an abundance maximum at $m/z = 217$ corresponding to an exchange of about 3 H per serine molecule. This does not fit to the amount of exchange at the same reaction period for population A (maximum at $m/z = 850$, corresponding to 1–2 H exchanged per serine molecule), but matches reasonably well that of population B (maximum at $m/z = 868$, corresponding to 3–4 H exchanged per serine molecule). In conclusion, previous reports on the lower stability of B [9–11] and its extreme sensitivity to CID/excess energy were fully confirmed by these experiments.

Note that the influence of the spraying solution's composition onto the B/A ratio is neglectable compared to the effect of electrospray ionization conditions: An increase of the percentage of $CH_3COOH$ in five steps from 0.1% up to 10% showed only a slight B/A ratio enhancement of about 60%. On the other hand, increasing the serine concentration from 0.2 mM in five steps up to 10 mM diminished the B/A ratio by 70%. Overall, population B's abundance reached 4–12% of population A's abundance. Thus, the observed abundance differences do not exceed the experimental error margins due to ESI fluctuations during the course of the experiments.

Addressing the seemingly contradictory results of ion-mobility and H/D exchange experiments (one [6–8] versus two [9–11] ion populations), we would like to point out that ion-mobility and H/D exchange experiments may well lead to different results. For example, two populations of 12-fold protonated ubiquitin are observed in H/D exchange experiments [14,15] but only one in ion-mobility experiments [16]. These observations have been attributed to nearly identical cross sections of two populations, which, therefore, are only visible in H/D exchange experiments [17]. The same argumentation may hold true for the serine case as well. In addition, the diverging results among the H/D exchange studies for the protonated serine octamer can be explained easily by the facile fragmentation of population B [10,12] as stated above.

## 4. Conclusions

In the study presented here, the stability of the protonated serine octamer was reinvestigated. Prior findings of two independent, non-interconverting populations [9–11] were confirmed by showing a bimodal H/D exchange reaction kinetics during forward HDX. Further, the very same bimodal behavior was observed during reverse HDX, indicating that the two populations do not interconvert and that HDX does not change the structures of serine octamer clusters. Therefore, H/D exchange rate constants should provide valuable data for structural assignments of the two serine octamer populations [10]. Even the slightest activation of the octamer, however, leads to substantial amounts of collision-induced decay during HDX, as evident from octamer mass-selection/HDX experiments. Confirming prior findings [11], the ions of population B were found to be less stable towards CID. On the other hand, formation of population B ions from metaclusters could be completely ruled out. Further investigations will be necessary to obtain a complete 3D picture of $[(ser)_8H]^+$ and its different ion populations.

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