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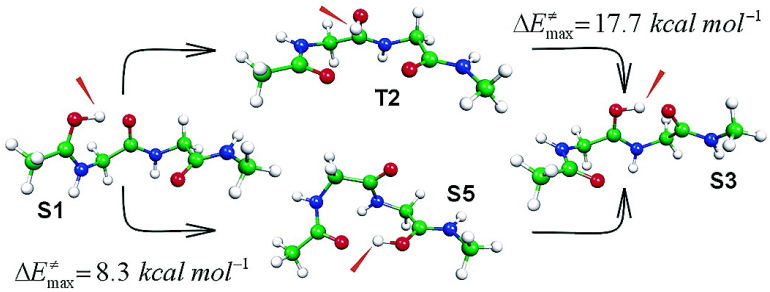

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A Novel Mechanism of Proton Transfer in Protonated Peptides

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Peptides are key biomolecules. Their role and functionality is broad. Enzymatic catalysis is one of their most well-known functions. They also form signal-processing systems or even act themselves as signal messengers. Peptides may contain a series of basic groups. Among them, the terminal amino group, the amino group of lysine, the aromatic ring of histidine, and arginine are the most important. These basic groups are usually protonated, and if so, they often become a part of bridges that stabilize the tertiary structure of proteins. Protonation can also change the conformational equilibria of peptides and, consequently, influence their biological activity. However, peptides also contain groups which are less basic. These are mainly oxygen and nitrogen atoms of peptide bonds. Proton interactions with such atoms are not so strong, but under certain circumstances, they may play an important role. Hydrolysis of a peptide bond in an acid solution[1] is a good example. In the gas phase, the importance of this type of interaction is increasing, as it is expected to play a key role in peptide fragmentation processes when peptides are analyzed by mass spectroscopy.[2,3] These methods are now becoming more and more important with the development of soft ionization methods.[4−6]

When a longer peptide chain is available, the proton can interact with more groups, and also a proton transfer may occur. This may not only influence the above-mentioned fragmentation processes, but it may have several other functions in living systems, e.g., information transfer. Therefore, proton transfer has several times been subjected to computational studies.[7−9]

Proton transfer from the oxygen to the nitrogen of a peptide bond exhibits a high energy barrier[7] of about 39.1 kcal mol^{-1}. On the other hand, processes[8] that involve only oxygen atoms show significantly smaller energy barriers (16.5 kcal mol^{-1}). This would imply that only interactions with oxygens of peptide bonds are important for proton transfer. In our study, we will focus on this idea, and we will present a mechanism of proton transfer with approximately half the energy barrier compared to that published so far.[8]

In theory, a particularly long peptide chain should be used as a model for the appropriate description of the proton transfer. However, the flexibility of such a chain would lead to a very complicated potential energy surface and, of course, very long calculations. Therefore, we decided to use *N*-acetylglycyl-*N*^{1}-methylglycinamide (AGA) as the model peptide (Figure 1).

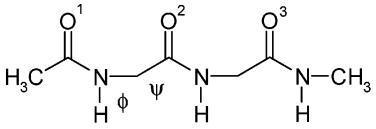

Figure 1. Peptide model.

We assume that the chemical and structural properties of the middle amidic group and the internal parts of the terminal amidic groups are very similar to the properties of peptide groups in a glycine polypeptide. If this is so, then the middle amidic group of AGA can be used as a model for proton transfer in polyglycine. The energies of structures on a proton-transfer pathway were calculated with density functional theory employing the hybrid functional B3LYP and the 6-31++G** basis set. It is known that the accuracy of this method should be, in this case, comparable with the accuracy of perturbation (MP2) or coupled clusters methods.[5]

First, the mechanism published in the literature[8] (mechanism A) will be applied to our model. It will be described in the **S1** → **S3** direction (Figure 1S, Supporting Information). At the beginning, the proton is situated between the first and the second oxygen atoms (structure **S1**). The proton is connected to the first oxygen by a bond that is formed by an interaction of the proton with a lone pair of the oxygen atom. This only slightly influences the bond order of the carbonyl C═O bond. The proton is also stabilized by interaction with the second oxygen. This stabilization creates a seven-membered ring in which the proton is situated near to both of the amidic group planes. The proton transfer is started by the proton jump between these two oxygens (**S1** → **T1** → **S2**). The situation in structure **S2** is very similar to that in **S1**, but here the proton is bonded to the second oxygen and stabilized by the first oxygen. The geometry difference between **S1** and **S2** is small, and the energy barrier for the proton jump is also very low (Figure 2).

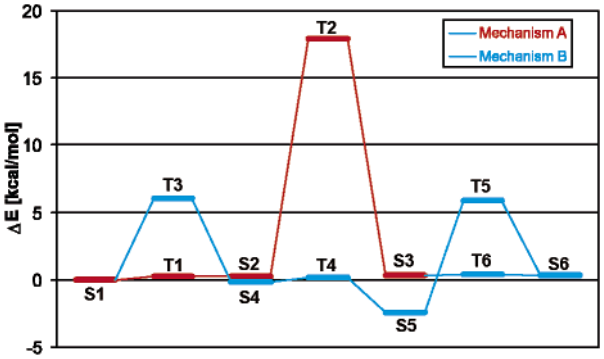

Figure 2. Relative electronic energies along proton-transfer pathways from **S1** to **S3**. Comparison of mechanisms A (**S1**−**T1**−**S2**−**T2**−**S3**) and B (**S1**−**T3**−**S4**−**T4**−**S5**−**T5**−**S6**−**T6**−**S3**).

The proton transfer proceeds by proton rotation around the second carbonyl double bond, reaching structure **S3** (**S2** → **T2** → **S3**). Here, the proton occupies a configuration similar to that in **S1**, but it is located between the oxygens of the second and the third amidic groups. So, during these two steps the proton is transferred along one amidic unit of the triamide chain. The rate-determining step of mechanism A is the isomerization process (rotation of the proton around the double bond of the carbonyl group) with transition state **T2** and a barrier of 17.7 kcal mol^{-1}. The proton is located

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Table 1. Comparison of Some Mechanism A and Mechanism B Geometry Parameters

	mechanism A				mechanism B		
	n(O)[a]	α (°)[b]	*β* (°)[c]		*n*(O)[a]	α (°)[b]	*β* (°)[c]
S1	1	160.6	19.4	**S1**	1	160.6	19.4
T1	2	8.4	8.4	**T3**	1	−157.6	22.4
S2	2	7.4	7.4	**S4**	1	−164.8	15.2
T2	2	88.4	88.4	**T4**	1	−134.8	45.2
S3	2	161.9	18.1	**S5**	3	−11.8	11.8
				T5	3	0.9	0.9
				S6	3	7.8	7.8
				T6	3	9.2	9.2
				S3	2	161.9	18.1
max			90.0[d]				45.2

[a] Oxygen number. [b] Dihedral angle C_α—C═O—H^+. [c] Deviation from the amidic plane (β = abs(α) if abs(α) ≤ 90°; β = 180 − abs(α) if abs(α) > 90°). [d] Due to nature of rotation.

in **T2** almost perpendicularly to the plane of the corresponding amidic group.

It is generally known that peptides are very flexible. The question is whether such a high flexibility would allow for another proton-transfer mechanism where the proton would all the time remain close to the planes of the amidic groups. A mechanism that meets such a feature has been found in our study (mechanism B).

We begin again with structure **S1**, in order to be able to compare both mechanisms. This proton transfer stops in structure **S3** like that in mechanism A. Dihedral angles φ and ψ between the first and second amidic groups are changed during the first step. Consequently, the stabilization of the proton by the second oxygen is substituted by the stabilization with the third oxygen (**S1** → **T3** → **S4**; Figure 2S, Supporting Information). The original seven-membered ring (**S1**) is transformed into the lower energy 10-membered ring (**S4**), and the proton still resides near the plane of the first amidic group. The decrease of energy may imply higher tension in the original seven-membered ring. During the second step, the proton jumps from the first to the third oxygen (**S4** → **T4** → **S5**). This process has a higher energy barrier than the similar proton jump between adjacent oxygens (**S1** → **T1** → **S2**). The reason is the higher geometry difference between structures **S4** and **S5** compared to that between **S1** and **S2** (mechanism A). Structure **S5** has then the lowest energy along the proton-transfer pathway. The penultimate step is very similar to the first step. The interaction of the proton with the first oxygen is broken, and a stabilization with the second oxygen occurs (**S5** → **T5** → **S6**). The original 10-membered ring is transformed back to the seven-membered ring. But now the proton is situated on the opposite side toward the second oxygen. The last step is the proton jump between the third and the second oxygens (**S6** → **T6** → **S3**). This leads to a structure where the proton occupies a similar configuration as in **S1** but is now bonded to the next oxygen in the chain. So the proton is transferred along the chain by one amidic unit.

The proton always stays close to the amidic plane of the oxygen that is closest to the proton. This is illustrated by data introduced in Table 1. The maximum deviation of the proton from the amidic plane is 90° in mechanism A and almost half in mechanism B.

Since the energies of structures **S1** and **S3** are similar, the proton transfer is not thermodynamically controlled in either of the two mechanisms. The situation is different from the kinetic point of view. While the energy barriers of all steps in mechanism A are almost symmetrical, mechanism B exhibits a different rate-determining barrier for each direction (the energy barrier is 8.3 kcal mol^{-1} for the **S1** → **S3** direction but only 6.3 kcal mol^{-1} for the opposite direction).

To achieve a more precise kinetic and thermodynamic description of the studied problem, one would need to calculate Gibbs energies. Their calculation seems to be very problematic in this particular case. We performed a thermodynamic analysis on the same level of theory that was used for geometry optimization, and discrepancies were found (Table 1S). The Gibbs energies of some transition states were found to be lower than the Gibbs energies of corresponding initial structures. This situation is described on similar systems in the literature.[9] In our opinion the reason is that some frequency modes, that are not well described as harmonic, are likely responsible for the inaccurate Gibbs energies. Further analysis would be necessary to fully understand this discrepancy. Therefore, one of the goals of our future effort will be the calculation of more precise values of the Gibbs energies and also tunneling effects that may play a significant role.

In summary, a novel mechanism of proton transfer has been found by this computational study. The mechanism exhibits an energy barrier of about 8 kcal mol^{-1}, which is approximately half of the barrier reported so far.[8] The predicted mechanism may kinetically prefer the proton transfer in the **S3** → **S1** direction (from C terminal to N terminal). The results may be strictly valid only for short peptides without side chains. In other cases, side chains can influence flexibility, which is necessary in this mechanism. In longer oligopeptides, the proton transfer can be influenced also by secondary and tertiary structures.

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Supporting Information Available: Tables containing absolute energies, enthalpies, Gibbs energies (in a.u.), dihedral angles φ and ψ for all structures, imaginary frequencies (in cm^{-1}) for all transition structures, Cartesian coordinates (in Å), and perspective view (Figure 1S and 2S for mechanism A and B, respectively) for all structures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hopkinson, A. C.; McClelland, R. A.; Yates, K.; Csizmadia, I. G. *Theor. Chim. Acta* **1969**, *13*, 65.
(2) Green, M. K.; Lebrilla, C. B. *Mass Spectrom. Rev.* **1997**, *16*, 53.
(3) Papayannopoulous, I. A. *Mass Spectrom. Rev.* **1995**, *14*, 49.
(4) Barber, M.; Boddoli, R. D.; Sedgwick, R. D.; Tyler, A. N. *J. Chem. Soc., Chem. Commun.* **1981**, 325.
(5) Hillenkamp, F.; Karas, J.; Beavis, R. C.; Chait, B. T. *Anal. Chem.* **1991**, *63*, 1193.
(6) Smith, R. D.; Loo, J. A.; Barinaga, C. I.; Edmonds, C. G.; Udseth, H. R. *J. Am. Soc. Mass Spectrom.* 1**990**, *62*, 882.
(7) Rodriquez, C. F.; Cunje, A.; Shoeib, T.; Chu, I. K.; Hopkinson A. C.; Siu K. W. M. *J. Phys. Chem. A* **2000**, *104*, 5023.
(8) Paizs, B.; Csonka, I. P.; Lendvay, G.; Suhai, S. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 637.
(9) Rodriquez, C. F.; Cunje, A.; Shoeib, T.; Chu, I. K.; Hopkinson A. C.; Siu K. W. M. *J. Am. Chem. Soc.* **2001**, *123*, 3006.

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