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COMMUNICATION

Do zwitterionic species exist in the non-enzymatic peptide bond formation?†

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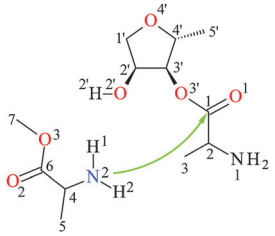
The use of proper computational methods and models has allowed answering the controversial question of whether zwitterionic species exist in the mechanism of peptide bond synthesis in aqueous solution. In fact, the different conformations of zwitterionic species open the door to different mechanistic paths.

The peptide bond synthesis mechanism in ribosomes has attracted much attention in the past years. In order to get information on this problem, aminolysis of esters in aqueous solution has been the subject of many experimental and theoretical studies. The main reason for this interest comes from the hypothesis that similar mechanisms are involved in both media. Experimental studies based on linear free-energy relationships[1] and isotope substitution effects[2] in solution support a stepwise mechanism with participation of different kinds of intermediates, ranging from anionic, cationic, neutral and zwitterionic species. Based on theoretical studies,[3–5] three different mechanisms have been proposed: (1) a concerted mechanism in which the nucleophilic attack on the amino group, the C–O bond cleavage and the proton transfer take place simultaneously; (2) a stepwise mechanism through a neutral intermediate; and (3) a stepwise mechanism through a zwitterionic intermediate. The latter mechanism has been the subject of controversy since even the presence of this intermediate has been questioned. Nevertheless, theoretical studies using a reduced number of water molecules to describe the solvent, or by mixed explicit-polarizable continuum models (PCM), despite their limitations,[6] have supported the existence of this intermediate.[7–9] Warshel and coworkers located a zwitterionic intermediate in the catalyzed methanolysis of formamide (formally the reverse reaction of the one studied in the present manuscript) by combined *ab initio*/Langevin dipole calculations,[10] and more recently the solute electron density model (SMD)[11] has also been used to stabilize zwitterionic species.[12]

The present communication is focused on the existence and relevance of the zwitterionic species in the aminolysis of esters in solution within a realistic molecular model similar to the natural substrate used by ribosomes, as shown in Scheme 1. The model of the substrate in our hybrid QM/MM calculations includes all the atoms involved in the different proposed mechanisms. It is important to point out that previous theoretical studies in solution have been performed with simpler molecular models ignoring, for instance, the constraint of the ribose ring that can govern the role of the hydroxyl group bound to C2′.

The substrate has been treated by semiempirical AM1[13] hamiltonian and by means of the M06-2X functional developed by Truhlar's group[14] with the standard 6-31 + G(d,p) basis set. The full molecule described quantum mechanically has been embedded in a cubic box of water molecules ($100 \times 80 \times 80$ Å^3) treated by the TIP3P[15] force field, as implemented in the fDYNAMO library.[16] Periodic boundary conditions were applied and a switched cut-off from 16 to 14.5 Å was employed for all non-bonded MM interactions. The water molecules were equilibrated by means of hybrid AM1/MM Molecular Dynamics (MD) in the reactant complex. Afterwards, the nucleophilic attack of the amino group, N2, on the carbonyl group of the ester, C1, was carried out to explore the formation of the zwitterionic intermediate with both hybrid potentials, AM1/TIP3P and M06-2X/TIP3P. An AM1/MM mono-dimensional PMF (Fig. 1) was computed at 300 K with the *NVT* ensemble using the antisymmetric combination of the forming and breaking bonds (N2–C1 and C1–O3′), with 81 simulation windows evenly distributed in the range from −1.76 Å to 2.24 Å. An umbrella force constant of 2500 kcal mol^{-1} Å^{-2} was applied to the distinguished reaction coordinate to allow a perfect overlapping



Scheme 1 Schematic model for the nucleophilic attack of the amino group on the carbonyl group of the ester.

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† Electronic supplementary information (ESI) available: Evolution of key distances obtained by the QM/MM MD simulation of the zwitterionic species, ball and stick representations, natural population analysis, 2D PES for the ester aminolysis at the AM1/MM level, and table of zwitterionic species optimized in continuum solvation models. See DOI: 10.1039/c2cc35409h

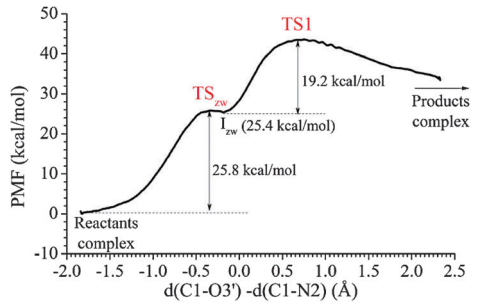

Fig. 1 Free energy profile of ester aminolysis at the AM1/MM level.

among the windows. The weighted histogram analysis method, combined with the umbrella sampling approach,[17] was employed to scan the reaction coordinate. 10 ps of relaxation and 20 ps of production, with a time step of 0.5 fs, using the velocity Verlet algorithm[18] to update the velocities, were run in each window.

As Fig. 1 shows, the reaction is a step-wise mechanism with a rate limiting TS1 described by a N1–C1 forming bond concomitant with an advanced C1–O3′ breaking bond and a proton transfer from N2 to O3′ (C1–N2 = 1.52 ± 0.03 Å, C1–O3′ = 2.26 ± 0.04 Å and H–O3′ = 2.44 ± 0.20 Å). TS1 is reached after crossing an intermediate, I_{zw}, with tetrahedral zwitterionic character as revealed by the averaged distances C1–N2 = 1.65 ± 0.03 Å, C1–O3′ = 1.44 ± 0.02 Å and C1–O1 = 1.29 ± 0.02 Å.

I_{zw} presents a very small backward free energy barrier of 0.4 kcal mol^{-1}. Thus, in order to further explore the nature of this meta-stable state, long QM/MM MD simulations have been carried out constraining the reaction coordinate at the value corresponding to this minimum. The time evolution of some geometrical parameters shows that many different conformers are involved in this intermediate zwitterionic state (see ESI†). Four representative structures of these families of conformers have been selected and optimized at the M06-2X/MM level. The resulting species are schematically shown in Fig. 2.

ZWa (Fig. 2a) corresponds to a structure where the H1 of the amino group and the O3′ acceptor are at a distance (2.57 Å) suggesting a possible direct proton transfer. The O1 atom establishes an intramolecular hydrogen bond with the C2′ hydroxyl group (1.68 Å) and two intermolecular hydrogen bonds with two water molecules (1.63 and 1.60 Å). These strong hydrogen bond interactions are related to a large negative charge developed on the O1 atom (−1.11 a.u.) and an elongation of the C1–O1

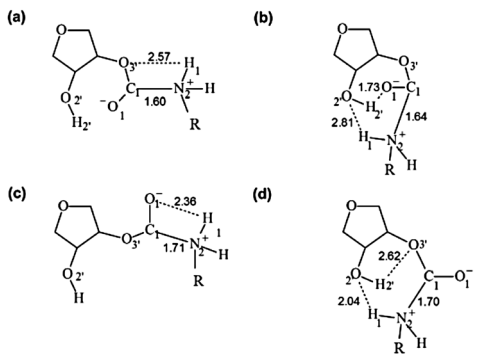

Fig. 2 Schematic representation of the zwitterionic intermediates located at the M06-2X/MM level. Key distances are reported in Å.

interatomic distance (1.30 Å), in between standard double and single bond distances. Thus, the C1 center presents a distorted tetrahedral geometry in this intermediate. The charge on NH_2 is +0.50 a.u. corresponding to an advanced nucleophilic attack defined by the C1–N2 interatomic distance (1.60 Å). Natural population analysis[19] shows that the positive charge developed on the NH_2 moiety is correlated with the negative charge developed on the O1 atom from reactants to the intermediate (see ESI†). This shows an electronic flux between the amino and the ester groups, which is related to changes in the geometries of the substrate along the reaction process, reinforced by the polarization effect due to the reaction field created by the water molecules of the solvent.

ZWb (Fig. 2b) is characterized by a long distance between the O3′ atom and any of the two hydrogen atoms of the attacking amine N2 group (2.93 and 3.22 Å) and a very unfavourable angle for a direct proton transfer (N2–H1–O3′ and N2–H2–O3′ angles equal to 47.5 and 30.6°, respectively). Instead, the H1 hydrogen atom is at a hydrogen bond distance from the oxygen atom of the C2′ hydroxyl group (2.81 Å), which together with a N2–H1–O2′ angle of 103° suggest a possible proton transfer to this atom. The fact that the C2′ hydroxyl group establishes a strong hydrogen bond interaction with the O1 atom (H–O1 distance equal to 1.73 Å) opens the possibility of a six membered ring TS describing a double proton transfer leading to a new neutral intermediate. The analysis of the charge evolution on the key atoms and the interatomic distance reveals a less advanced nucleophilic attack: a slightly longer C1–N2 distance (1.64 Å) than in ZWa. The less negative charge on the O1 atom in ZWb (−1.03 a.u.) is reflected in weaker hydrogen bonds established with the hydrogen atom of the C2′ hydroxyl group and with two water molecules (found at 1.65 and 1.85 Å).

ZWc (Fig. 2c) is characterized by a long distance between both two hydrogen atoms of the attacking amine NH_2 group and the O2′ atom (4.82 and 4.85 Å) and also an unfavourable orientation to be directly transferred to the O3′ atom (H–N2–C1–O3′ dihedral angles equal to 143.6 and −103.4°). Instead, the conformation of ZWc opens the possibility of a direct transfer of one hydrogen atom from N2 to O1 (distance equal to 2.36 Å) through a four-membered ring TS, in contrast to a six-membered ring TS that ZWb suggests. The nucleophilic attack is less advanced than in previous intermediates (the N2–C1 distance is 1.71 Å), which is associated with a less charge transfer from NH_2 to O1. As before, the negative charge developed on the O1 atom (−1.03 a.u) is stabilized by the interaction with two water molecules (H–O1 distances equal to 1.69 and 1.54 Å). This ZWc structure presents the largest dipole moment of the four analyzed structures (9.40 D). It must be pointed out that the dipole moment depends on the zwitterionic character, the polarization effect of the solvent (as observed from the comparison to gas phase values) and the global conformation (extended structures are related to larger dipole moments).

Finally, ZWd (Fig. 2d) presents a conformation where the N2–H1 bond points towards the oxygen atom of the C2′ hydroxyl group (H1–O2′ distance equal to 2.04 Å), and its hydrogen atom is oriented towards the O3′ atom (H2′–O3′ distance equal to 2.62 Å). As a consequence, this conformation could progress towards a six membered ring TS for the aminolysis

reaction, involving the C2′ hydroxyl group. Nevertheless, the distance between H1 of the NH_2 group and O3′ is also relatively short (2.36 Å) and then progress to a four membered ring TS, involving C1, N2, H1 and O3′ atoms, would also be possible. The nucleophilic attack is more advanced than in ZWc (C1–N2 distance equal to 1.70 Å), but less than in ZWa and ZWb, which is associated with a lower negative charge transfer on the O1 atom (−1.00 a.u.) and lower positive charge on NH_2 (+0.24 a.u.). These values, together with a short C1–O1 distance (1.28 Å), reveal less zwitterionic character on this intermediate than in ZWa and ZWb intermediates. Again, the negative charge on the O1 atom is stabilized by the strong intermolecular hydrogen bond with two water molecules and a weak intramolecular hydrogen bond established with the amino group of the vicinal aminoacid (H–O1 distances equal to 1.60, 1.60 and 2.62 Å).

These different conformations of the zwitterionic intermediate can be considered as the origin of different mechanisms of the aminolysis of esters. In particular, ZWa would precede a four-membered ring TS that will directly render products. ZWc would also give a four-membered ring TS but now leads to a neutral intermediate. ZWb and ZWd would be the previous step to arrive at a six-membered ring TS but, while the former describes a double proton transfer leading to a new neutral intermediate, the mechanism from the ZWd would involve a TS directly conducting to the products. This last mechanism has been termed by Green and Strobel as a substrate-assisted catalysis.[20] Similar mechanisms for the aminolysis of esters involving water-assisted catalysis, through the six-membered ring TS, to the ones studied by theoretical calculations in aqueous solution by Warshel and coworkers[5] and Yang and Drueckhammer[21] could be possible. Furthermore, the proton transfer from the C2′ hydroxyl group to the O3′ atom could be assisted by a water molecule thus forming an 8 membered-ring TS, as initially suggested by Schmeing *et al.*[22] From our results, it is inferred that ZWd could be a candidate to evolve through this conformation (a water molecule is at 2.26 Å from the hydrogen atom of the C2′ hydroxyl group). Obviously, keeping in mind that all water molecules in our calculations are treated by a classical force field, exploration of mechanisms assisted by water molecules is excluded. In addition, positive or negative intermediates will not appear in our simulations since they are carried out at neutral pH, in the absence of additional base or acid species.

In this communication it is shown that the main solvent effect is the stabilization of the tetrahedral zwitterionic intermediate and, in particular, the large negative charge developed on the carbonylic oxygen atom of the ester. This is achieved by interactions with two water molecules and, in some cases, also by an intramolecular hydrogen bond. The intermediate is described as an average of significantly different conformers, each of them opening the door to different molecular mechanisms for the ester aminolysis reaction. Our results suggest, in agreement with experimental data, that the first step of the reaction in aqueous solution at neutral pH always implies the initial formation of an additional zwitterionic species. The solvent environment effects have to be properly introduced to successfully find and describe these transient structures. In fact, while we were able to re-optimize ZWa and ZWb at the M06-2X level with PCM and SMD models, ZWc was located just with the latter model and any attempt to localize ZWd failed (see ESI† for details). In this regard, the computational methodology employed in this communication appears to be a promising tool for studying the reaction not only in aqueous solution but also in the active site of the ribosome. Our results suggest that the hypothesis that similar mechanisms are involved in the ribosome cannot be then concluded since even the presence of these zwitterionic species depends on specific interactions with the ribosome. The existence and the role of these possible intermediates must be explored with an adequate method and model to get insight into this reaction in the ribosome, which is a key step in the biosynthesis of proteins.

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Notes and references

1 (*a*) W. P. Jencks and G. M. Blackburn, *J. Am. Chem. Soc.*, 1968, **90**, 2638; (*b*) W. P. Jencks, *Acc. Chem. Res.*, 1976, **9**, 425; (*c*) A. C. Satterth-wait and W. P. Jencks, *J. Am. Chem. Soc.*, 1974, **96**, 7018; (*d*) M. M. Cox and W. P. Jencks, *J. Am. Chem. Soc.*, 1981, **103**, 572.
2 (*a*) J. F Marlier, B. A. Haptonstall, A. J. Johnson and K. A. Sacksteder, *J. Am. Chem. Soc.*, 1997, **119**, 8838; (*b*) J. F. Marlier, *Acc. Chem. Res.*, 2001, **34**, 283; (*c*) A. C. Seila, K. Okuda, S. Núñez, A. F. Seila and S. A. Strobel, *Biochemistry*, 2005, **44**, 4018; (*d*) D. A. Hiller, M. Zhong, V. Singh and S. A. Strobel, *Biochemistry*, 2010, **49**, 3868.
3 S. Ilieva, B. Galabov, D. G. Musaev, K. Morokuma and H. F. Schaefer III, *J. Org. Chem.*, 2003, **68**, 1496.
4 B. Galabov, Y. Atanasov, S. Ilieva and H. F. Schaefer III, *J. Phys. Chem. A*, 2005, **109**, 11470.
5 P. K. Sharma, Y. Xiang, M. Kato and A. Warshel, *Biochemistry*, 2005, **44**, 11307.
6 S. C. L. Kamerlin, M. Haranczyk and A. Warshel, *ChemPhysChem*, 2009, **10**, 1125.
7 S. Chalmet, W. Harb and M. F. Ruiz-López, *J. Phys. Chem. A*, 2001, **105**, 11574.
8 D. A. Singleton and S. R. Merrigan, *J. Am. Chem. Soc.*, 2000, **122**, 11035.
9 D. D. Sung, I. S. Koo, K. Yang and I. Lee, *Chem. Phys. Lett.*, 2006, **426**, 280.
10 M. Sýtrajbl, J. Florián and A. Warshel, *J. Am. Chem. Soc.*, 2000, **122**, 5354.
11 A. V. Marenich, C. J. Cramer and D. G. Truhlar, *J. Phys. Chem. B*, 2009, **113**, 6378.
12 C. Acosta-Silva, J. Bertran, V. Branchadell and A. Oliva, *J. Am. Chem. Soc.*, 2012, **134**, 5817.
13 M. J. S. Dewar, E. G. Zoebisch, E. F. Healy and J. J. P. Stewart, *J. Am. Chem. Soc.*, 1985, **107**, 3902.
14 (*a*) Y. Zhao and D. G. Truhlar, *Theor. Chem. Acc.*, 2008, **120**, 215; (*b*) Y. Zhao and D. G. Truhlar, *Acc. Chem. Res.*, 2008, **41**, 157.
15 W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey and M. L. Klein, *J. Chem. Phys.*, 1983, **79**, 926.
16 M. J. Field, M. Albe, C. Bret, F. Proust-De Martin and A. Thomas, *J. Comput. Chem.*, 2000, **21**, 1088.
17 (*a*) S. Kumar, D. Bouzida, R. H. Swendsen, P. A. Kollman and J. M. Rosenberg, *J. Comput. Chem.*, 1992, **13**, 1011; (*b*) G. M. Torrie and J. P. Valleau, *J. Comput. Phys.*, 1977, **23**, 187.
18 L. Verlet, *Phys. Rev.*, 1967, **159**, 98.
19 A. E. Reed, R. B. Weinstock and F. Weinhold, *J. Chem. Phys.*, 1985, **83**, 735.
20 J. S. Weinger, K. M. Parnell, S. Dorner, R. Green and S. A. Strobel, *Nat. Struct. Mol. Biol.*, 2004, **11**, 1101.
21 W. Yang and D. G. Drueckhammer, *Org. Lett.*, 2000, **2**, 4133.
22 T. M. Schmeing, K. S. Huang, D. E. Kitchen, S. A. Strobel and T. A. Steitz, *Mol. Cell.*, 2005, **20**, 437.