Theor Chem Acc (2000) 104:461–470
DOI 10.1007/s002140000170

Theoretical
Chemistry Accounts

Regular article

A density functional theory study of a concerted mechanism for proton exchange between amino acid side chains and water

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Received: 25 October 1999 / Accepted: 5 April 2000 /

Abstract. A concerted mechanism for proton exchange between water and the amino acid side chains of cysteine, serine, arginine and glutamic acid has been investigated with hybrid density functional theory. The models used include, besides the amino acid side chain, a number of water molecules ranging from one to five in some cases. The modeling of the amino acids without their backbones is shown to be an excellent approximation. Long-range polarization effects were incorporated through a dielectric cavity method allowing a better comparison to existing measurements for free amino acids in water. The barriers converge rather fast with the number of water molecules for all the present amino acids and the converged values are in reasonable agreement with experiments with discrepancies in the range 2–6 kcal/mol. The dielectric effects were found to be small for all systems except cysteine, where there is a lowering of the barrier by 3–5 kcal/mol. The transition states for these concerted pathways form rings in which the separated charges can be stabilized.

Key words: Proton exchange – Density functional theory – Transition states – Amino acids – Mechanism

1 Introduction

Theoretical studies of enzyme reactions have so far been performed using essentially two different approaches. In the first approach, normally termed the quantum mechanical (QM)–molecular mechanics (MM) model, the protein surrounding the active site has been modeled in detail usually by placing point charges at the individual atoms and sometimes also having dielectric regions with different dielectric constants depending on the actual situation. The active site has usually been treated at a moderate level of accuracy often with a semi-empirical parameterization of some type [1–4]. That type of modeling has been by far the dominating type in the past few decades. In the second approach, where there is considerably less experience, the active site is modeled as accurately as possible using modern density functional theory (DFT) methods [5, 6]. In this approach, the protein surrounding has usually been modeled at a rather approximate level using a continuous dielectric medium. It is obvious that in this approach the quantum chemical model needs to be larger than in the QM–MM model but it is not clear at this stage how large it must be. For systems containing metals, it has been demonstrated that results in good agreement with experiments can be obtained for models with 30–40 atoms and sometimes even for smaller models [5, 6]. Examples from systems without transition metals point in the same direction [7]. It is important to note in this context that the dielectric effects computed in this approach have given almost negligible contributions to the results. If the dielectric effects had been large, the dependence of this part on the dielectric constant, which is chosen in a semiempirical way, would have represented a significant uncertainty of the method, but this is not found to be the case.

In order to shed light on the question of how large a quantum chemical model needs to be, and how accurately the surrounding solvent needs to be described, a simple chemical problem is studied in detail in the present study. The question studied is how the rate of proton exchange between water and an amino acid side chain for a free amino acid in water solvent converges with the number of water molecules in the model. This problem highlights the modeling requirements for a situation where the description of charge separation might be critical. It should be emphasized that the question of how nonuniform charge distributions, as can occur in enzymes, might affect the results is not addressed in this model study; however, that situation has been addressed recently in a model study of the photosynthetic reaction center [8], where it was shown that the highly nonuniform dielectric medium around the oxygen of the quinone Q_A was very well modeled by adding a single water, hydrogen-bonded to the quinone oxygen. Only a concerted mechanism for proton ex-

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change is considered here since this type of mechanism has often been found to be preferred in previous studies [5–7]. For some of the present systems, a stepwise mechanism can furthermore be excluded and it is unlikely for the others. The focus on concerted mechanisms does not mean that any constraint is put on the geometry optimizations, but rather that the molecular models are chosen to describe only the concerted situation. This means models where the water molecules included bridge the starting and end points for the proton transfer. A stepwise proton transfer would probably be better described by grouping the water molecules to optimally stabilize separated charges. Many chemical reactions are described in terms of proton transfers and this is therefore also a relevant and typical problem of interest for a biochemical system. If it is found that a large number (much greater than ten) of water molecules is needed to obtain a reasonable proton exchange rate then it is clear that the type of small models described previously, as used in many recent DFT applications, will at least be incapable of describing this important class of charge-separation problems. If, in contrast, it is found that a reasonable rate is obtained using only a few water molecules then this would give some support for the use of small models for studying biochemical systems, even if the surrounding protein is only very approximately described. The energetic requirements in the present context are set by the accuracy of DFT, which could be estimated to have error bars in the range 3–5 kcal/mol. The amino acids chosen for the present study are represented by their functional groups only, which means that formic acid is used to model glutamic or aspartic acid, formamidine to model arginine, methanol to model serine or threonine and methanethiol to model cysteine. The effect of adding the backbone of the amino acid is tested for serine. The results are presented here for comparison to future gas-phase experiments, but comparisons are also made to results obtained for a water solution, in which case dielectric effects are added based on a simple cavity model.

Experimentally, proton-exchange rates between water and serine, arginine and cysteine molecules have been measured in a pH range from 0.5 to 8.5 and at temperatures of 4, 10, 20, 30 and 36 °C [9,10]. The exchange rates of amino and hydroxyl protons at pH 7.0 and 36 °C were found to be in the range from 700 to about 10000 s^{-1}. Using transition-state theory, the most relevant rates can be translated to barriers, which are all quite similar for the amino acids considered here, in the range 13.1–13.5 kcal/mol. For glutamic acid no barrier is measured. It is important in this context to note that in the experiments catalysis of the proton exchange by the α-amino and α-carboxyl groups was prevented by using acetylated α-amino groups and amidated α-carboxyl groups (or these were protected as methyl ester).

2 Computational details

The calculations were performed in two steps. First, an optimization of the geometry was performed using the Becke's three parameter hybrid method with the Lee, Yang and Parr correlation functional (B3LYP) [11] with double-zeta (D95) basis sets in most cases. For the cysteine reaction the somewhat larger D95(d,p) basis with one polarization function on each atom was used. In the second step the B3LYP energy was evaluated for the optimized geometry using the large 6-311 + G(2d,2p) basis sets, which include diffuse functions and two polarization functions on each atom. All degrees of freedom were optimized. The transition states obtained were confirmed to have only one imaginary frequency of the Hessian. Zero-point vibrational and entropy effects were added based on B3LYP calculations using the same basis sets as for the geometry optimization. The dielectric effects from the surrounding environment were obtained using the self-consistent reaction field method [12]. The self-consistent isodensity polarized continuum model as implemented in the GAUSSIAN94 program was used with a dielectric constant for water of 80. It should be added in this context that the computed dielectric effects were found to be rather small and the overall results are thus not very sensitive to the method used or to the value chosen for the dielectric constant. All calculations were made using the GAUSSIAN94 program [13].

3 Results and discussion

The calculations of the barrier heights for a concerted proton exchange between selected amino acid side chains and water are described in the following sections. No constraint was put on the geometry optimizations, but the chemical models were chosen to optimally describe only the concerted process. The results are presented both with and without dielectric effects included in order to make comparisons to both water solvent and gas-phase experiments possible. For the comparison between calculated barrier heights and measured rates in water solvent, transition-state theory was used. The amino acids are represented by their functional groups only since the study only concerns proton exchange involving the side chain. For serine, the effect of adding the backbone was investigated and was found to be extremely small. The number of water molecules in the model was increased from one to five in some cases. For the models with one, two and three water molecules the possibility of multiple minima does not represent a serious problem, but for the larger models it is clear that this question has to be addressed in some way. In the present cases this was dealt with by choosing the same type of transition state as found for the smaller models, even though other transition states cannot be excluded. The corresponding minima were taken to be those that lie closest geometrically to the transition states obtained (Figs. 1, 2). It should be added in this context that in most cases investigated the rate is found to be reasonably well converged already for three water molecules. The effect of optimizing the geometry including the dielectric was made for one case of the cysteine reactions, since this system has the largest dielectric effects. The effect on the geometry and the geometry effect on the energy were found to be extremely small.

3.1 Cysteine

Concerted proton exchange between cysteine and water was studied for models with up to five water molecules. The barrier heights are given in Table 1 and the

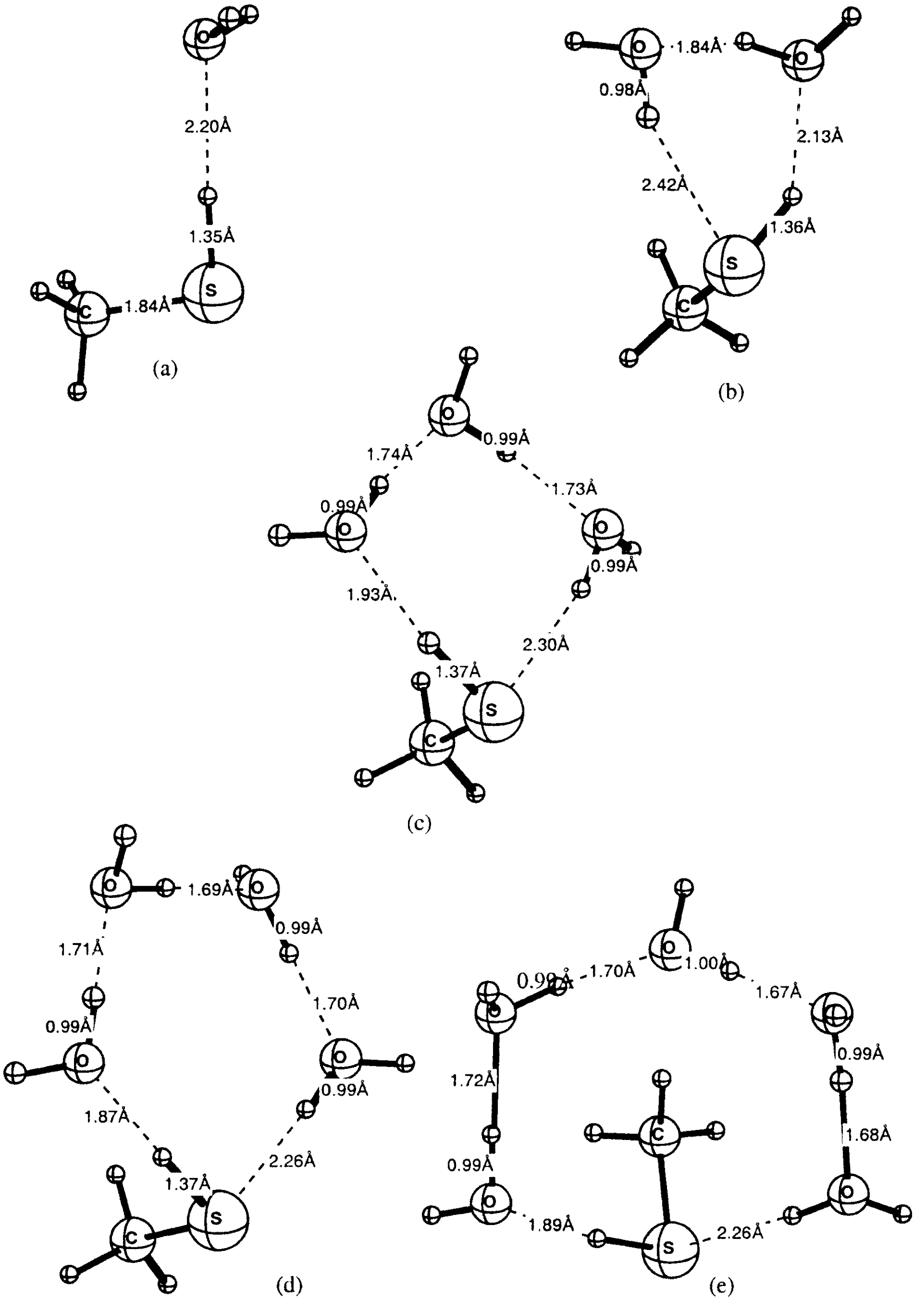

Fig. 1. Equilibrium structures for cysteine

transition states are shown in Fig. 2. The transition-state structures are as expected on the basis on previous investigations on similar processes [14]. Together, the amino acid and the water molecules form a ring in which the separated charges of the thiolate and the protonated water molecules can be stabilized. This stabilization is larger the larger the ring is, until convergence is reached. The second effect of increasing the ring is to relieve the strain caused by nonoptimal bond angles for the smaller rings. At the transition state for the case of cysteine the positive charge is stabilized among the water molecules, while the negative charge resides on cysteine. This is best seen on those O—H bonds that are closest to the sulfur of the cysteine. This distance decreases gradually as the number of water molecules is increased, starting with 1.13 Å for one water molecule, going to 1.10 Å for two water molecules, to 1.08 Å for three water molecules and to 1.06 Å for four water molecules. The corresponding S—H distance increases from 1.85 Å for one water molecule to 1.89 Å for four water molecules. The structures with four and five water molecules are quite similar and the geometric changes at this stage are well converged. The best way to view these ring-type model structures is to consider them as the essential part of the actual structure of an amino acid molecule solvated in water. This actual structure can be regarded as a cluster containing a large number of water molecules around the amino acid. In the model, only those water molecules that essentially affect the barrier for proton exchange are selected. This means that reaching the structures of the

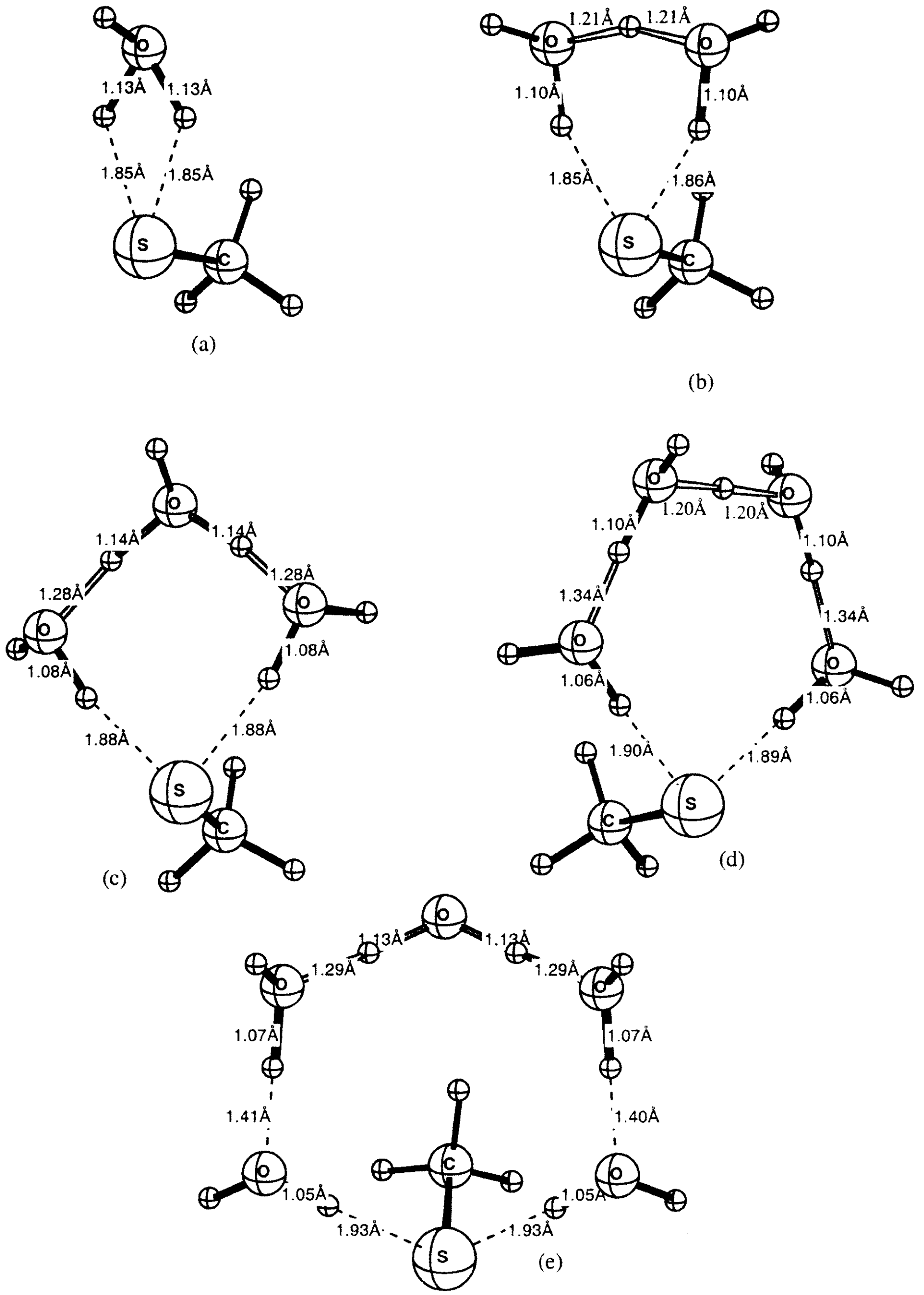

Fig. 2. Transition-state structures for cysteine

present transition states in reality does not require major rearrangements since in the actual water cluster around the amino acid there will always be water molecules that are already close to the required positions.

As can be seen in the table, there are dramatic changes in the barrier height as the number of water molecules is increased. The gas-phase barrier using one water molecule is as high as 44.3 kcal/mol, which is very different from the experimental barrier height in water solution of only 13.1 kcal/mol. Dielectric effects do not decrease this gas-phase value very much, only by 1.0 kcal/mol, and at this stage a representation of the water solvent by a dielectric cavity model must be considered extremely poor. The addition of a second water molecule decreases the barrier considerably by 18.8 kcal/mol to 25.5 kcal/mol. When dielectric effects of −2.9 kcal/mol are added the barrier height becomes 22.6 kcal/mol, which is still too high compared to the experimental solution result of 13.1 kcal/mol. The addition of a third water molecule also has a notable effect on the barrier, decreasing the gas-phase value by 3.4 kcal/mol to 22.1 kcal/mol. When dielectric effects are added the barrier becomes 18.6 kcal/mol, which is still 5.5 kcal/mol higher than the experimental water solution value. The addition of a fourth and a fifth water molecule does not change the barrier significantly and the results must therefore be considered as converged. There can be several reasons for the remaining discrepancy to

Table 1. Barrier heights, ΔE (kcal/mol), for proton exchange between different amino acids and water as a function of the number (n) of water molecules in the model

Amino acid	n*H_2O	ΔE gas phase	ΔE water solution
Cys	1	44.3	43.3
	2	25.5	22.6
	3	22.1	18.6
	4	22.1	18.3
	5	24.2	19.4
	Exp.		13.1
Ser	1	45.5	50.9
	2	24.1	24.2
	3	15.1	15.8
	4	14.4	15.1
	Exp.		13.4
Arg[a]	1	13.6	13.2
	2	9.4	8.2
	3	10.1	9.3
	Exp.		13.6
Arg[b]	1	45.5	37.8
	2	26.3	21.3
	3	16.0	12.2
	4	10.8	11.1
	Exp.		13.6
Arg-H^+	1	46.1	42.2
	2	31.0	32.0
	3	23.5	24.7
	4	18.3	18.5
	5	17.0	18.7
	Exp.		13.6
Glu	1	14.0	14.2
	2	8.6	7.6
	3	8.1	7.0
	Exp.		–

[a] Two-center mechanism, see text and Fig. 5
[b] One-center mechanism, see text and Fig. 6

experiments. One reason could be that the dielectric model is too simplified and that a more detailed model of the water solvent is required for better agreement. Reasons that involve a cost to reach the ordered transition states in Fig. 2 are unlikely since this cost would only raise the calculated barriers still further. There is thus no indication in the present results that it should be very costly to take the water molecules out of their ordered structure in the water solvent. An explanation of the discrepancy to experiments based on an inaccuracy of B3LYP is possible but rather unusual since barriers using this method tend to be too low rather than too high [15, 16]. The most likely explanation for the remaining discrepancy to experiments is therefore another one. Even though a heterolytic step, yielding $X^- + H^+$, will probably have a high barrier (see discussion for serine later), it may still contribute to increasing the rate by generating ions that could catalyze the proton exchange and therefore lower the measured effective barrier. To test this type of mechanism theoretically is quite difficult and is beyond the scope of the present study.

The dielectric effects for the larger clusters of cysteine are notable and are the largest ones in the present study. For five water molecules the effect is −4.8 kcal/mol, bringing the result into considerably better agreement with the experimental water solution result. The reason for the large effect is that there is a very clear charge separation in the transition states in Fig. 2, with a cysteinate anion and a proton bound between the water molecules. The transition states for the other amino acids discussed later have considerably smaller charge separations. However, even if the effect of the dielectric medium is rather large for cysteine, it is possible that it is not as large as it should be since the calculated barriers are still too high. One possibility to improve the solvent description could be to place additional water molecules specifically around the position where the proton is. A few such attempts were made by adding one or two water molecules, but the effects were very small.

In the present study all the structures were optimized without a dielectric surrounding. Even though the total dielectric effect on the energy is not negligible, it is not expected that an optimization of the structure in the dielectric should have significant effects. First, the effect of an improved level of geometry optimization on the final energy is usually found to be very small [17]. Since the effect of the dielectric on the geometry only has secondary effects on the energy, it is furthermore expected to be an order of magnitude smaller than the direct effect. Since the largest direct effect is 4.8 kcal/mol, an effect on the order of 0.5 kcal/mol might be expected. This was tested for the case of two water molecules where the optimization using the dielectric only changed the barrier height by 0.1 kcal/mol, from 22.6 to 22.7 kcal/mol. This correction is much smaller than the uncertainty of B3LYP of 3–5 kcal/mol and can therefore be neglected. Since the dielectric effects are rather small for the present neutral systems, as usual, it can be of interest to also look at the change in the dipole moments. For cysteine and two water molecules the dipole moment for the reactant is 2.1 D, which increases to 4.6 D at the transition state due to the charge separation. These values increase to 2.3 and 6.1 D, respectively, when the system is optimized with the dielectric medium. For the total dielectric effects, the shape of the cavity and higher moments also contribute.

With transition states such as those in Fig. 2, which are very ordered, it might be expected that entropy changes should be important for the barrier height; however, the differential entropy effects calculated from the molecular Hessians are not very large, about 2.5 kcal/mol (independent of the size of the models in Fig. 2), raising the barrier heights. One reason for these rather small effects is clearly that the structures of the minima are almost as equally well ordered as the transition states. It should be added in this context that the ordering of both the minima and the transition states is only apparent from the models. In reality, these molecules are part of a large water cluster where water molecules are already more or less in place without any ordering (see previous discussion). Concerning the accuracy of the entropy evaluation, it is possible that the estimate of the increase in translational entropy could be somewhat uncertain. The entropy effects are anyway not expected to be very much larger using a more accurate treatment taking into account nonharmonic effects, since this would increase the deviation from the experimental water solvent result still further.

3.2 Serine

The proton-exchange barrier heights obtained for serine are given in Table 1 and the transition state for four water molecules is shown in Fig. 3. Serine is modeled here by methanol, which could almost equally well be used as a model for threonine. Models with up to four water molecules were used. The transition-state structures are of the same ring type as discussed previously for cysteine. Since serine is chemically more similar to water than cysteine is, the transition-state structures for serine are more symmetric. For the cases with three and four water molecules, all O—H distances are about 1.20 Å, and it can be noted how remarkably concerted the proton transfers are. Unlike the case of cysteine, there is thus no charge separation between the water molecules, on the one hand, and the amino acid, on the other hand, for serine. The negative charge is spread equally among the oxygens and the positive charge among the protons. The dielectric effects are therefore found to be much smaller than for cysteine.

The convergence of the barrier height with the number of water molecules is very similar for serine and cysteine. With one water molecule the barrier height is very high with a value of 45.5 kcal/mol for serine compared to 44.3 kcal/mol for cysteine. For serine the barrier decreases to 24.1 kcal/mol for two water molecules, to 15.1 kcal/mol for three water molecules and to 14.4 kcal/mol for four water molecules, where the value appears converged just as for cysteine. The dielectric effects are very small and positive and when they are added the results are 15.8 and 15.1 kcal/mol for three and four water molecules, respectively, in quite good agreement with the experimental solution result of 13.4 kcal/mol. A few points should be stressed. Firstly, the convergence of the barrier height is very fast and gives no indication that large models including a large number of solvent molecules should be needed for studying proton exchange. Secondly, since the dielectric effects are so small, the final results are quite insensitive to the choice of dielectric constant, which is otherwise a valid argument against the use of the dielectric cavity model. Thirdly, a stepwise model for proton exchange can be ruled out for serine, since the dissociation constant indicates a much higher energy for proton loss than the barrier of 13.4 kcal/mol for proton exchange. As a comparison, the barrier for separation of water into H^+ and OH^- can be estimated to be 24 kcal/mol [18].

Since the results for serine are expected to be quite accurate, it was decided to test the model further by also adding the backbone. The effect is not expected to be significant, on the basis of previous vast experience of modeling reactions, where it has been very common to model peripheral methyl groups by hydrogen atoms, for example. In almost all model studies to date, the backbone has also been left out for amino acid ligands to metals for good reasons. As an example of the effect of the backbone, the S—H bond strength in cysteine was only changed by 0.2 kcal/mol when the backbone was removed [19]. The resulting structure for serine with four water molecules is shown in Fig. 4. The effect of the

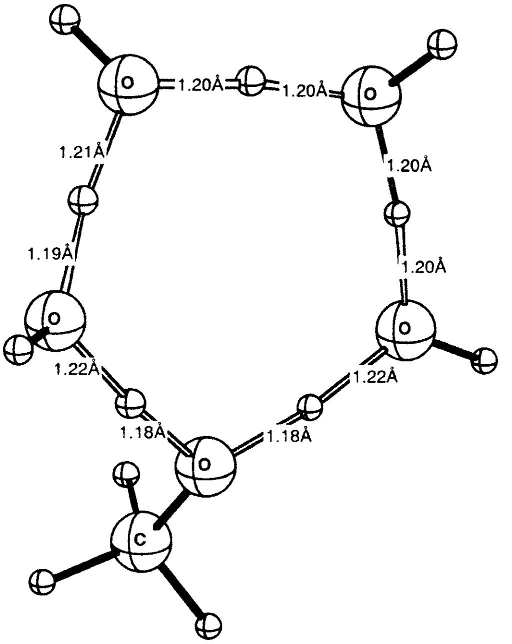

Fig. 3. Transition-state structure for serine and four water molecules

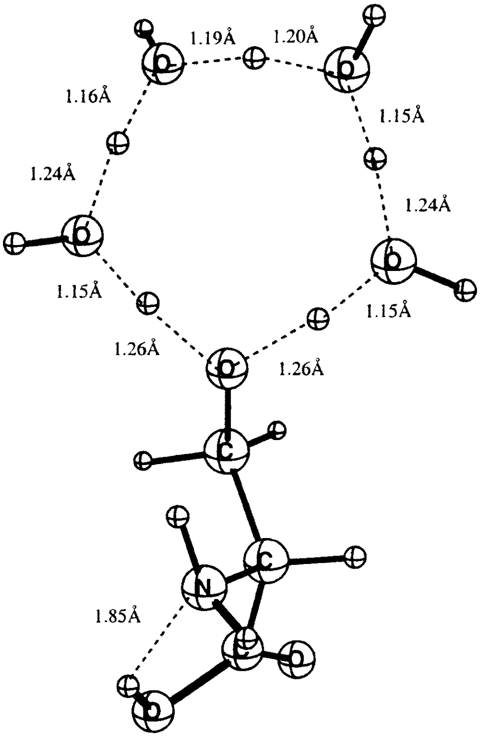

Fig. 4. Transition-state structure for the four-center mechanism for serine and four water molecules including the amino acid backbone

backbone on the barrier is very small, with an increase from 15.1 to 15.3 kcal/mol, similar to the effect mentioned previously for cysteine. Again, any effect smaller than 1 kcal/mol is considered negligible in comparison to the uncertainty of B3LYP of 3–5 kcal/mol.

3.3 Arginine

Arginine belongs to a different class of amino acids, where the functional group contains two equivalent centers. This leads to two quite different possibilities for proton exchange. Firstly, proton exchange could involve both the equivalent centers. Secondly, proton exchange could also be made following the same mechanism as discussed earlier for cysteine and serine involving only one of these equivalent centers. The results for unprotonated arginine using the two-center mechanism are given in Table 1 and the transition state for three water molecules is shown in Fig. 5. In these model systems arginine is modeled by formamidine. The transition states are quite symmetric as expected, with the positive charge located on the more basic arginine. The protons between arginine and water are much closer to arginine, with distances of 1.14 Å to the nitrogen of arginine and of 1.38 Å to the water oxygen for the model with three water molecules. The convergence of both structures and barrier heights is very fast. In fact, the results can be considered as converged already for two water molecules. The barrier height for two water molecules is 8.2 kcal/mol and for three water molecules 9.3 kcal/mol, with inclusion of very small dielectric and entropy effects. These values are somewhat lower than the experimental water solution result of 13.6 kcal/mol. However, modeling solvated arginine is in a certain aspect more complicated than modeling cysteine and serine, since arginine could be protonated in water. In fact, since the experimental result is for pH 7, the most common form of arginine should be protonated (see later).

The one-center mechanism, mentioned earlier, was also tested for neutral arginine. The results are given in Table 1 and the transition-state structure with three water molecules is shown in Fig. 6. As seen in Table 1, the converged result is very similar to the two-center mechanism; however, the convergence of the barrier heights is very different and it is much more similar to that discussed previously for cysteine and serine. This is as expected on the basis of the similarity of these transition, states. For arginine the converged result is 11.1 kcal/mol including dielectric effects, which is in quite good agreement with the experimental result of 13.6 kcal/mol. However, again it should be remembered that unprotonated arginine should not be the most common form at pH 7.

For protonated arginine only the one-center proton exchange mechanism is applicable. The results using this mechanism are given in Table 1 and the structures are shown in Figs. 7 and 8. Models with up to five water molecules were used and formamidine was again used to model arginine. This is the system that has the least satisfactory convergence of all the cases studied here. One reason for this could be that in this case there are actually local minima which are both geometrically and energetically very close to the transition states. These local minima appear for four and five water molecules and are 0.4 and 0.1 kcal/mol, respectively, below the transition states. For the structures shown in the figures there are, partly for this reason, still considerable changes on going from four to five water molecules. For example, one O—H distance between the water molecules changes from 1.40 to 1.45 Å. Another reason for

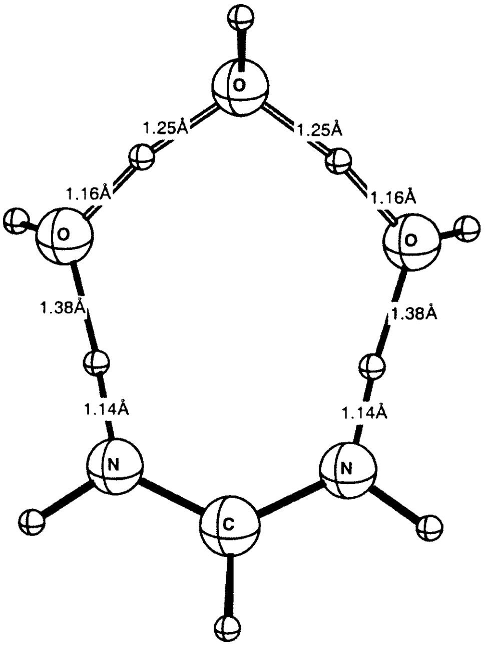

Fig. 5. Transition-state structure for the two-center mechanism with unprotonated arginine and three water molecules

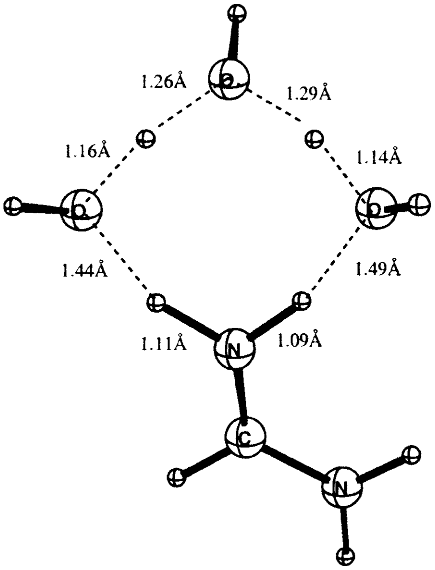

Fig. 6. Transition-state structure for the one-center mechanism with unprotonated arginine and three water molecules

the slower convergence might be that in this case the transition-state structures have to be compared to a slightly different type of reactant structure (Fig. 7), where one water molecule in the chain binds to both N—H protons. For one water molecule the gas-phase barrier height is 46.1 kcal/mol, similar to the corresponding model results for cysteine and serine. This value decreases to 31.0 kcal/mol for two water molecules, to 23.5 kcal/mol for three water molecules, to 18.3 kcal/mol for four water molecules and to 17.0 kcal/mol for five water molecules. When the small dielectric effects are added the result for five water molecules goes from 17.0 to 18.7 kcal/mol, which is actually in somewhat worse agreement with the water solution result of 13.6 kcal/mol. The agreement is about the same as for cysteine. However, for arginine, besides the protonated forms, a comparison to experiment really should also consider the unprotonated forms, where the calculated barrier is too low rather than too high. Even though arginine is most commonly protonated, it could with a rather small cost get rid of its additional proton and use one of the unprotonated mechanisms for proton exchange described previously. Overall, the agreement between the calculated results and experiments for arginine must therefore be considered satisfactory.

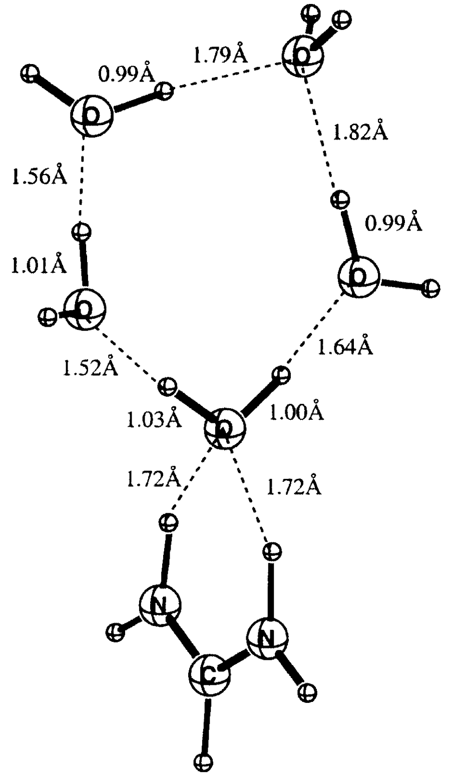

Fig. 7. Equilibrium structure for protonated arginine and five water molecules

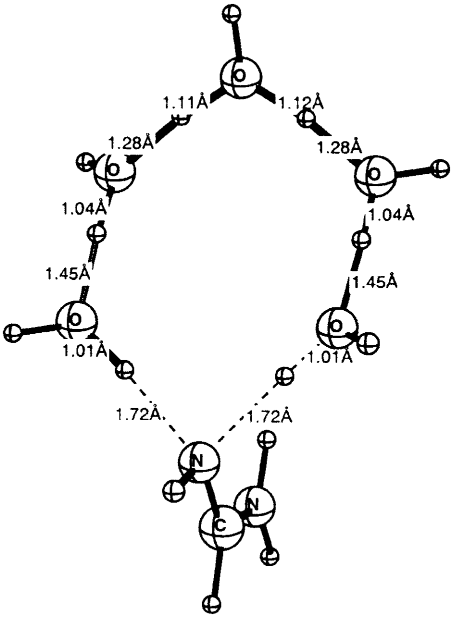

Fig. 8. Transition-state structure for the one-center mechanism with protonated arginine and five water molecules

For arginine, the protonation state of the guanidine functional group in the gas phase is actually not obvious. It could be that a proton is abstracted by the functional group from the carboxyl group of the amino acid. To test if this occurs a geometry optimization was performed for the full model of arginine. The result is shown in Fig. 9, and, indeed, the guanidine group becomes protonated even in the gas phase. Therefore, if the results for arginine were to be compared to gas-phase experiments, the results for protonated arginine in Table 1 could be the most relevant ones for the comparison. It should be noted that in the water experiments [9], to which the present results are compared, the protonation from the α-carboxyl group was prevented by using amidated carboxyl groups.

3.4 Glutamic acid

Glutamic acid is similar to arginine in the sense that it has two equivalent centers in the functional group. The

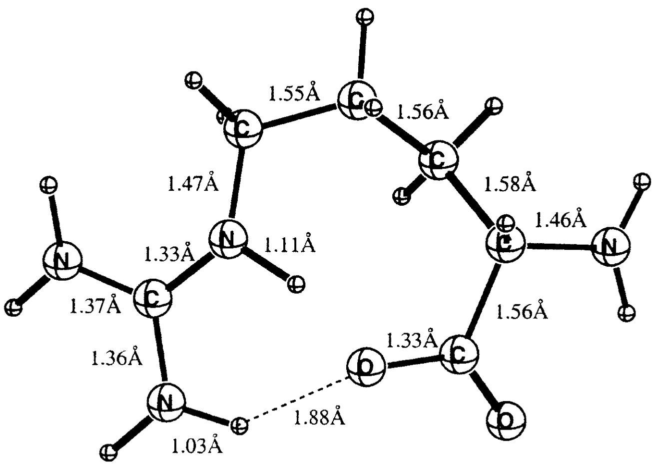

Fig. 9. Equilibrium structure for the full model of arginine

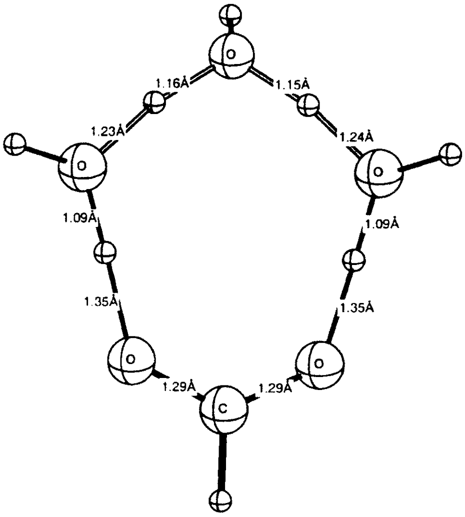

Fig. 10. Transition-state structures for glutamic acid

results for a neutral glutamic acid using the two-center mechanism are given in Table 1 and the transition state for three water molecules is shown in Fig. 10. The structures of the transition states are quite similar to the corresponding ones for neutral arginine except that the proton now resides on the water molecules instead of on the amino acid. This is, of course, expected since glutamic acid is more acidic than water and arginine is more basic than water. The O—H distance to water is 1.09 Å and to the glutamic acid oxygens it is 1.35 Å for the structure with three water molecules. The structure with two water molecules is quite similar and these structures must therefore be considered as nearly converged with the number of water molecules in the model already after two water molecules as in the arginine case. This is also the case for the barrier heights, which follow a very similar pattern to those for neutral arginine. The calculated value for three water molecules is 7.0 kcal/mol including dielectric effects, which is even lower than for the neutral models of arginine. For glutamic acid there is no experimental value available for the rate of proton exchange.

4 Conclusions

Simple models of concerted proton exchange between water and amino acid side chains have been studied using hybrid DFT methods. The calculations show that the barrier for proton exchange converges quite fast with the number of water molecules in the model. Reasonably converged results are in most cases obtained already with three water molecules. For serine, arginine and glutamic acid, the dielectric effects are very small and do not improve the results for the larger models. In contrast, for cysteine the effects are significant in the range 3–5 kcal/mol for the larger models and improve the agreement with experiments. The dielectric effect on the geometries is very small, as expected, and this is also true for the effect of adding the backbone of the amino acid. The exchange mechanism is essentially the same for cysteine, serine and protonated arginine and the convergence behavior is also similar in these cases. The transition states can be described as ring structures with one amino acid side chain atom participating in the ring. This ordered ring structure should be regarded as a part of the actual structure in water, where each water molecule and the amino acid are surrounded by a large number of additional water molecules. These additional water molecules are left out of the present models. Such ring-type structures are likely to be automatically present for selected water molecules in water, and the cost to reach these ordered structures should therefore be small. For neutral arginine and glutamic acid, where there are two equivalent atoms on the amino acid that can participate in the reaction, a mechanism involving these two centers is also possible. The converged results for serine and unprotonated arginine are in quite reasonable agreement with experiments, while the results for cysteine and protonated arginine are somewhat high by 5–6 kcal/mol. Even though the present study concerned concerted proton exchange and used specifically constructed models for this purpose, the alternative with a simple stepwise mechanism can be ruled out for serine and is unlikely for the other amino acids. Serine should be quite similar to water, where the barrier to dissociation into H^+ and OH^- can be predicted to be as high as 24 kcal/mol, which is much higher than the measured barrier for proton exchange in serine of only 13.4 kcal/mol. However, a heterolytic step, could still contribute to increasing the rate for proton exchange by generating ions that could catalyze the proton exchange and therefore lower the measured effective barrier.

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