Internal Rotation of 1,2-Dichloroethane in Haloalkane Dehalogenase. A Test Case for Analyzing Electrostatic Effects in Enzymes

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1,2-Dichoroethane (DCE) is a prototypical molecule for studying electrostatic solvent effects on molecular conformation as far as rotation around the carbon−carbon bond notably changes the electric properties of the molecule and especially the dipole moment. While the apolar trans conformation is the absolute free energy minimum in the gas phase, solvents of increased polarity relatively favor the population of the gauche conformers. DCE is also a substrate of haloakane dehalogenase from *Xanthobacter Autotrophicus* (DhlA), an enzyme that catalyzes the conversion of DCE to 2-chloroethanol. We here investigate the nature of substrate−enzyme interactions, obtaining the free energy profiles of rotation around the C−C bond in the gas phase, in aqueous solution, and in the enzymic environment. In the enzyme only the gauche conformers are free energy minima, the trans conformer being a free energy maximum. Differences between the aqueous solution and enzyme energy profiles are rationalized taking into account the different magnitudes and orientations of the electric field created by the environment in both cases. In aqueous solution DCE feels a reaction field several times lower in modulus than in the enzyme active site. Consequences on enzyme catalysis are also discussed.

1. Introduction

The ability of enzymes to enhance reaction rates by several orders of magnitude has been the subject of numerous interpretations since the seminal idea of Pauling.[1] However, a clear consensus still has not been reached about the origin of enzyme catalysis. Even more, it is not at all clear if a general theory of enzyme catalysis can be established or, on the contrary, different enzymes work with different strategies. The original Pauling proposal about the fit between the reaction transition state (TS) and the protein environment has found support in the extensive work of Warshel and co-workers.[2−5] Following these works, the enzyme preferentially stabilizes the TS with respect to the counterpart process in aqueous solution. However, other authors have preferred to emphasize the effect of the enzyme on the reactants side, showing that the protein environment could favor some particular conformations of the reactants prepared to follow the chemical process.[6−9] In this sense, several concepts, such as the near attack conformation,[6,7] have been used to analyze this possible effect. In some recent works, we have shown that, at least in some cases, these two views on the origin of enzyme catalysis could have a common origin, if the flexibilities of both the substrate and the enzyme are taken into account.[10−12]

An adequate knowledge of the origin and magnitude of enzyme−substrate interactions is needed for a rational design of new and more powerful enzymes and/or inhibitors. The physical nature of enzyme interactions with the substrate, either in its reactant state or in the transition state, is also discussed. While some authors stress the importance of electrostatic interactions,[3] other contributions have also been invoked to explain enzyme activity (see ref 13 for a recent revision). In this work we try to evaluate the electrostatic interactions in the active site of an enzyme as compared to aqueous solution. For this purpose, we have selected a prototypical molecule, 1,2-dichloroethane (DCE), in the active site of the haloakane dehalogenase from *Xanthobacter Autotrophicus* (DhlA), an enzyme that catalyzes the conversion of DCE to 2-chloroethanol.[14] DCE is a very good candidate to serve as a test for electrostatic effects, because its conformational equilibrium can be to a large extent determined by electrostatic interactions with the environment. Effectively, in the gas phase the trans form of DCE (presenting a ClCCCl dihedral angle of 180°) predominates over the two equivalent gauche conformers (with about +60° and −60° for the ClCCCl dihedral angle). The larger population of the trans conformer is due to the minimization of chlorine−chlorine repulsions. When DCE is solvated, the population of gauche conformers is increased because the dipole moment of DCE continuously grows when the dihedral angle diminishes. The relative population of trans/gauche conformers can thus be controlled by changing the polarity of the solvent. As far as experimental data exist for DCE in several solvents,[15] this system has been usually employed as a test for different solvation models, and in general, experimental tendencies are correctly reproduced by both continuum[16] and discrete solvent[17] models.

As said above, haloalkane dehalogenase catalyzes the conversion of DCE to 2-chloroethanol and chloride anion, and thus, it has received much experimental[18,19] and theoretical[20−23] attention as a nonaggressive ant pollutant. The reaction involves an S_N2 displacement of chloride anion by Asp124 and subsequent hydrolysis of the ester intermediate to yield the final product.[24] For the substrate−enzyme complex, X-ray coordinates[25] obtained at 4 °C and pH 5 display a trans conformation for the DCE, although the observed density for the nonreactive chorine atom was weak. Bruice et al.[20,21] have recently shown that this conformation is kept during molecular mechanics energy minimizations, but when molecular dynamics are run at 300 K, DCE rotates to gauche conformations, remaining in that form most of the simulation run. A change to this gauche conformer

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is important for the reaction to proceed as far as in the trans conformation, where the nonreactive chlorine atom can hinder the approach of the Asp124 CO_2^- group.

In this work we compare the free energy profiles associated with the rotation around the carbon−carbon bond of DCE in the gas phase, in aqueous solution, and in the enzyme active site. The differences found in these profiles will be analyzed in terms of the electrostatic interaction of the DCE dipole moment with the electric field due to the environment. Differences between the enzyme and the solvent will be explained on the basis of the different magnitudes and orientations of the electric field acting on the substrate. Although we will not explore here the effect of the enzyme along the reaction coordinate, our results will be connected with recent analysis of the electrostatic contributions to catalysis.

2. Methodology

To study the influence of the medium in the conformational equilibrium of DCE, we obtained the potential of mean force (PMF) of the system as a function of the ClCCCl dihedral angle in the gas phase, in aqueous solution, and in the active site of DhlA. All calculations have been done using the DYNAMO[26] library. QM/MM calculations were carried out using the AM1 semiempirical Hamiltonian for the QM subsystem and OPLS-AA force field[27] for treating the MM subsystem. The molecular system used in the gas phase was the DCE molecule (eight QM atoms). For QM/MM calculations in solution, DCE was placed in a cavity deleted from a 31.4 Å side box of water molecules described by the TIP3P empirical potential.[28] The resulting system had 3098 atoms in total and 1030 water molecules. For the QM/MM enzyme calculations the starting structure was the crystal structure of DhlA with 1,2-dichloroethane, PDB entry 2DHC. DCE and part of Asp124 are taken as the QM subsystem (15 atoms). The carbon−carbon bond between CA and CB of this residue is broken to separate the QM and MM subsystems, and a link atom is added to the QM subsystem to complete the valence. The MM subsystem is thus formed by the enzyme chain and the crystallization water molecules (4851 enzyme atoms plus 121 nonrigid water molecules) introduced in a 55.8 Å side box of TIP3P water molecules, resulting in a total of 17109 atoms. Periodic boundary conditions and a smoothed cutoff radius of 12 Å were used during the molecular dynamics (MD) simulations. The starting points were in all cases the gauche conformer of DCE, which is the stable one inside the active site of the enzyme. The umbrella sampling approach[29] was used to keep the system in a particular value of the ClCCCl dihedral angle by means of the addition of a parabolic potential. The value of the force constant used for the umbrella sampling (0.5 $kJ \cdot mol^{-1} \cdot deg^{-2}$) was determined to allow a full overlapping of the different windows traced in the PMF evaluation. The length of each window (10 ps) and the total number of windows (60 in solution and the gas phase and 120 in the enzyme) was proved to be long enough to sample the complete range of the dihedral angles at a reference temperature of 300 K. Variations of the dihedral angle are stored during molecular dynamics simulations. Then, these variations are used together to obtain the PMF, using the weighted histogram analysis method (WHAM).[29] The canonical thermodynamical ensemble (NVT) was used all around the calculations.

For each window a trajectory file is stored, to make then an analysis of the window, and obtain averaged values. To compare the magnitude of the electrostatic interactions in different media, they were obtained describing all the system, but DCE, with the MM potential and using the same cutoff region (12 Å). The averaging process was carried out for different biased values of the ClCCCl dihedral angle.

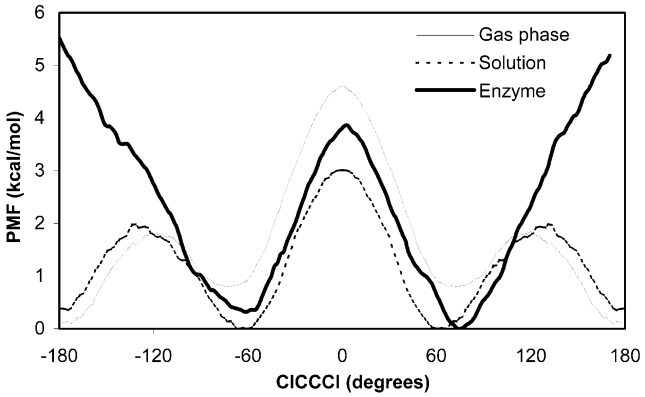

Figure 1. Potential of mean force (kcal/mol) obtained for rotation about the C−C bond in 1,2-dichloroethane in the gas phase (thin line), in aqueous solution (dashed line), and in the enzyme active site (bold line).

3. Results and Discussion

Figure 1 shows the PMFs obtained in the gas phase, in aqueous solution, and in the enzyme active site as a function of the ClCCCl dihedral angle. In the gas phase the PMF contains three different minima, those corresponding to the trans and gauche conformers (with dihedral angles of 180° and ±72°, respectively). The free energy difference between these two forms is about 0.80 kcal/mol, favorable to the trans conformer. This result compares well with the experimental free energy difference of 1.20 kcal/mol.[15] In aqueous solution, the free energy difference between the trans and the two equivalent gauche conformers (with ClCCCl dihedral angles of ±63°) is −0.38 kcal/mol, the gauche form now being more stable. Experimental free energy differences between trans and gauche conformers have been measured in solvents of increasing dielectric constant, being 0.11, −0.14, and −0.22 kcal/mol in tetrahydrofuran ($\epsilon = 7.58$), acetone ($\epsilon = 20.7$), and acetonitrile ($\epsilon = 36.0$), respectively. A recent theoretical calculation by Jorgensen et al. of the gauche−trans free energy difference by means of Monte Carlo simulations provided −0.62 kcal/mol.[17] Thus, our theoretical estimations in the gas phase and aqueous solution seem reasonable and validate our computational procedure.

The free energy profile found in the enzyme active site is completely different. Only two free energy minima corresponding to the two gauche conformers appear on the PMF, while the trans form is now the absolute free energy maximum, in agreement with the observations of previous classical simulations.[20,21] Moreover, the protein environment is anisotropic, and thus, the two gauche conformers are no longer equivalent. The gauche conformer presenting a dihedral angle of 75° (gauche1) is the absolute free energy minimum, while the one with a dihedral angle of −61° (gauche2) has a free energy of 0.32 kcal/mol relative to the former. Interestingly, in a limited potential energy surface exploration, gauche1 has been found to present an enhanced reactivity as compared to gauche2.[21] The trans conformer is 5.50 kcal/mol above gauche1. The disagreement with the reported X-ray data,[25] which show the substrate bound in the trans conformation, is probably due to the fact that at low pH DhlA is not active because the neighboring His289 residue is present as an imidizolium ion. Therefore, this X-ray structure would not be an adequate representation of the true Michaelis complex.[30] Figure 2 shows two snapshots correspond-

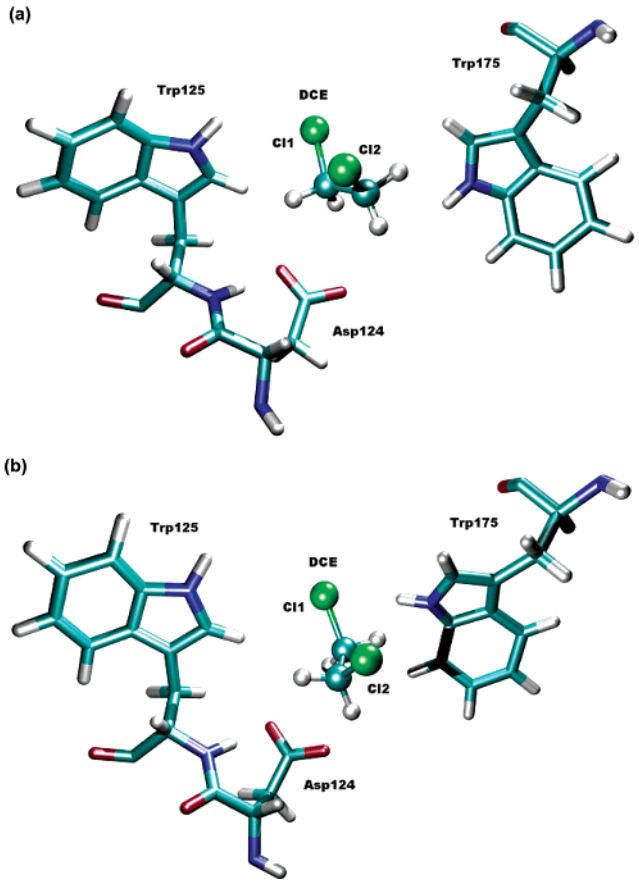

Figure 2. Snapshots of the two free energy minima found for DCE in the enzyme active site: (a) gauche1 (ClCCCl dihedral angle of 75°) and (b) gauche2 (ClCCCl dihedral angle of −61°). For the sake of simplicity, only some key residues are shown.

ing to the dynamics of the two gauche conformers. It can be observed that, when the dihedral angle is changed from the value corresponding to gauche1 to that of gauche2, the position of Cl1 (the reactive chlorine atom) remains essentially unchanged, most probably due to its favorable interactions with the HN groups of Trp125 and/or Trp175 and the repulsion of the carboxylate group of Asp124, which attacks the carbon atom from the opposite direction during the subsequent reaction. The position of the second chlorine atom (Cl2) is more flexible, in agreement with the analysis of crystallographic data.[25]

To analyze the origin of the differences in the aqueous solution and enzymic PMFs, we have investigated electrostatic effects in both media. In particular, we have computed the dipole moment of DCE and the electric field created by the surroundings in the center of mass of DCE. The scalar product of these two vector quantities gives a good idea of the magnitude of the electrostatic interaction in aqueous solution and in the enzyme active site. Figure 3 shows the dipole moment of DCE as a function of the ClCCCl dihedral angle in the gas phase, in aqueous solution, and in the enzyme active site. The dipole moment in the gas phase increases from 0 to 0.98 au when the dihedral angle rotates from 180° to 0°. In aqueous solution the dipole moment is larger because the solute polarizes the solvent, a reaction field appearing, which in turn polarizes the solute. It is interesting to note that even at 180° the DCE dipole moment is not zero because the solute can polarize the solvent by means of higher order electric moments, a solvent reaction field consequently appearing. Thus, in solution, the averaged dipole moment of DCE increases from 0.13 to 1.26 au as the dihedral angle goes from 180° to 0°. The increase in DCE's dipole

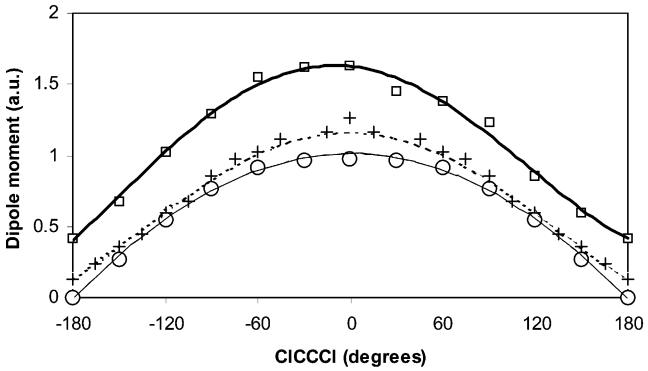

Figure 3. Dipole moments (au) of DCE as a function of the ClCCCl dihedral angle in the gas phase (circles and thin line), in aqueous solution (plus signs and dashed line), and in the enzyme (squares and bold line). Lines display the best fourth-order polynomial fit.

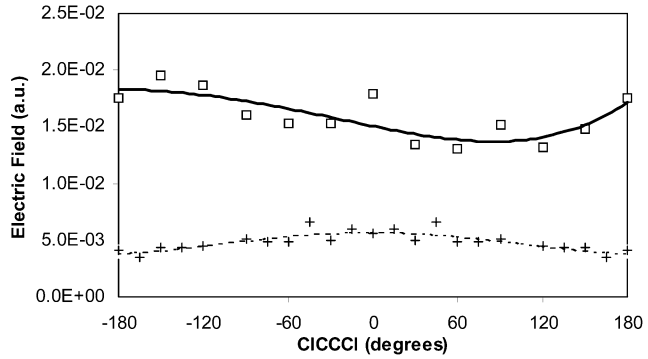

Figure 4. Electric field (au) created by the environment on DCE's center of mass as a function of the ClCCCl dihedral angle. Symbols are as in Figure 3.

moment is still larger in the enzyme active site. The dipole moment is 0.43 au for the trans conformer and 1.63 au for the cis one. These values already give an idea about the importance and magnitude of the electrostatic effects in the enzyme active site. The dipole moment found for the trans conformer in the enzyme, which can be attributed to substrate polarization by the environment, is equivalent to the dipole moment obtained after rotation of about 60° in the gas phase.

The modulus of the averaged electric field created by the environment (water molecules or protein residues) on the center of mass of DCE is depicted in Figure 4. It is important to stress the different magnitudes, of about 1 order, of the electric field in the enzyme and in aqueous solution. In the enzyme the electric field ranges between 1.5×10^{-2} and 2.0×10^{-2} au. In aqueous solution the solvent reaction field increases from 4.0×10^{-3} au for the trans conformation to 6.5×10^{-3} au for the cis one. Moreover, in aqueous solution the electric field shows a strong correlation with the dihedral angle, increasing in magnitude as the dihedral angle goes from ±180° to 0°. A linear fit between the electric field and DCE's dipole moment in aqueous solution gives a correlation coefficient of 0.81. The same linear fit in the enzyme gives a very poor inverse correlation ($R = -0.35$). This means that while in aqueous solution the electric field is a reaction field caused by the solute polarity; in the enzyme we find a permanent field, whose variations are not essentially due to the substrate polarity. This permanent character of the enzymic electric field is observed not only in the modulus but also in the orientation. The angular deviations of the averaged electric fields found in the enzyme for different conformations of DCE are not larger than 20° in any case. This means that the electrostatic field created by the enzyme in the active site remains essentially unchanged in

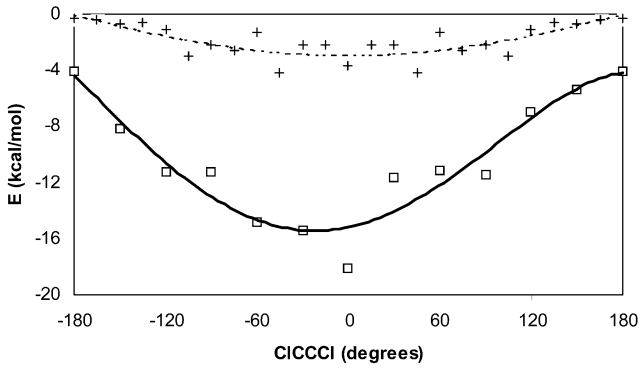

Figure 5. Electrostatic interaction (kcal/mol) between the DCE dipole moment and the environmental electric field as a function of the ClCCCl dihedral angle. Symbols are as in Figure 3.

orientation and magnitude for different DCE conformers. Obviously, the enzyme active site is not fully rigid and can be adapted to different substrates, but this flexibility has nothing to do with water, where the electrostatic answer of the medium is completely determined by the solute. The change from a reaction to a permanent field can have important consequences for catalysis.

Finally, we have calculated the electrostatic interaction energy between the electric field (F) created by the surroundings and the substrate/solute dipole moment (μ) as the scalar product between the two vector quantities:

$$E_{\text{int}} = -\vec{\mu} \cdot \vec{\mathbf{F}} = -(\mu_x F_x + \mu_y F_y + \mu_z F_z)$$

The interaction energy is shown in Figure 5 as a function of the ClCCCl dihedral angle. In aqueous solution this interaction energy ranges from −0.3 to −2.5 kcal/mol, the absolute values increasing monotonically from ±180° to 0°. In the enzyme we have the same qualitative behavior, but the energy differences are much more important. The interaction energy is about −2.6 kcal/mol for the trans conformer and about −18.0 kcal/mol for the cis one. This means that the electrostatic interaction contribution to the gauche−trans energy difference amounts to more than 15 kcal/mol in the enzyme but only 2.2 kcal/mol in aqueous solution. Moreover, it should be taken into account that, in the global energy balance in a polarizable medium, such as aqueous solution, about half of the interaction energy would be spent in the solvent polarization. Thus, energy differences due to electrostatic effects are much more pronounced in the enzyme than in aqueous solution and are decisive to determine the absolute free energy minimum of DCE in the active site of haloalkane dehalogenase.

4. Conclusions

We have studied the free energy profiles associated with the conformational equilibria of DCE in the gas phase, in aqueous solution, and in the enzyme active site of DhlA. The PMFs show three different minima in the gas phase and aqueous solution, corresponding to the trans and two gauche conformers. While in the gas phase the trans conformer is the absolute minimum, in aqueous solution the gauche forms are more stable. In the enzyme active site the situation is completely different. Only the gauche conformers are free energy minima, and moreover, these two structures are no longer equivalent because the environment is anisotropic. Analysis of the solute/substrate dipole moment and of the electric field due to the surroundings shows that electrostatic effects are decisive to understand the changes appearing in the PMFs when passing from aqueous solution to the enzyme active site. In solution, we have a reaction field whose magnitude and orientation depends on the polarity of the solute. As the dipole moment of DCE decreases with the ClCCCl angle, solvent effects relatively favor the gauche forms with respect to the trans one. In the enzyme active site the electric field is larger in magnitude than in aqueous solution, as reflected by the larger induced dipole moment in DCE. Moreover, this electric field is a permanent one, and its magnitude and orientation are essentially independent of the conformational changes taking place in the substrate.

The enzymatic electric field can also be seen as a reaction catalyst. In this sense, it is interesting to comment on the recent results of Warshel et al. on the catalytic effect of this enzyme.[22] These authors have shown that for this enzyme catalysis with respect to the aqueous solution reaction is the result of transition-state stabilization caused mainly by electrostatic contributions. As a consequence, this electrostatic effect also leads to a steric strain on the reactant state, keeping Asp124 at an adequate distance and orientation for the nucleophilic attack. Our contribution in this work would be then complementary, showing the role of the electrostatic effects on the rotation around the CC bond. Moreover, from our analysis we can highlight the different natures of the electrostatic effects in water solution and in the enzyme active site. Effectively, in the enzyme we have found a permanent electric field that would be correctly oriented to favor the transfer of a negative charge from the nucleophilic oxygen atom of Asp124 to the leaving group (the chlorine atom). The averaged angle between the electric field and the line joining the attacking and leaving groups in gauche1 is only about 30°. So, the same enzymatic feature, the electric field in this case, which is prepared to favor the reaction progress promoting the charge-transfer process, would also play an additional role in preorganizing the substrate. A similar dual role has also been found in other enzymatic processes.[10−12]

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References and Notes

(1) (a) Pauling, L. *Nature* **1948**, *161*, 707−709. (b) Pauling, L. *Chem. Eng. News* **1946**, *24*, 1375−1377. (c) Pauling, L. *Am. Sci.* **1948**, *36*, 51.

(2) Warshel, A. *Computer Modeling of Chemical Reactions in Enzymes and Solutions*; John Wiley and Sons: New York, 1991.

(3) Warshel, A. *Acc. Chem. Res*. **2002**, *35*, 385−395.

(4) Strajbl, S.; Florián, J.; Warshel, A. *J. Am. Chem. Soc.* **2000**, *122*, 5354−5366.

(5) Villà, J.; Warshel, A. *J. Phys. Chem. B* **2001**, *105*, 7887−7907.

(6) (a) Lau, E. Y.; Bruice, T. C. *J. Am. Chem. Soc.* **1998**, *120*, 12387−12394. (b) Torres, R. A.; Schiøt, B.; Bruice, T. C. *J. Am. Chem. Soc.* **1999**, *121*, 8164.

(7) (a) Lightstone, F. C.; Bruice, T. C. *J. Am. Chem. Soc.* **1996**, *118*, 2595−2605. (b) Bruice, T. C.; Lightstone, F. C. *Acc. Chem. Res*. **1999**, *32*, 127−136. (c) Bruice, T. C.; Benkovic, S. J. *Biochemistry* **2000**, *39*, 6267−6274.

(8) Mesecar, A. T.; Stoddard, B. L.; Koshland, D. E., Jr. *Science* **1997**, *227*, 202−206.

(9) (a) Stanton, R. V.; Peräkylä, M.; Bakowies, D.; Kollman, P. A. *J. Am. Chem. Soc.* **1998**, *120*, 3448−3457. (b) Kollman, P. A.; Khun, B.; Donini, O.; Peräkylä, M.; Stanton, R. V.; Bakowies, D. *Acc. Chem. Res.* **2001**, *34*, 72−79. (c) Kollman, P. A.; Kuhn, B.; Peräkylä, M. *J. Phys. Chem. B* **2002**, *106*, 1537−1542.

(10) Martí, S.; Andrés, J.; Moliner, V.; Silla, E.; Tuñón, I.; Bertrán, J.; Field, M. J. *J. Am. Chem. Soc.* **2001**, *123*, 1709−1712.

(11) Martí, S.; Andrés, J.; Moliner, V.; Silla, E.; Tuñón, I.; Bertrán, J. *Chem.−Eur. J.*, in press.

(12) Roca, M.; Martí, S.; Andrés, J.; Moliner, V.; Tuñón, I., Bertrán, J.; Williams, I. H. *J. Am. Chem. Soc.*, in press.

(13) Warshel, A. *J. Biol. Chem.* **1998**, *273*, 27035−27038.

(14) Keuning, S.; Janssen, D. B.; Witholt, B. *J. Bacteriol.* **1985**, *163*, 635−639.

(15) Wiber, K. N.; Keith, T. A.; Frisch, M. J. Murcko, M. *J. Phys. Chem.* **1995**, *99*, 9072−9079.

(16) Wong, M. W.; Frisch, M. J.; Wiberg, K. N. *J. Am. Chem. Soc.* **1991**, *113*, 4776−4782.

(17) (a) Jorgensen, W. L.; McDonald, N. A:; Selmi, M.; Rablen, P. R. *J. Am. Chem. Soc.* **1995**, *117*, 11809−11810. (b) Kaminski, G. A.; Jorgensen, W. L. *J. Phys. Chem. B* **1998**, *102*, 1787−1796.

(18) Fetzner, S.; Lingens, F. *Microbiol. Rev.* **1994**, *58*, 641−685.

(19) Leisinger, T. *Curr. Opin. Biotechnol.* **1996**, *7*, 295−300.

(20) (a) Maulitz, A. H.; Lightstone, F. C.; Zheng, Y. J.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6591−6595. (b) Lightstone, F. C.; Zheng, Y. J.; Bruice, T. C. *J. Am. Chem. Soc.* **1998**, *120*, 5611−5621.

(21) Lau, E. Y.; Kahn, K.; Bash, Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 9937−9942.

(22) Shurki, A.; Strajbl, M.; Villà, J.; Warshel, A. *J. Am. Chem. Soc.* **2002**, *124*, 4097−4107.

(23) Devi-Kesavan, L. S.; Gao, J. *J. Am. Chem. Soc.* **2003**, *125*, 1532−1540.

(24) Verschueren, K. H. G.; Franken, S. M.; Rozeboom, H. J.; Kalk, K. H.; Dijkstra, B. W. *J. Mol. Biol.* **1993**, *232*, 856−872.

(25) Verschueren, K. H. G.; Seljée, F.; Rozebomm. H. J.; Kalk, K. H.; Dijkstra, B. W. *Nature* **1993**, *363*, 693.

(26) Field, M. J.; Albe, M.; Bret, C.; Proust-de Martin, F.; Thomas, A. *J. Comput. Chem.* **2000**, *21*, 1088−1100.

(27) (a) Jorgensen, W. L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, *110*, 1657. (b) Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1996**, *118*, 11225−11236.

(28) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926−935.

(29) Torrie, G. M.; Valleau, J. P. *J. Comput. Phys.* **1977**, *23*, 187−199.

(30) Schindler, J. F.; Naranjo, P. A.; Honaberger, D. A.; Chang, C. H.; Brainard, J. R.; Vanderberg, L. A.; Unkefer, C. J. *Biochemistry* **1999**, *38*, 5772−5778.