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REACTION FIELD EFFECTS ON PROTON TRANSFER IN THE ACTIVE SITE OF ACTINIDIN

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The feasibility of the inclusion of reaction field effects in accurate ab initio self-consistent field–molecular orbital calculations was studied in the case of proton transfer in the active site of actinidin. The effects of the polarizability of the environment were included, using the direct reaction field model, which treats the environment as a set of interacting polarizable atoms. Up to 1000 of these atoms could be treated but about 300 were sufficient. The full geometry of the active site and the environment was taken into account. The stabilization of the ion pair was calculated to be 3.5 kcal, but this value may be 10 kcal depending on the geometry used. The effect of the static field from the long α-helix present in the enzyme was also studied. Dispersion effects are shown to be unimportant. The orientational polarizability of side chains and water molecules was not included.

1. Introduction

An important step in the resolution of the problem of environmental effects on enzymatic mechanisms is a realistic treatment of the polarizability of the environment. This problem has been attacked in studies of proton transfers in systems such as Cys-25–His-159–Asn-175 in papain or in the lactate dehydrogenase proton-relay system [1]. Because of the complexity of the mathematical treatment of dielectric effects in complex systems, often approximate methods are used, in which a molecule and its environment are treated as a limited number of charges in a spherical or ellipsoidal cavity in a continuum dielectric [2–4]. The method of mirror charges [5] is approximate and still treats the environment as a continuum. Noncontinuum methods have been used recently [1,6] but only in the context of approximate quantum-mechanical calculations. In order to avoid the approximations of these methods, we developed the direct reaction field method [7] which treats the environment as a large set of interacting polarizable atoms in an arbitrary geometry. The polarizability of the atoms, and the form of the interaction between the induced dipoles are chosen such that molecular electronic polarizabilities are reproduced realistically [8], thus avoiding the choice of the value for a dielectric constant. In the quantum-mechanical calculation approximations are made which produce errors of about 10% or smaller [9]. The total error in this part of the reaction field effect is most probably well within 50%. It is also possible to calculate a quantity, V^{disp}, which is an upper limit for the dispersion interaction between the system considered and its environment. This upper limit is typically too large by a factor of 2 [9], but basis set effects commonly result in it being calculated too small by a factor of up to 2. This value can be used to give an impression of the effects of dispersion.

This paper will demonstrate the feasibility of accurate calculations of reaction field effects in the case of proton transfer in the active site of

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actinidin, which is very much like that of papain [10,11], but its X-ray structure is more accurate [12]. Since Cys S^- is a stronger base than His, special environmental effects are needed in order to make Cys-25 S^- ---H^+ His-162–Asn-182 the more stable structure (fig. 1). Indeed, previous work on papain [13,14] provided strong evidence that the electric field of the α-helix [15], comprising residues 24–43, has a large effect, by pushing the sulfhydryl proton towards $N_{\delta 1}$ of His-162, stabilizing the ion pair by 15 kcal [13]. Together with smaller contributions from neighbouring units [16], this makes the presence of the zwitterion very probable. This result is supported by experimental evidence [17–19].

Reaction field effects on this system are expected for two reasons. First charge separation is expected to be stabilized by a polarizable environment because the environment can relax more strongly in the stronger field from the ion pair. Second, the field from the helix will be changed by the environment. In the simplest approach it will be divided by the dielectric constant, for which values of 2–120 [2,20] may be appropriate, depending on the amount of free water present, and on the geometry. The relatively accurate calculations presented here will probably settle many of the discussions and uncertainties about the magnitude of dielectric effects on proton transfer-type mechanisms in proteins. Note, however, that we will be concerned here only with the polarizability of protein atoms. The orientational polarizability of water molecules or of protein side chains is not taken into account. Some preliminary results of the calculations have been discussed in refs. 21 and 22.

2. Calculations

Fig. 1 shows two models for the active site of actinidin, systems I and II. Methanethiol, imidazole and formaldehyde represent Cys-25, His-162 and Asn-182, respectively. Fig. 2 shows the results from previous ab initio Hartree-Fock calculations on system II. Details on these calculations can be found in refs. 13 and 14. We only note here that a double zeta basis set was used and that the S–N distance was 3.00 Å, a distance which we then considered as a lower limit compatible with the papain X-ray data [14]. Fig. 2 shows that the electric field from the helix stabilized the ion pair by about 10 kcal.

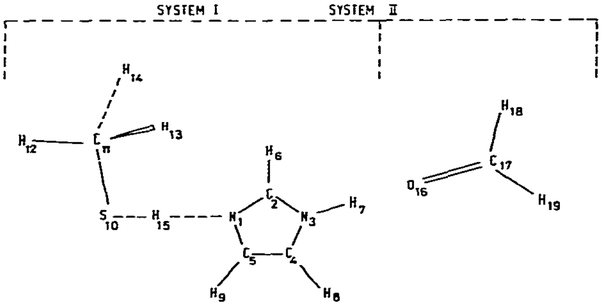

Fig. 1. Model systems for the active sites of papain and actinidin.

For the present calculations of the dielectric effects on these curves we used coordinates taken from the X-ray structure of actinidin [12] instead of papain, because actinidin's X-ray structure is more accurate. In the following the actinidin numbering will be used. We followed Baker's [12] suggestion to rotate the ring of His-162 over 35° such that the Cys-25 sulfur atom was nearly in the plane of the ring. The distance between S_γ-25 and $N_{\delta 1}$-162 was found to be 2.94 Å. This is rather short compared to the distances of 3.00 Å (fig. 2) and 3.35 Å used in the papain calculations, and this fact is the cause of the differences between our results for these molecules. In this paper, however, we are only interested in the effects of a polarizable environment, and these can be studied apart from the question of the correct geometry. The curves in fig. 3 for the system without a polarizable environment can be compared to those of fig. 2. The shorter S–N distance in fig. 3 makes the minimum near N disappear completely, even when the helix field is applied, and it will be particularly hard to find the effects which make the ion pair structure the most stable one. Note that fig. 3 gives the curves for system I, which contains no formaldehyde.

After the application of the helix field the second effect we add is the polarizability of the rest of the environment. The helix field was generated by the charges 0.42, −0.42, −0.20 and 0.20 [5] on C, O, N and HN from C of Gly-24 to HN of Gly-43. In order to avoid excessive polarization, those atoms in close contact with the helix charges or with the active site model system had to be removed from the list of polarizable atoms. At distances shorter than approx. 2 Å our model breaks down because it does not treat short-range effects properly. Thus, apart from the charged atoms of the helix backbone, also the C_α's of units 24–43, and C_α and C_β of His-162, together with their hydrogens, were deleted. Note that the side chains of the helix were included in the list of polarizable atoms. No water molecules were considered. In order to study the distance dependency of the dielectric effects we chose from the environment those atoms not farther than a certain distance from the closest atom in the active site model (system I). In fig. 3 this distance was 24 a.u. (12.7 Å) which was the largest distance we could use without running into excessive computation times (more than a few hours on a Cyber 170/760). Within this radius there were 1047 polarizable atoms. The curves in fig. 3 give the energy of the complete system in which the active site model is treated quantum mechanically, including the effect of the helix field on the active site and the polarization of the environment by the active site and by the helix.

The ion pair in itself was expected to be stabilized by the polarizable environment. This is confirmed by the curve for the system with polarizabilities and without helix field (curve 2). The effect of the helix field was expected to decrease in a polarizable environment. The latter effect is calculated to be smaller than the former, stabilizing effect. This follows from the fact that curve 4 is lower than curve 3 at the ion pair side. But the effect of the helix field is indeed decreased from 7.0 to 5.3 kcal at the S–H distance of 2 Å, from which we may derive an effective dielectric constant of 1.3 for this effect. For the system with the helix field the polarization gives an extra stabilization of the ion pair of 3.5 kcal. This value is small compared to values of the order of 50 kcal obtained by Tapia and Johannin [1], which is probably due to their large approximations, especially the neglect of electropoles of higher order than dipoles. On the other hand, our S–N distance is rather small and the proton is thus transferred over a corresponding short distance. Smaller changes in the active site are expected to give smaller changes in the reaction field. The effect of the helix field on the stabilization of the ion pair for S–N distances of 2.94, 3.00 and 3.35 Å is 7, 10 and 15 kcal, respectively. This effect should be approximately proportional to the change in dipole moment caused by the transfer of the proton.

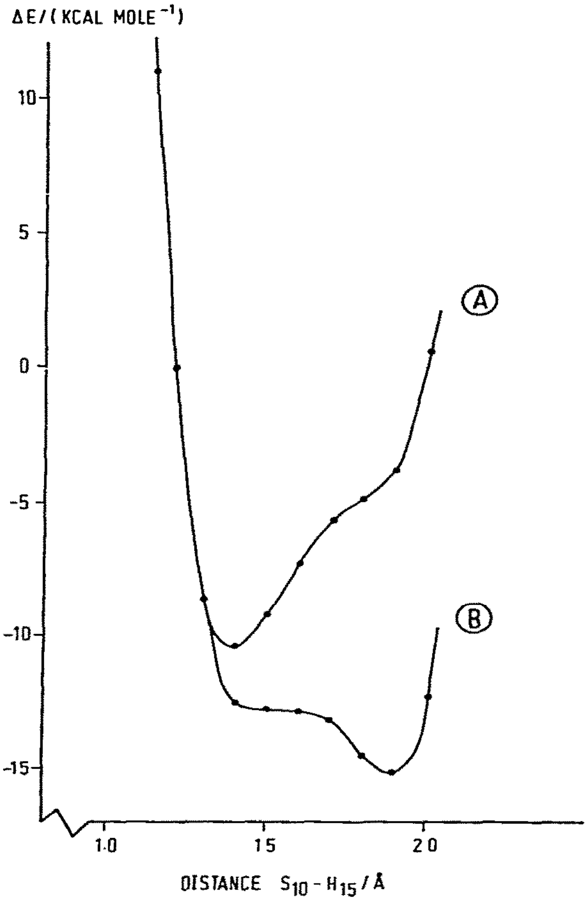

Fig. 2. Results of ab initio SCF calculations on model II of fig. 1. $S_{10}–N_1 = 3.00$ Å. Coordinates from ref. 14. Double zeta basis; cf. appendix.

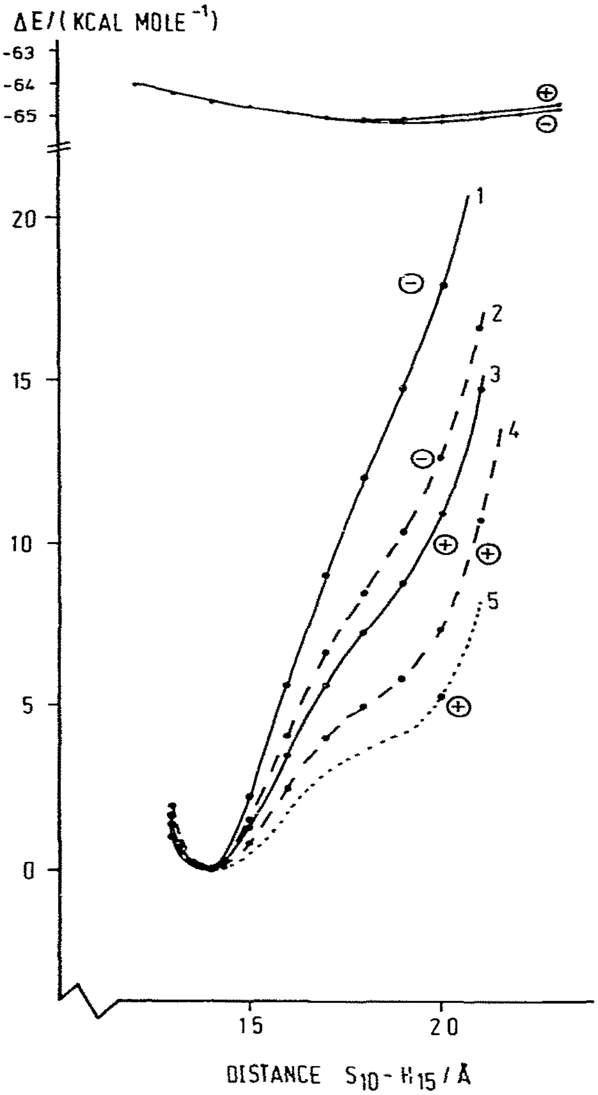

Fig. 3. Results of calculations for system I with various combinations of environmental effects. S–N = 2.94 Å. Coordinates from ref. 12. cf. text. Double zeta basis. cf. appendix. Lower part: (———) without polarizability, (- - - - - -) with polarizability (cutoff 12.7 Å), (· · · · · ·) estimated for system with polarizability, and point charges for Asn-182, (⊕) with helix field, (⊖) without helix field. All curves shifted to zero energy for S–H = 1.4 Å. Upper part: dispersion contribution to the curves with polarizability.

The reaction field effect is expected to be quadratic in the change in dipole moment, giving for the aforementioned distances 3.5, 7 and 16 kcal. This quadratic response is valid for a dipole in a cavity of constant radius [3]. Concomitant enlargement of the cavity will lower the effect giving a less than quadratic response. Thus, the 7 and 16 kcal are probably upper limits and 5 and 8 kcal may be more realistic values.

In the top part of fig. 3 the value of the dispersion term is plotted. As expected, it is large, but the fluctuations with the position of the proton are small. The dispersion stabilizes the ion pair by 0.5 kcal. Note, however, that this value is only an estimate. We may, however, conclude that dispersion effects are unimportant as far as the relative stability of the ion pair is concerned.

In order to estimate how much of the polarizable environment has to be taken into account we also carried out calculations with other cutoff radii. Some results are collected in table 1. The calculation with 7.9 Å gave about the same curves as those with 12.7 Å while they included only 311 atoms. Within 0.3 kcal they showed the same trends and were shifted upward by about 1.5 kcal. The calculations with a cutoff radius of 3.7 Å showed significant differences in the induction and dispersion effects. In following calculations 8 Å is therefore sufficient and 5 Å may be a lower limit. The time required for the calculation of the transfer matrix of the environment is a third-power function of the number of atoms. With a cutoff radius of 8 Å (311 atoms) this calculation time is still far from being a bottleneck in the whole quantum-mechanical calculation. Thus, there is no reason to use a smaller radius and so the complete accuracy of the model is easily achieved. V_{HEH} is the induction energy released when the helix is immersed partly into the sphere containing the environment, in the absence of the active site model. This term is included for completeness and gives an impression of the magnitude of this interaction. Note that the side chains of the helix are counted as environment.

Table 1

Dependency of reaction field effects on cutoff radius

All energies are given in kcal; cf. the appendix for exact definitions.

Cutoff [a] (Å)	N [b]	Induction [c]				Dispersion [d]				V_{HEH} [e]	CPU time [h] (s)
		+ helix [f]		− helix [g]		+ helix		− helix			
		S [i]	N [j]	S	N	S	N	S	N		
2.1	1	−0.57	−0.91	−0.46	−0.79	−4.30	−4.16	−4.32	−4.18	−0.00	2
2.4	3	−0.68	−1.83	−0.69	−2.14	−15.71	−15.99	−15.69	−16.03	−0.07	2
2.6	7	−0.76	−2.09	−0.83	−2.55	−21.96	−22.36	−21.99	−22.46	−0.17	2
3.7	41	−1.09	−3.93	−1.61	−5.09	−48.31	−48.66	−48.35	−48.84	−0.60	10
7.9	311	−0.56	−3.61	−2.21	−6.75	−63.07	−63.53	−63.09	−63.70	−4.78	204
12.7	1047	−0.43	−3.43	−2.30	−7.03	−64.55	−65.07	−64.60	−65.19	−6.90	6600

[a] Atoms within this distance to the closest atom in the active site model are taken as polarizable.

[b] Total number of polarizable atoms.

[c] Stabilization of active site model by polarization of the environment, calculated as the drop in SCF energy on introduction of the direct reaction field energy term into the Hamiltonian, minus the dispersion term. V_{HEH}, which is independent of the proton position, is not included.

[d] Dispersion interaction between active site model and polarizable atoms.

[e] Energy of interaction between the helix and the polarizable atoms.

[f] Calculation with point charges on helix backbone atoms (see text).

[g] Calculation with zero charges on helix.

[h] CPU time, required on CDC Cyber 170/760 computer, for calculation of the quantities, given in the appendix of ref. 7, which describe the polarizability of the environment. The major contribution is from the Cholesky decomposition of the $3N \times 3N$ interaction matrix which takes about $3N^3$ multiplications.

[i] Proton position near S of Cys-25. S–H = 1.4 Å. Neutral configuration.

[j] Proton position near N of His-162. S–H = 2.0 Å. Ion pair configuration.

3. Discussion

The present calculations show that it is feasible to perform reaction field calculations with a model which treats the environment as a set of interacting polarizable atoms. A set of 300 atoms is sufficient and can be treated conveniently. The model gives full regard to the geometry of the active site and of the environment. Furthermore, an estimate of the dispersion effects is obtained. Assuming that we may divide a system into a quantum-mechanical and a classical part, deleting short contacts and adding H atoms to the quantum part where necessary, the model gives the reaction field effects with an error which is probably well below 50%. Thus, future research on reaction field effects will be much less determined by speculations on the errors in the theoretical calculations. In protein work the next step now will to be study the effects of the orientational polarizability of side chains and of water. These effects are potentially very large, as is indicated by the large dielectric constant of water which can reduce all electrostatic effects to about zero. On the other hand, water near the surface of an enzyme is partially bound and restrained in its movement. Moreover, if necessary, the enzyme may be especially designed to reduce the effects of water, or apply them for its own needs. Many enzymes are made just to catalyze reactions which will not proceed in an aqueous environment.

The orientational polarizabililty of bulk water can possibly be simulated by assigning a polarizability to each water molecule which reproduces the correct dielectric constant. Water near the surface, and side chains, can probably only be studied by molecular dynamics, Monte Carlo and other statistical methods, which may perhaps supply effective polarizabilities. Also, a method like that of Bolis et al. [16] may be used, which minimizes the orientational energy of the environment for each configuration of the quantum-mechanical part.

With the S–N distance of 2.94 Å, the ion pair configuration is still unstable by 7 kcal after introduction of the helix field and of a polarizable environment which give stabilizing effects of 5.3 and 3.5 kcal. From a calculation with point charges for Asn-182 without a polarizable environment, we estimate the stabilization by the static field from Asn to be 2 kcal. Other groups will give some more stabilization (Ala-163). Also, the field from the helix, as calculated by the point charges we used, is underestimated by 30–40% [23,24]. Therefore, its effect is underestimated by 2 or 3 kcal. Thus, even with this S–N distance the ion pair is about stable. This precarious balance shows that accurate coordinates from X-ray measurements are very desirable for this type of calculation, in order to avoid the tedious geometry optimizations which are needed to settle the problem.

Appendix: Details of the calculations

The Hamiltonian used for the calculations on the active site Q including the effects of the field from the helix H and the polarizable environment E, may be written, in analogy to ref. 9:

$$H_{\mathrm{tot}} = H_{\mathrm{QQ}} + \sum_{ir} \frac{Q_i Q_r}{r_{ir}} - \sum_{rpqi} f_{rp}^{\dagger} A_{pq} f_{qi} - \tfrac{1}{2} \sum_{ijrs} f_{ip}^{\dagger} A_{pq} f_{qj}$$

$$= H_{\mathrm{QQ}} + V_{\mathrm{HQ}} + V_{\mathrm{HEQ}} + V_{\mathrm{QEQ}} \qquad \text{(A1)}$$

where H_{QQ} is the 'normal', internal energy of Q, Q_i and Q_r the charges of the particles in Q (electrons and nuclei) and H, respectively, f_{rp} the field of particle r in point p, and A the transfer matrix of E. Subscripts ij, pq and rs denote particles in Q, E and H, respectively. Terms V_{HH} and V_{HEH}, occurring in the total energy of the complete system, have not been included, because they do not contain coordinates of Q. V_{HEH} is, however, displayed in table 1.

We define $|\phi^0\rangle$ as the Hartree-Fock ground state of $H_{\mathrm{QQ}} + V_{\mathrm{HQ}}$ and $|\phi^{\mathrm{SC}}\rangle$ as the ground state of H_{tot}. Further, we use for shorthand:

$$V_{\mathrm{QEQ}} = -\tfrac{1}{2} \tilde{F}^{\dagger} \tilde{A} \tilde{F}, \qquad \text{(A2)}$$

where $\tilde{F}$ and $\tilde{A}$ are supermatrices. Then

$$V^{\mathrm{disp}}(\phi) = -\tfrac{1}{2}\langle\phi|\tilde{F}^{\dagger}\tilde{A}\tilde{F}|\phi\rangle + \tfrac{1}{2}\langle\phi|\tilde{F}^{\dagger}|\phi\rangle\tilde{A}\langle\phi|\tilde{F}|\phi\rangle \qquad \text{(A3)}$$

$$V^{\mathrm{ind}}(\phi) = \langle\phi|H_{\mathrm{tot}}|\phi\rangle - \langle\phi^0|H_{\mathrm{QQ}} + V_{\mathrm{HQ}}|\phi^0\rangle - V^{\mathrm{disp}}(\phi) \qquad \text{(A4)}$$

The quantities in table 1 are then defined as $V^{\mathrm{ind}}(\phi^{\mathrm{SC}})$ and $V^{\mathrm{disp}}(\phi^{\mathrm{SC}})$.

The basis set used for the SCF-LCAO calculations is the (7/3) Gaussian basis of Roos and Siegbahn [25] contracted to a (4111/21) 'double zeta' atomic basis. For hydrogen we use the 3s basis of Jonkman et al. [26] contracted to (21).

Matrix elements of the reaction field and the helix field terms in the Hamiltonian are calculated in a multicenter dipole expansion by the methods described in refs. 7 and 9. As expansion centers we used all the atoms of the active site plus all the 12 positions of H_{15}. The 12 proton positions were taken along the line connecting S_{10}–N_1 at distances indicated in fig. 3. The extra computation time needed for the calculation of the quantities given in the appendix of ref. 7 is given in table 1. Further, the two-electron integrals of H_{QEQ} require about 20% of the time needed for the coulomb electron repulsion integrals. The SCF procedure is equal to the normal procedure and does not require extra computation time. V^{disp} is calculated separately afterward in the time needed for one SCF iteration.

For the construction of A we used the model described in the appendix of ref. 8. For the atomic polarizability of sulfur atoms we used $\alpha_{\mathrm{S}} = 23.22$ a.u. $= 3.44$ Å^3. This value was obtained from variation perturbation theory by Fraga et al. [27], which seems justified because atomic polarizabilities obtained from a fit to experimental molecular polarizabilities differ only by 20–30% from Fraga's values. Moreover, there are no sulfur atoms sufficiently near the active site to be significant.

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