Simulations of the T ↔ R conformational transition in aspartate transcarbamylase

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Aspartate transcarbamylase (ATCase) from Escherichia coli is one of the best known allosteric enzymes. In spite of numerous experiments performed by biochemists, no consensus model for the cooperative transition between the tensed (T) and the relaxed (R) forms exists. It is hypothesized, however, that changes in the quaternary structure play a key role in the allosteric properties of oligomeric proteins such as ATCase. Previous normal mode calculations of the two states of ATCase illustrated the type of motions that could be important in initiating the transition.

In this work four pathways for the transition were calculated using the targeted molecular dynamics (TMD) method without constraint on the symmetry of the system. The most important quaternary structure changes are the relative rotation and translation of the catalytic trimers and the rotations of the regulatory dimers. The simulations show that these quaternary changes start immediately and finish when about 70% of the transition is completed whereas there are tertiary changes throughout the transition. In agreement with the work of Lipscomb et al., it was found that the relative translation between the catalytic trimers appears to play a central role in allowing the transition to occur. In all the simulations differences are observed in the opening and closing behaviours of the domains in the catalytic and regulatory chains that could provide a structural interpretation for the results of certain site-directed mutagenesis experiments. Overall the motions of the subunits are concerted even though the constraint imposed on the TMD method does not explicitly require that this be so.

Keywords: allosteric transition/aspartate transcarbamylase/mutant proteins/targeted molecular dynamics simulations/tertiary and quaternary structural changes

Introduction

Aspartate transcarbamylase (ATCase) from Escherichia coli (EC 2.1.3.2) is one of the most thoroughly studied enzymes. It catalyses the carbamylation of the L-aspartate amino group by carbamylphosphate, which is the first step in the pyrimidine pathway. The binding of aspartate induces the cooperative transition from the low affinity–low activity T state (tense) to the high affinity–high activity R state (relaxed). This homotropic transition is fully compatible with the classical Monod–Wyman–Changeux theory of allostery (Monod et al., 1965). The enzyme’s activity is modulated by allosteric effectors—there is a feedback inhibition by cytosine 5′-triphosphate (CTP) and an activation by adenosine 5′-triphosphate (ATP).

Several crystal structures of ATCase from Escherichia coli have been determined by Lipscomb and co-workers (Ke et al., 1988; Stevens et al., 1990). Unligated structures exist for the two allosteric states and also for complexed structures with different effectors and/or substrate analogues (for a review, see Lipscomb, 1992, 1994). The holoenzyme is a dodecamer with C₃ symmetry. There is a slight deviation from D₃ symmetry which is due to the existence of low- and high-affinity CTP-binding sites (Kim et al., 1987; Lipscomb, 1992). The protein is organized into two catalytic trimers and three regulatory dimers. The trimers are composed of catalytic chains (C₁–C₆) of 310 residues each and the dimers of regulatory chains (R₁–R₆) of 153 residues each. The catalytic chains have two ligand-binding domains, the N-terminal carbamylphosphate domain (CP domain) and the C-terminal aspartate domain (ASP domain), which are linked by helices H₅ and H₁₂. The regulatory chains are also composed of two domains, namely the allosteric domain (ALLO domain), which binds the effectors, and the zinc domain (ZN domain), which contains a structural zinc atom. The structure of ATCase is shown in Figure 1.

The T → R transition in the ATCase dodecamer involves large quaternary changes, notably a 12 Å opening of the intercatalytic trimer distance, a 5° rotation of these trimers in opposite directions relative to the C₃ axis and a 15° global rotation of the regulatory dimers around their pseudo-twofold rotation axes. There are also considerable changes in the tertiary structure including the closure of the active site in each catalytic chain by the rigid-body movement of the ASP domain towards the CP domain and the displacements of the 240s loops (residues 230–245) which play a crucial role in the quaternary structural transition by modifying the C₁:C₄ interfaces and removing the C₁:R₄ interfaces (Ladjimi et al., 1988).

The work described in this paper is part of an ongoing project to investigate the mechanism of the allosteric transition in ATCase using theoretical simulation techniques. In particular, we want to learn more about the type of quaternary and tertiary motions and the order in which they occur. In three recent publications we calculated the normal modes of each state to obtain an idea of the movements that could be important in the initial stages of the transition (Thomas et al., 1996a, b, 1999). In this work we attempted a more direct approach by generating pathways for the complete transition using a molecular dynamics simulation technique. It is not possible to study the transition with free molecular dynamics (MD) because the time-scale for the transition is of the order of 1 µs whereas the maximum times currently accessible by MD simulations are of the order of 1 ns (for a protein system in water). To circumvent this problem, we employ a targeted molecular dynamics (TMD) approach, in which a single
The initial crystal structures were taken from the Brookhaven Protein Data Bank (Bernstein \textit{et al}, 1977). The coordinates of the T state protein were those of the most recently refined structure of Kosman \textit{et al} (1993) obtained at a resolution of 2.5 Å. The coordinates of the R state protein correspond to the structure resolved at 2.8 Å by Gouaux \textit{et al} (1990). In both structures there are CTP molecules that bind to each allosteric site. There are no water molecules in the R structure, so the 81 water molecules present in the T state were omitted. There is also no substrate in the T state, so the N-(phosphonoacetyl)-L-aspartate (PALA) molecules present in the R state were removed. The removal of the PALA molecules is necessary because the TMD algorithm (like all other reaction path-finding algorithms) requires the same numbers of atoms for both the initial and reference structures. All polar hydrogen atoms were included explicitly in the calculations whereas the others were treated with a united atom model. In the R state crystal structure, the first seven residues of each regulatory chain are missing because they are disordered. These were left out of our calculations, giving 146 residues per regulatory chain only. Our final model protein had 2736 amino acids and 26 370 atoms.

All calculations were performed with version 23 of the CHARMM molecular modelling software and with the CHARMM 19 polar hydrogen force field (Brooks \textit{et al}, 1983). The CTP parameters were obtained as a combination of ATP and cytosine parameters from version 21 of the QUANTA force field (Molecular Simulations, San Diego, CA). The programs VMD (Humphrey \textit{et al}, 1996) and Raster3D (Merritt and Murphy, 1994) were used for graphical display.

An explicitly solvated ATCase molecule requires $\sim 10^5$ water molecules. These, together with the $\sim 3 \times 10^4$ atoms of the protein, comprise a system which is too large for our current computational resources and so we employed an implicit model for the solvent, which, of course, is a strong approximation. The choice and justification of the model were fully discussed previously (Thomas \textit{et al}, 1996a) but, to summarize what was said there, we approximated the effect of the solvent with a linear distance-dependent dielectric constant (Loncharich and Brooks, 1989), a shifted electrostatic potential and a switched van der Waals potential (Steinbach and Brooks, 1994). The cut-off for non-bonding interactions was taken to be 9 Å. The screening effect of the solvent was modelled by weighting the atomic charges of the ends of the charged amino acid side chains (Lys, Asp, Glu, Arg) by 0.3 (Mouawad and Perahia, 1996). The charges of CTP were treated similarly. The histidine residues were taken as neutral and their enantiomeric states were the same as those assigned by Kosman \textit{et al} (1993). The coordinates of the polar hydrogens were determined using the HBUILD algorithm (Brunger and Karplus, 1988).

Both the R and T state structures were minimized using the same model until their r.m.s. gradient norms were <0.0001 kcal/mol. The r.m.s. coordinate differences (r.m.s.c.d.s) of the minimized structures from the corresponding crystallographic structures were 2.65 and 3.40 Å, respectively. The minimized structures, which we denote as $R_{\text{min}}$ and $T_{\text{min}}$, were used both as reference structures in the TMD simulations and as starting points for the generation of the dynamics structures described below.

The starting dynamics structures

To generate structures suitable for the TMD calculations, molecular dynamics simulations without constraints were per-
formed on the minimized structures described earlier. For each structure, there was a 4 ps heating phase in which the temperature was increased from 0 to 300 K followed by a 96 ps equilibration phase. During the heating phase, a harmonic constraint was placed on all the atoms of each structure to prevent too strong a deviation from the minimized structure. The starting value of the constraint force constant was 400 kcal/molÅ² but this was reduced every 400 fs. The full protocol is detailed in Table 1. The time step for all dynamics simulations was 1 fs. No constraints were put on the symmetry of the system so the structures were free to deviate from C3 symmetry. The two structures resulting from the simulations were used as both starting and reference structures for the TMD simulations and we call them R300 and T300.

The TMD protocol

The TMD protocol that we use is similar to that developed by Schlitter et al. (1993) and employed in several studies (Jacoby et al., 1996; Diaz et al., 1997; Ma and Karplus, 1997; Wroblowski et al., 1997). In the scheme, the r.m.s.c.d. between the structure undergoing the dynamics simulation (X) and a reference structure (Ref) is constrained to have a particular value. The (mass-weighted) r.m.s.c.d., d, between structures X and Ref is defined as

$$d = \sqrt{\frac{\sum_{i=1}^{N} m_i (x_i^X - x_i^{Ref})^2}{\sum_{i=1}^{N} m_i}}$$

(1)

where N is the number of atoms in the system and \(m_i\) and \(x_i\) are the mass and the position vector of atom i, respectively.

The r.m.s.c.d. constraint, by itself, is not sufficient to prevent global rotations and translations of the structure X with respect to Ref. It is therefore necessary to impose an additional set of six linear constraints which for the rotations take the form

$$\sum_{i=1}^{N} m_i (x_i^X \wedge x_i^{Ref}) = 0$$

(2)

where \(\wedge\) indicates a cross or vector product. The corresponding constraints for the translations are

$$\sum_{i=1}^{N} m_i (x_i^X - x_i^{Ref}) = 0$$

(3)

The strategy for performing a TMD simulation with these constraints is as follows:
1. Choose a starting structure and a reference structure for the TMD simulation.
2. Fit the starting structure to the reference structure so that the r.m.s.c.d. between the two is minimized. Well known algorithms, such as that developed by Kabsch (1976, 1978), exist for doing this. The resulting value of \(d\) is the starting value for the constraint defined in Equation 1.
3. Perform a simulation enforcing the seven constraints defined above and keeping the value of the r.m.s.c.d. between the dynamic and reference structure constant. This is to allow the dynamic structure to ‘equilibrate’ in the presence of the constraints.
4. Continue the simulation enforcing the rotational and translational constraints by decrementing the target value of the r.m.s.c.d. for the constraint in Equation 1 by a small amount at each dynamics step. Continue the simulation until the distance between the two structures has the desired value.

There are a number of ways to enforce the constraints, but we chose to use the SHAKE method developed by Ryckaert (1985). The implementation is straightforward and experience showed that the constraints could be satisfied to a high degree of precision (typically with a deviation of \(10^{-10}\)) in a small number of iterations. The method described above was implemented as a module (TMD) into version 23 of the CHARMM program. The simulations were performed with the Verlet algorithm (Allen and Tildesley, 1987) with a time step of 1 fs at a temperature of 300 K.

Four trajectories were generated. Each consisted of 1 ps of equilibration in which the r.m.s.c.d.s between the dynamic and reference structures were held constant and then 200 ps of dynamics in which the r.m.s.c.d.s were reduced at each step. Two of the 200 ps simulations went from R → T and two from T → R. As the r.m.s.c.d. between the R and T structures is of the order of 8 Å, the target distance for the constraint was reduced by about \(4 \times 10^{-5} \) Å per dynamics step. In each case the starting structure for the simulations was the appropriate dynamics structure, either R300 or T300, but different reference structures were used for the pair of TMD simulations in each direction. In one the reference structure was the minimized structure of the appropriate form (R\(_{min}\) or T\(_{min}\)) and in the other it was the dynamics structure (R\(_{300}\) or T\(_{300}\)). This was done to test the effect of a different target on the calculated transition. Each simulation took about 1500 h (2 months) of CPU time on an HP 735 workstation. Subsequently we have performed a simulation of 500 ps with the TMD algorithm to see how the generated path changes with trajectory length. Our analysis shows that the results of the longer calculation are fully coherent with those of the 200 ps simulations.

### Table 1. Heating and equilibration protocols used to produce the initial structures for the TMD simulations starting from the minimized R and T state structures

<table>
<thead>
<tr>
<th>Time (fs)</th>
<th>Temperature (K)</th>
<th>Harmonic constraint force constant (kcal/molÅ²)</th>
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<tr>
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</tr>
<tr>
<td>100000</td>
<td>300</td>
<td>0</td>
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</table>

Analysis of structural and energetic changes during the transitions

All four trajectories were analysed independently and then the results were compared. As no constraint was put on the symmetry of the system, the analysis was performed for all the chains (six catalytic chains and six regulatory chains) within the protein.

To indicate the extent to which the transition between the two forms has occurred, we define a reaction coordinate or ‘transition progress’ parameter, \(f(t)\), of the form
\[ f(t) = 100 \left( 1 - \frac{\text{r.m.s.c.d.} \left[ X(t), \text{Ref} \right]}{\text{r.m.s.c.d.} \left[ X(0), \text{Ref} \right]} \right) \] (4)

where \( X(t) \) is the TMD simulation structure at time \( t \) and Ref is the reference structure. It is to be noted that the TMD method, in common with other reaction path-finding algorithms, gives no indication about the rate of passage of the system along the reaction coordinate and so it is not possible to draw any conclusions from the calculated paths concerning the time it takes for particular events to occur. In contrast, however, the order in which structural changes take place during the transition can be identified.

The simulations were analysed for the first 90% of each trajectory. For structures too close to the reference structure, there is a conflict between the constraint and the thermal fluctuations of the atoms that causes the potential energy of the system to rise considerably. In any case, at a progress parameter of 90%, the r.m.s.c.d.s between the dynamic and reference structures are <1 Å and the dynamic structures of all four trajectories have the correct characteristics of the reference structures.

The most important analyses that we performed were the examination of the global rotation and translation of the trimers and the dimers of each intermediate structure with respect to the reference structures and the determination of the relative movements of the domains and their axes of rotation. The tools that we used for these analyses were algorithms for the superposition of molecular structures using either the Kabsch algorithm or an algorithm based upon quaternions (Kneller, 1991). These functions were implemented in the program CHARM and in the Molecular Modeling Tool Kit (Hinsen, 1997), respectively.

The exact definitions of the quantities discussed in the Results section are as follows:
1. The relative intertrimer rotation angle corresponds to the difference in the intertrimer rotation angles of two structures. In practice, this was calculated by rotating and translating one of the structures so that its C1C2C3 trimer was superposed on the C1C2C3 trimer of the other structure. Another superposition was then made for the C4C5C6 trimers and the angle of rotation obtained from this fit was the required angle.
2. The relative intertrimer translation is the difference in the translations between the trimers of two structures. It was calculated following the same procedure as the relative intertrimer rotation angle.
3. The dimer rotation angle is the angle by which it is necessary to rotate a particular dimer of one structure to superpose it on the equivalent dimer in a second structure. To a good approximation in all cases studied, the axes for these rotations were the pseudo-twofold axes of ATCase. Note that before performing these fits the entire ATCase molecule was first superposed to remove any spurious contribution from global rotations and translations.
4. The relative interdomain angles are the differences in the angles between the domains in the catalytic or the regulatory chains of two structures. The domains are the CP (residues 7–132) and ASP (residues 155–283) domains for the catalytic chains and the ALLO (residues 7–92) and ZN (residues 96–143) domains for the regulatory chains. The angles were calculated in a similar way to the relative intertrimer rotation angle. First the entire chain (C or R) of one structure was rotated and translated so that its N-terminal domain (CP or ALLO) was superposed on the equivalent domain of the chain of the second structure. A second fit was then made to superpose the C-terminal domains of the two structures (ASP or ZN) and the angle necessary for this fit was the one desired. Note that there is a reduction (closing) of the catalytic chain interdomain angle on going from the T state to the R state whereas there is an increase (opening) of the regulatory chain interdomain angle in the same direction.

### Results and discussion

#### The minimized reference structures

After the minimization, the r.m.s.c.d. between the T state crystallographic and minimized structures was equal to 3.40 Å if the analysis was performed for all atoms and 3.12 Å if the analysis was carried out for the heavy atoms of the backbone only (N, Cα, C). The corresponding values for the R state were 2.65 and 2.42 Å, respectively. These changes have been fully discussed in previous papers (Thomas et al., 1996a,b). Despite their large structural differences, the R and T minimized structures have total potential energies that differ by only 13 kcal/mol (mainly in the non-bonding interactions).

#### The 300 K initial structures

After the 100 ps dynamics simulation (both heating and equilibration), the r.m.s.c.d. between the structures, \( T_{\text{min}} \) and \( T_{300} \), had stabilized at 4.28 Å for all the atoms of the protein and 3.82 Å for the backbone atoms. For the R state structures the differences were slightly larger at 5.46 Å (all atoms) and 5.11 Å (backbone atoms). In both cases the deformation was distributed over the whole protein. The r.m.s.c.d. for the backbone atoms was 1.91 and 1.56 Å on average for the catalytic chains of the T and R states, respectively, and 2.69 and 2.47 Å for the regulatory chains. The larger changes in the regulatory chains can be related to the higher mobility of the dimers which is observed in the crystallographic structures.

A more detailed analysis of the energetic and structural differences between the minimized and dynamic R and T state forms is contained in Tables II and III. Table II lists the different energetic contributions for the structures and Table III lists geometric parameters that highlight global, quaternary and tertiary structural differences between pairs of R and T state structures. The quantities listed in Table III are fully defined in the Methods section.

In contrast to the minimized structures, the R state dynamic structure is more stable than the T state structure, but only by about 30 kcal/mol. This may be related to the larger reduction in the radius of gyration of the R state structure after the

<table>
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<th>Potential energy terms</th>
<th>Minimized structures</th>
<th>300 K structures</th>
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<td></td>
<td>T state</td>
<td>R state</td>
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<tr>
<td>Total</td>
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<td>–42 694</td>
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<tr>
<td>Bond</td>
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<td>431</td>
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<tr>
<td>Angle</td>
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<td>2 507</td>
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<tr>
<td>Dihedral</td>
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<td>5 923</td>
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<tr>
<td>Improper Electrostatic</td>
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<td>592</td>
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<tr>
<td></td>
<td>–18 691</td>
<td>–18 829</td>
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Fig. 2. The r.m.s.c.d.s between the backbone atoms of the intermediate and initial and reference structures for the $T_{300} \rightarrow R_{300}$ and the $R_{300} \rightarrow T_{300}$ pathways.

Table III. Geometric parameters characterizing some global, quaternary and tertiary structural differences between pairs of T and R state structures

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<th>r.m.s.c.d.:</th>
<th>$T_{cry}$</th>
<th>$T_{min}$</th>
<th>$T_{300}$</th>
<th>$T_{300}$</th>
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<td>Radius of gyration (Å)</td>
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<td>2.02</td>
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<td>18.4</td>
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<td>18.4</td>
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<td>15.8</td>
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<td>14.7</td>
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The trimer, dimer and domain quantities are defined in the Methods section.

The trimer, dimer and domain quantities are defined in the Methods section.

The simulation which goes from 43.8 to 42.4 Å. The corresponding values for the T state structures are 41.8 and 41.1 Å, respectively. There are also changes in the relative rotations and translations of different elements of the tertiary and quaternary structures, although these changes are mostly minor and do not affect the overall nature of the structures. The loss of $C_3$ symmetry is also obvious as the rotation angles for chains of the same type are different.

The transition pathways

The four transitions studied were $T_{300} \rightarrow R_{300}$, $T_{300} \rightarrow R_{min}$, $R_{300} \rightarrow T_{300}$ and $R_{300} \rightarrow T_{min}$. The only differences between the simulations were the initial and the reference structures as the same simulation protocol was used.

Figure 2 shows the r.m.s.c.d.s between the intermediate structures and both the initial and the reference structures for the $T_{300} \rightarrow R_{300}$ and the $R_{300} \rightarrow T_{300}$ pathways. That the

TMD's r.m.s.c.d. constraint works as desired is clear from the linearity of the variation of the r.m.s.c.d.s between the intermediate and the reference structures along the paths. In contrast, the variation of the r.m.s.c.d.s between the intermediate and the starting structures is initially steeper, although approximately linear, but then slows as the transition progresses (at ~40 and ~60% of the transition for the R$_{300} \rightarrow T_{300}$ and the T$_{300} \rightarrow R_{300}$ pathways, respectively).

**Quaternary changes**

**Rotation and translation of the trimers.** The relative positions of the two trimers (C1C2C3, C4,C5,C6) of ATCase change substantially during the allosteric transition. For the T $\rightarrow$ R pathway the intertrimer distance between the crystallographic structures increases by 12 Å whereas there is a $10^\circ$ rotation around the threefold axis (and vice versa for the transition in the other direction).

The relative rotations of the trimers with respect to the reference structures during the four transition pathways are shown in Figure 3. There are differences, but the overall qualitative behaviour is similar. There is an initial increase in the rotation angle (i.e. a moving away from the reference structure) before the angle decreases monotonically to zero. The relative rotation of the trimers is finished at about 70% of the transition in all the transitions. There is a similar behaviour in Figure 4 which shows the relative rotation angle of the trimers during the transition with respect to the initial simulation structures. Also apparent from this figure is a brief period (~5%) at the beginning of the simulations during which there is no rotation.

Let us examine in more detail the increase in the rotation angle of the trimers away from the value in the reference
structure. Considering the $R_{300} \rightarrow T_{300}$ transition pathway as an example, we observe that the difference in the rotation angle between the R and T state structures is about 4° which increases to about 10° at $f(t) = 20\%$ before decreasing during the remainder of the simulation. This behaviour is illustrated in Figure 5a and b, which plots, as vector fields, the magnitude and direction of the rotation angles that the atoms of a single trimer undergo during different portions of the pathway. It is clear that the rotations occur in essentially opposite directions. A possible explanation for this behaviour is that the initial rotation that moves the trimers away from their position in the reference structure is needed to destabilize the starting structure so that the transition can occur. It is worth noting that the largest rotations visible in Fig. 5b are the reorganizations that occur at the interfaces, especially at the interfaces of type C1C4R.

Fig. 6. Relative translation between the trimers during the transitions.

As a complementary analysis, we tried to rotate the trimers directly to their final positions and observed that this was effectively impossible owing to the large forces acting on the 240s loops located at the C1C4 interfaces. This means that steric constraints on the 240s loops will prevent the direct transition. It appears, therefore, that a combination of an indirect rotation preceded by a translation of the trimers (see below) is necessary for an energetically favourable transition. This observation is consistent with the results of Ha and Allevet (1998) and of Svergun et al. (1997), who have shown that the T and R states can display more pronounced conformational differences than those seen between the crystallographic structures.

Figure 6 plots the relative intertrimer translations for each of the transition pathways. The main translation occurs along the threefold axis of symmetry. There is no significant translation along the other axes (data not shown). The overall qualitative behaviour of the changes is the same with the translations diminishing monotonically to zero. The detailed behaviours of the pathways in the two directions, however, are slightly different with the $R \rightarrow T$ translations decreasing more rapidly than those of the $T \rightarrow R$ pathways.

One striking feature of these results is that the translation starts before and finishes after the rotational movement has either started or finished. The values of the translation that occur in each of the simulations before the rotations begin are 0.20, 0.45, 0.88 and 1.12 Å in the $T_{300} \rightarrow R_{300}$, $T_{300} \rightarrow R_{\text{min}}$, $R_{300} \rightarrow T_{\text{min}}$ and $R_{300} \rightarrow T_{300}$ pathways, respectively. Note that the required translations are larger for the $R \rightarrow T$ transitions, indicating that a reduction of the intertrimer distance is easier than an increase. After the rotations have finished, about 1 Å of translation is still needed in each of the simulations to attain the values in the reference structures. The fact that a small displacement (~1 Å) along the threefold axis of symmetry appears to be necessary (in either direction) before a rotation can occur is consistent with the results of Lipscomb (1994), who solved the structures of ATCase complexed with ATP and CTP and compared them with the unligated enzymes. They noticed a small, but significant, variation in the intertrimer distances but no variation in the trimers’ relative rotation angles. The ATP ligated to the T state ATCase increases the intertrimer distance by about 0.4 Å (Stevens et al., 1990) and CTP ligated to the R state decreases the intertrimer distance by about 0.5 Å. These very slight quaternary perturbations appear to modulate the allosteric transition (Gouaux et al., 1998).
As the behaviour of these intermediate structures of each pathway and the pathway's is to calculate the r.m.s.c.d.s for the trimers between the remaining 30% of the transition. One way to investigate thisments of the trimers appear to be complete after about 70%

Thus, the translation seems to act as a trigger that initiates the transition by altering the interactions between the trimers and modifying the stability of the structure.

As already remarked, the rotational and translational movements of the trimers appear to be complete after about 70% of the transition. It is pertinent to ask what happens in the remaining 30% of the transition. One way to investigate this is to calculate the r.m.s.c.d.s for the trimers between the intermediate structures of each pathway and the pathway’s starting and reference structures. As the behaviour of these results for all the simulations is similar, only the results for the T_{300} → R_{300} pathway are shown. The r.m.s.c.d.s for the trimers with respect to the reference structure, R_{300} (Figure 7a), are roughly constant for the first 60% of the transition but decrease rapidly thereafter. The behaviour with respect to the initial structure, T_{300}, is different (Figure 7b). There is a rapid increase in the first steps of the simulation (this is a typical property of r.m.s.c.d.s for simulation structures) and, subsequently, a gradual increase. These results indicate that whereas there are movements within the trimer throughout the transition, it is the large amplitude quaternary motions that are primarily responsible for bringing the dynamics structures towards the reference structure and it is only at the end of the transition that the more localized tertiary rearrangements occur.

Rotation of the dimers. A second important quaternary motion characteristic of the allosteric transition in ATCase is the rotation of the regulatory dimers (R1R6, R2R4, R3R5) around their pseudo-twofold axes. The difference in the rotations between the crystallographic structures is about 18°.

In contrast to the case of the rotation angles of the catalytic trimers where there were large differences in quantitative behaviour, the behaviour of the dimer rotations is more uniform (Figure 8). The rotations all decrease in a relatively steady way, with a few oscillations, until the rotation in the reference state is reached at f(t) ≈ 75%. Four of the 12 curves show a slight increase in the rotation angle in the first ~10% of the transition, but these are proportionately much smaller than those observed for the trimers.

An examination of the data for the dimer rotations in Table III shows that a large range of dimer rotation angles is possible, from 10 to 26°. This is a manifestation of the fact that the regulatory dimers have much weaker structural constraints on their conformations than the catalytic trimers. The data in Table III also indicate that the C₃ symmetry of the structure is lost, which is unsurprising given the fact that no such constraint was imposed. In general, there should be no overriding reason to suppose that the transitions should be either symmetrical or synchronous. However, it does appear from Figure 8 that the rotation of the dimers is indeed concerted, as even those dimers for which the rotation angle is largest rapidly move to conformations with rotation angles closer to those of the other dimers in the structure. Thus, for example, the regulatory dimers in the R_{300} → T_{min} transition (Figure 8a) have similar rotation angles after about 40% of the transition, even though the value for the R1R6 dimer is much greater initially. The data in Figure 8b and d show similar behaviours but after 20 and 25% of the transition have been completed, respectively. This disparity in the way the rotation angles decrease is also reflected in the absence of a preferential order in which dimers reach their reference positions, i.e. those that start with the largest rotations do not necessarily attain their reference positions last.

Tertiary changes. In addition to quaternary motions, changes in the tertiary structure are also important during the transition. The most notable of these are the variations in the relative positions of the domains within the catalytic and regulatory chains. Thus, for example, the CP and the ASP domains of the catalytic chains come together during the T → R transition. There is also an opening of up to 15° between the ZN and ALLO domains of the regulatory chains (Lipscomb, 1994), although in our simulations we can obtain larger values depending upon the pairs of structures that are being compared (see Table III).

Other important tertiary structure changes are the rearrangements that occur at the interfaces of the subunits. These movements are not analysed in this paper but will be discussed in a future paper along with the results obtained with additional simulations of the transition pathways. There we will also compare our results with the normal mode data generated by Thomas et al. (1996a,b, 1999).

Catalytic domain closure. The domain closure associated with the binding of substrate and substrate analogues has been observed in the R state by Krause et al. (1985, 1987). We omitted the substrate in our simulations so we will not be able to see any substrate-induced changes. However, we should see some of the motions that are possible in these domains during the transition.

The variations in the angles between the domains of the catalytic and regulatory chains as a function of the transition progress parameter for the R_{300} → T_{min} pathway are shown in Figure 9. The data for the other transitions are similar and are not shown. The concerted aspect of the transition, already noted above, is confirmed by these graphs, which indicate that the changes for the catalytic and regulatory chains occur in a synchronous fashion.
Fig. 8. Rotations of the regulatory dimers for the structure along the transition pathways with respect to the reference structure. (a) $R_{300} \rightarrow T_{min}$; (b) $R_{300} \rightarrow T_{300}$; (c) $T_{300} \rightarrow R_{min}$; (d) $T_{300} \rightarrow R_{300}$.

Fig. 9. Angle between the domains in (a) the catalytic chains and (b) the regulatory chains for the $R_{300} \rightarrow T_{min}$ transition pathway.

A clearer image of the overall behaviour of the interdomain angles can be obtained by plotting the average interdomain angle for the catalytic or regulatory chains versus the transition progress for each of the simulations. The results are given in Figure 10. The interdomain angles of the catalytic chains undergo little change for the first 60% of the transition and then change more rapidly afterwards. The variations in the angles are between 11 and 15° at 90% of the transition. These later motions agree with the r.m.s.c.d. data plotted in Figure 8.

By comparing Figures 3 and 10, we see that the largest variations in the catalytic chain interdomain angles occur after about 60% of the transition, when the relative intertrimer rotations are almost, but not fully, completed. This observation is consistent with results obtained for ATCases with mutated catalytic chains. The mutations, Glu50 $\rightarrow$ Ala, Ser171 $\rightarrow$ Ala (Dembowski et al., 1990) and Arg234 $\rightarrow$ Ser, inhibit the domain closure of the catalytic chains but a partial quaternary conformational change is observed in small-angle X-ray scattering experiments (Newton and Kantrowitz, 1990; Tauc et al., 1994). These mutant R states have greatly reduced catalytic activity (Kantrowitz and Lipscomb, 1990). We propose that they are locked in an intermediate state (at $\sim$60% of the transition) and could correspond to the kinetic I state reported in biochemical experiments (Ho Lee et al., 1995) or to the intermediate observed in time-resolved small-angle X-ray scattering experiments of the Glu50 $\rightarrow$ Ala mutant enzyme bound with natural substrates (Tsuruta et al., 1998). This intermediate state has undergone only a partial quaternary transition and it displays only weak activity because full closure of its catalytic domains is prevented (see position E50A in Figure 11).

Regulatory domain opening. The changes in the interdomain angles of the regulatory chains, like those of the catalytic chains, are concerted (Figure 9b), but their interdomain angles show larger fluctuations, which is probably due to the fact that the dimers are less structurally constrained. In contrast to the angles of the catalytic chains, however, the regulatory domain angles increase rapidly at the beginning of the transition and occur simultaneously with the quaternary structure changes (Figure 10).
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Fig. 10. Average interdomain angles for the regulatory and catalytic chains in each of the transition pathways. (a) $R_{300} \rightarrow T_{\text{min}}$; (b) $R_{300} \rightarrow T_{300}$; (c) $T_{300} \rightarrow R_{\text{min}}$; (d) $T_{300} \rightarrow R_{300}$.

Fig. 11. Summary of the chronology of the various quaternary and tertiary motions that take place during the T → R simulated transition pathway. There are two types of domain motions—the light shading corresponds to the opening of the regulatory domains and the dark shading to the closure of the catalytic domains. For the trimer rotations, the light shading represents the indirect rotation and the dark shading the direct rotation. The arrows Y77F and E50A indicate where along the transition pathway these mutants are proposed to have their observed effect.

We hypothesize that this difference in behaviour is consistent with the separation of the action of the allosteric effectors and of aspartate, which forms the basis of the ‘primary–secondary effects’ (Thiry and Herve, 1978; Herve, 1989) and the ‘effector modulated transition’ (Xi et al., 1991) mechanisms of regulation. The effectors cannot induce the transition but change the quaternary structure to make the transition easier (ATP increases the intertrimer distance by 0.4 Å) or more difficult (CTP decreases the intertrimer distance by 0.5 Å) whereas aspartate is necessary for the transition to the high activity–high affinity state of the enzyme (Stevens et al., 1990). Thus, in our model, the fact that the regulatory domain and the quaternary motions occur before the catalytic domain motions means that the former can modulate the latter. These results are also in agreement with those of Tanner et al. (1993), who performed a molecular dynamics simulation and a rigid-body analysis of the R state structure and observed that there is a decoupling of the allosteric domains from the rest of the enzyme and, hence, a separation of the catalytic and regulatory effects.

These observations could also give a structural explanation of the results obtained for ATCases with a Tyr77 → Phe mutation in the hydrophobic pockets of the regulatory chains. This mutation converts ATP into an inhibitor of ATCase instead of an activator and reduces the activity of the enzyme (Van Vliet et al., 1991). It is possible that this mutant inhibits, although does not entirely stop, the opening of the regulatory domains. This prevent the full transition from occurring and means that the mutant enzyme displays predominantly weak activity–weak affinity T state characteristics (see position Y77F in Figure 11).

Conclusion

The aim of our work has been to achieve a better understanding of the quaternary and tertiary motions that occur during the allosteric R ↔ T transition in ATCase. We have calculated four independent trajectories using a TMD simulation methodology that constrains neither the symmetry nor the synchronization of the motions. Four trajectories are insufficient to obtain statistically reliable results but the similarities in the pathways indicate that we can make a number of qualitative observations about the way in which the transition occurs. Even if the physical basis of the TMD simulation method can be debated,
several recent studies have shown that they can provide useful results for the analysis of transition motions in very large proteins (Jacoby et al., 1996; Daz et al., 1997; Ma and Karplus, 1997; Wrobloswki et al., 1997).

Figure 11 provides a summary of the order of the motions that take place in the transitions that we calculated but, as noted above, we cannot draw any conclusions about how fast each of these events occurs. The other main points that we would like to emphasize from our study are:

1. The motions, whether quaternary or tertiary, are extremely complex involving many, if not all, the atoms in the protein.
2. The movements of the subunits appear to occur in a synchronous or concerted fashion even though the TMD constraints do not require this.
3. The quaternary structure changes start immediately at the onset of the transition and finish when ~70% of the transition has been completed, whereas the tertiary structure changes continue throughout the transition.
4. The rotation of the regulatory dimers is uniform and concerted throughout the first 75% of the transition.
5. The relative translation between the catalytic trimers starts before and ends after their relative rotation. The translation appears to serve to destabilize the structure before the rotation can occur and can be related directly to the observations of Lipscomb (1992, 1994), who measured the effect that binding allosteric effectors has on the intertrimer distance (the relative rotations of the trimers being unchanged).
6. Most of the closing of the catalytic subunits’ CP and ASP domains occurs when the quaternary structure changes have almost finished. This can be linked to the results of mutant studies done on the closure of the catalytic domains (Dembowsi et al., 1990; Tauc et al., 1994) which show that the T → R transition can partially occur even if the catalytic domains are locked in an open conformation and that the catalytic activity of the E50A mutant is very weak. Our results suggest that the E50A mutant is an intermediate state (see Figure 11) in which the quaternary motions are nearly completely but the inhibition of closure of the catalytic domains prevents the appearance of a fully active catalytic state.
7. The regulatory domain opening occurs at the beginning of the transition. The disparity between the behaviours of the two interdomain angle motions is consistent with the ‘effector modulated transition’ model (Xi et al., 1991), which postulates a separation between the effects of binding of the allosteric effectors and the substrate. This is in agreement with the study of Tanner et al. (1993). Our results also suggest a structural explanation for the results of experiments on Y77F mutant proteins by Van Vliet et al. (1991) (see Figure 11).

Clearly, there is a lot more work to do even to be able to trace the structure of this complex problem. Currently we are completing the analysis of other transition pathways that we have calculated with a variety of alternative reaction path finding algorithms using the same molecular mechanical representation of the protein. These results will be presented in due course. In the near future we would like to improve the model that we use for the ATCase molecule and investigate more fully the effects that the binding of effector and substrate molecules have on the transition pathways that we obtain.

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References
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