

Computational Studies of Essential Dynamics of *Pseudomonas cepacia* Lipase

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Abstract

In order to investigate the interfacial activation of a lipase from *Pseudomonas cepacia* (PcL), molecular dynamics (MD) simulations and essential dynamics (ED) analysis were performed in different solvent environments: vacuum and explicit water solvents. Starting from the active (open) structure of PcL, the essential dynamics analysis of the simulations revealed large correlated motions, which may be responsible for the activation of the enzyme. Fluctuations in the U1 (active-site lid) and U2 domains are found to be important in the activation of PcL. In contrast, the catalytic triad exhibits very little displacement. These results are consistent with the previous X-ray structural determination. A combined analysis of the trajectories showed some differences for the simulations in different solvent environments. It was found that the region around the helix $\alpha 5$ showed larger displacements in the water simulations. It can be concluded that the open structure of PcL becomes unstable in water solvents, leading to the closing of the so-called 'lid' region. The simulations and ED analysis on the closed structure of PgL provided additional information concerning the structural changes involved in the activation of the lipases. It was found that structural changes for PcL and PgL, which are responsible for the essential motions of the protein, showed contrasting behavior in the different solvent environments.

Introduction

Triacylglycerol lipases, present in diverse organisms including animals, plants, fungi, and bacteria, catalyze the hydrolysis of triglycerides into free fatty acids and glycerol. A unique property of lipases is their enhanced activity at an oil-water interface, which is known as 'interfacial activation' (1). Lipases have been used as models for studying the mechanism of interfacial enzyme-catalyzed reactions. Considerable insight has been obtained from the X-ray studies for the crystal structures of many different lipases, both on their own and in complex with inhibitors (2).

Lipases are, in general, highly variable in size and the sequence similarity between them is limited to short spans located around the active-site residues. However, the three-dimensional structures of lipases, in their cores, share a common motif, known as α/β -hydrolase fold (3,4), which consists of an eight-stranded, mostly parallel β sheet flanked by six α helices, with a catalytic triad (Ser/Asp/Cys-His-Asp/Glu). In the so-called 'closed' structures, the catalytic triad is buried underneath a helical segment, called a 'lid' or a 'flap'. Crystallographic analyses of lipase-inhibitor complexes showed that, when an inhibitor is bound, the active site becomes accessible to the solvent and a hydrophobic surface is exposed by the movement of the lid (5,6). The conformational changes range from a simple rigid body hinge-type motion to complex reorganizations involving changes in the secondary structures. Generally speaking, the overall catalytic process involves adsorption of the enzyme from the bulk aqueous phase onto the lipid surface and subsequent catalysis at the interface (1). Previous structural studies suggest that the

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hydrophobic lipid-binding site is opened up by the rolling back of the lid away from the active site at an oil-water interface. However, even in the absence of an oil-water interface, there may be a subtle equilibrium between the two conformations of the enzyme. It is believed that the opening of the lid is essential but not sufficient to explain the interfacial activation (2).

Many mechanisms have been proposed for the activation of lipases at a lipid-water interface. A surface-mediated mechanism ('substrate theory') suggests that a pre-existing surface structure and/or different mesophases and crystalline phases of the lipid substrate may have considerable influences on the behavior of lipolytic enzymes (2). An 'enzyme theory' emphasizes the conformational changes in the enzyme upon adsorption onto the interface (7). Theoretical studies of the catalytic process based on both perspectives have been done by Peters *et al.* (8-11). As shown by these studies, the two models are merely conceptual extremes and are not mutually exclusive. The interfacial character of the lipid/water phase as well as the distribution of charged residues on the enzyme surface influences the lipase action.

The crystal structure of a lipase from *Pseudomonas cepacia* (PcL) has been determined in the absence of a bound inhibitor (12). The enzyme shares several structural features with homologous lipases from *Pseudomonas glumae* (PgL) (13) and *Chromobacterium viscosum* (CvL) (14). The structure of PcL revealed a highly open conformation with a solvent-accessible active site. This is in contrast to the structure of PgL or CvL in which the active site is buried under a closed or partially opened 'lid', respectively. These structures suggest that the interfacial activation of lipase involves the reorganization of secondary structures and a large movement of the lid to expose the active site.

Analyses and comparisons of X-ray structures provide important but predominantly static information about enzyme dynamics. The actual motion of the enzyme can only be studied by analyzing protein dynamics. Molecular dynamics (MD) simulations can describe the dynamics of various biomolecules including enzymes. In particular, the essential dynamics (ED) method has been proven useful in extracting large concerted atomic motions from a finite-duration MD trajectories, which are supposed to be related with the function of enzymes (15-19). Investigations of protein dynamics in collective coordinate space have also been done by others (20-24). In the present study, we have performed the ED analysis on the MD simulations for PcL. The main focus is on the examination of the motion of flexible loops in the enzyme, which is responsible for the opening of the active-site lid. The effect of a lipid interface on the activation of the enzyme may be inferred from the changes in dynamics in different solvent environments.

Method

Model and Simulation Details

The high resolution crystal structure of a triacylglycerol lipase from *Pseudomonas cepacia* (PcL) was used as a model for the active form of lipase. Figure 1 shows the topology diagram of PcL. MD simulations were performed using the GROMOS program (25). Simulations were performed for different solvent environments. Vacuum simulations were done using very small dielectric constant ($\epsilon_s=1$) for the continuum medium. Simulations including explicit solvent molecules were also done in a truncated octahedral box filled with 8856 SPC water molecules. A cut-off distance of 8Å was used for the non-bonded interactions and the long-range electrostatic interactions were truncated at 10Å. The SHAKE algorithm (26) was applied to constrain bond lengths. Simulations were performed at 298K with a time step of 2 fs and the equations of motion were solved using the leap-frog algorithm (27). The temperature of the system was controlled by use of a weak coupling to an external bath, using the method of Berendsen *et al.* (28) with the coupling constant of 0.1 ps.

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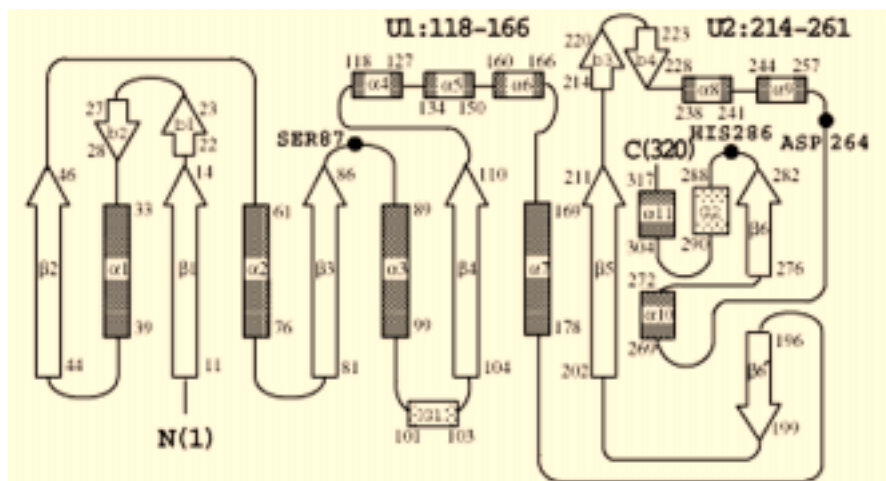


Figure 1: Topology diagram of PcL. The secondary structures are defined and labeled as in ref. 12; β strands are shown as arrows, α helices and 3_{10} helices (G1 and G2) are shown as rectangles. The three residues in the catalytic triad, Ser87, Asp264, and His286, are marked as black circles.

Simulations were started from the X-ray structure of the active (open) form of PcL and subjected to a steepest descent energy minimization to relax possible strains in the model. The minimization was followed by a period of equilibration MD simulation, for which the initial velocities were taken from a Maxwellian distribution. Simulations were continued for 1 ns, whose trajectories were used for analysis including essential dynamics method. The length of the simulations is known to be sufficient for the ED analysis. The stability of a simulation was examined by monitoring energies and geometrical properties of the system.

Essential Dynamics Analysis

The ED method (15) is based on the diagonalization of the covariance matrix built from atomic fluctuations in an MD trajectory from which overall translations and rotations have been removed:

$$C_{ij} \equiv \langle (x_i - x_{i,0})(x_j - x_{j,0}) \rangle, \quad [1]$$

where x_i are separate Cartesian coordinates of the atoms with corresponding average values denoted by $x_{i,0}$. $\langle \rangle$ represents an average over the whole MD trajectory. Diagonalization of the covariance matrix yields a set of eigenvectors and eigenvalues, which are sorted by the size of eigenvalues. The eigenvectors indicate directions in the total configuration space, representing correlated displacements of groups of atoms in the system. The corresponding eigenvalues indicate the total mean square fluctuations, i.e., the amplitude of the correlated motions, along these directions. The basic idea of essential dynamics is that only the correlated motions represented by the eigenvectors with large corresponding eigenvalues are important in describing the overall motion of the protein, closely related with its specific function. The ED method has been found to be useful for revealing functionally significant fluctuations in various protein systems.

A useful improvement in the ED analysis is the so-called ‘combined analysis’ (16). MD trajectories of similar systems are concatenated to form one big trajectory and a covariance matrix is constructed. The resulting eigenvectors now indicate concerted motions of atoms, which are common in the separate trajectories. The separate trajectories are then projected onto the common eigenvectors and the properties of these projections are compared to investigate the dynamic differences. The mean square fluctuation in the projection reveals a difference in dynamics along the direction described by the eigenvector. The ED analysis was carried out using the WHAT IF modeling program (29).

Results and Discussion

Simulations on Different Solvents

The main focus of the present study was to examine the so-called ‘interfacial acti-

vation' of lipases. We started with the 'active' (open) structure of PcL as revealed by the previous X-ray study (12). The relative stability of such an open form of lipase is expected to vary depending on the different solvent environments: hydrophobic or polar (aqueous). Starting from the open structure of PcL, we have carried out MD simulations for a vacuum ($\epsilon_s = 1$) condition and explicit water solvents. Vacuum simulations may be considered to model a lipase at interface, i.e., under hydrophobic environment. The ED analysis of the simulations in various solvent environments is likely to reveal the large correlated motions important for the activation of the lipase.

The stability of the simulations was checked by computing several structural properties. Using the stable trajectories, the main analysis performed in this study is the determination of the essential dynamics properties. As in other studies, only a few eigenvectors are found to represent the essential motions in the protein. For the vacuum simulations, the components of the first six eigenvectors as a function of residue number are shown in Figure 2. The eigenvectors show that there are some regions of the protein which are more flexible (larger displacements) than others. In the figure, we indicated two domains: U1 domain (residues 118-166) and U2 domain (residues 214-261), which are supposed to play an important role in the activation of the lipase. The U1 domain, composed of three α helices, forms the 'lid', the opening of which signals the activation of the lipase. The U2 domain, composed of two antiparallel β strands and two α helices, also plays a role in the opening of the active site. The eigenvectors clearly show that movements of these two domains represent essential motions of the protein.

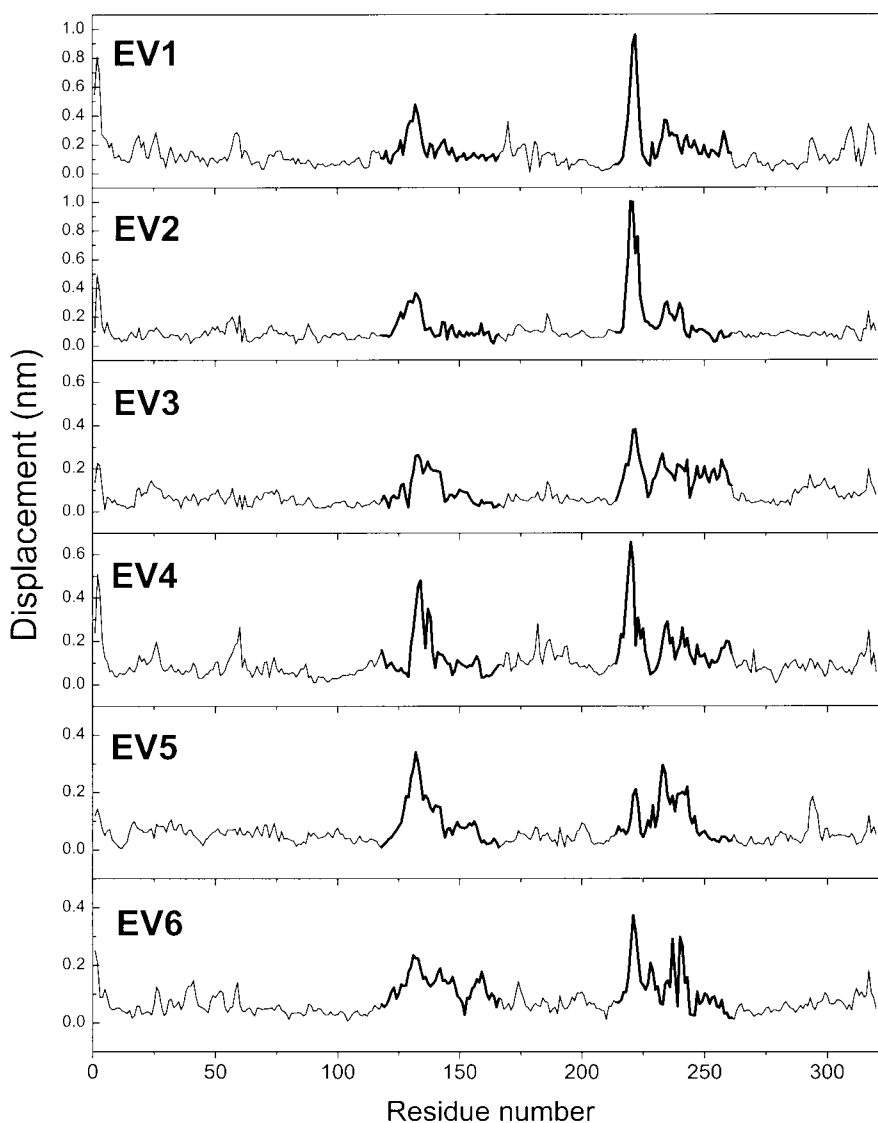


Figure 2: Absolute value of the components of the first six eigenvectors (EV1-EV6) obtained from the C_α coordinate covariance matrix of the trajectories of the vacuum simulations as a function of residue number. U1 domain (residues 118-166) and U2 domain (residues 214-261) are highlighted with thick solid lines.

The components of the first six eigenvectors for the explicit water solvent simulations are displayed in Figure 3. The eigenvectors show that the U1 and U2 domains describe main components of essential motions. The relative importance of the two domains in the essential dynamics is more pronounced in the explicit water solvent simulations. The water simulations also indicated some other regions of the protein with important concerted motions.

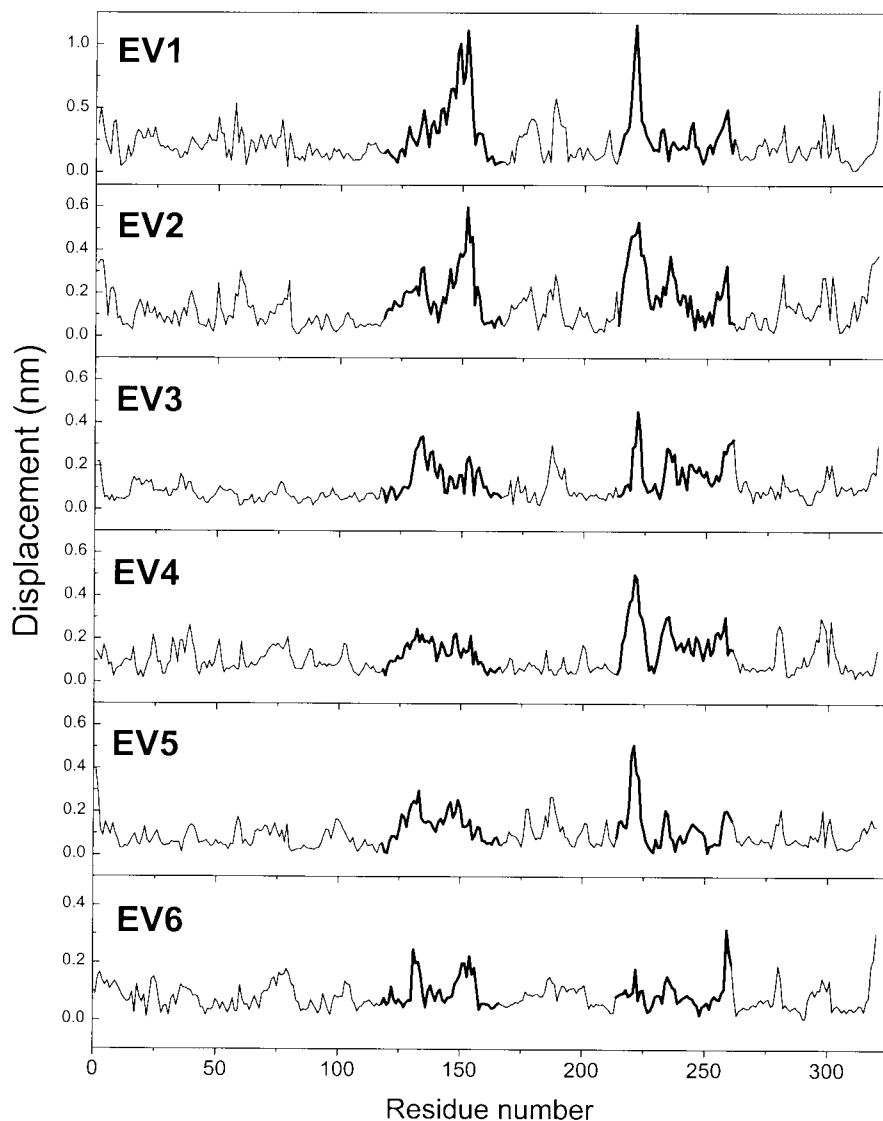


Figure 3: Absolute value of the components of the first six eigenvectors (EV1-EV6) obtained from the C_{α} coordinate covariance matrix of the trajectories of the explicit water solvent simulations as a function of residue number. U1 domain (residues 118-166) and U2 domain (residues 214-261) are highlighted with thick solid lines.

Figure 4 shows the displacements as a function of residue number averaged over the first six eigenvectors for the vacuum and the water solvent simulations. The overall structure of the deviation is similar for the two simulations, although there exist some differences in the detailed features. As discussed above, the U1 and U2 domains represent the major components of the essential motions of the lipase. In particular, the loop region between helices α_4 and α_5 (residues 128-133) and that between strands b3 and b4 (residues 221-222) show larger deviations. We suggest that these two regions play important roles in the flexibility of the U1 and U2 domains. The flexibility of the loop region between helices α_4 and α_5 indicates that rigid body motions of these helices are responsible for the opening and closing of the lid.

Most notable difference between the vacuum and the water solvent simulations is in the region of residues 135-155, which are located at the helix α_5 and its vicinities. The helix α_5 shows larger displacements in water solvent simulations compared with vacuum environments. This suggests that the movement of helix α_5 ,

Figure 4: Absolute value of the components averaged over the first six eigenvectors as a function of residue number for the vacuum (dashed-line) and the water solvent (solid line) simulations. U1 domain (residues 118-166) and U2 domain (residues 214-261) are indicated inside the figure. Solid circles represent the catalytic residues (Ser87, His286, and Asp 264). The inset shows the displacements in the 'oxyanion loop' region (residues 17-27): solid circles for the water simulations and solid triangles for the vacuum simulations.

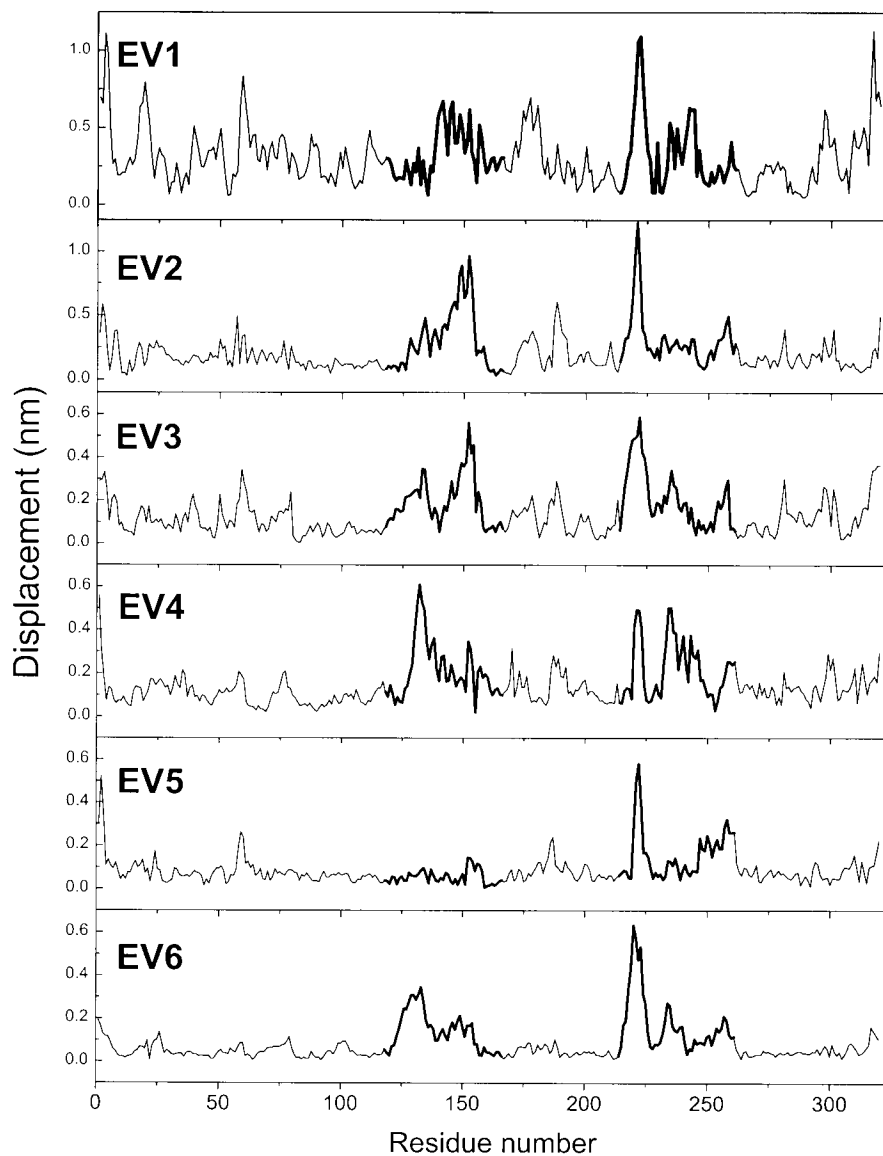
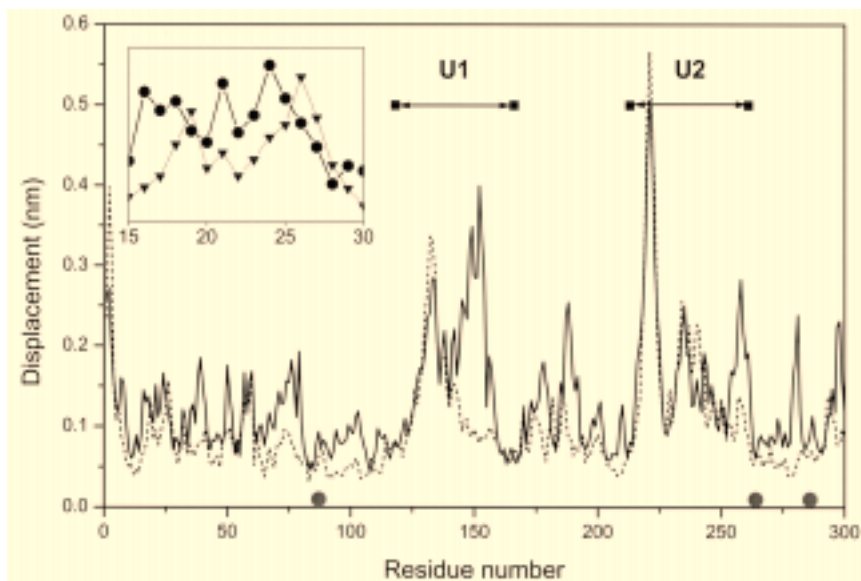
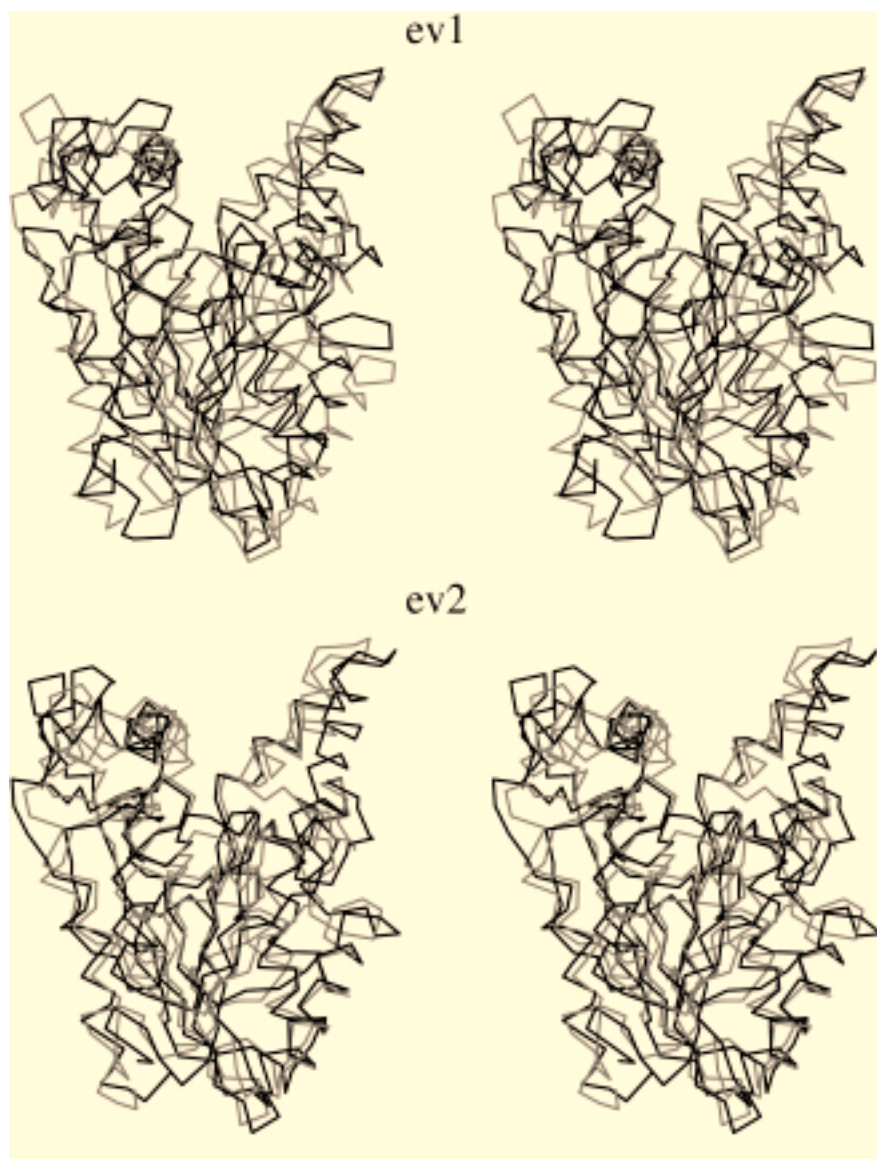


Figure 5: Absolute value of the components of the first six eigenvectors (EV1-EV6) obtained from the C_{α} coordinate covariance matrix of the concatenated trajectories of the two different simulations as a function of residue number. U1 domain (residues 118-166) and U2 domain (residues 214-261) are highlighted with thick solid lines.

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leading to the opening or closing of the active site, is essential for the activation of the lipase. The open (active) form of PcL is supposed to be stable in the hydrophobic interface as represented by vacuum environment. The open structure becomes unstable in polar (aqueous) solvents, resulting in the closing of the lid (helix $\alpha 5$). Another interesting observation is that the catalytic triad (Ser87, His286, and Asp264) shows very little displacement. The displacements in the 'oxyanion loop' region (residues 17-27) are not as large as in other regions such as U1 and U2. The region around the oxyanion-forming residue Leu17 shows some difference in vacuum and water solvent simulations (see the inset of Figure 4).

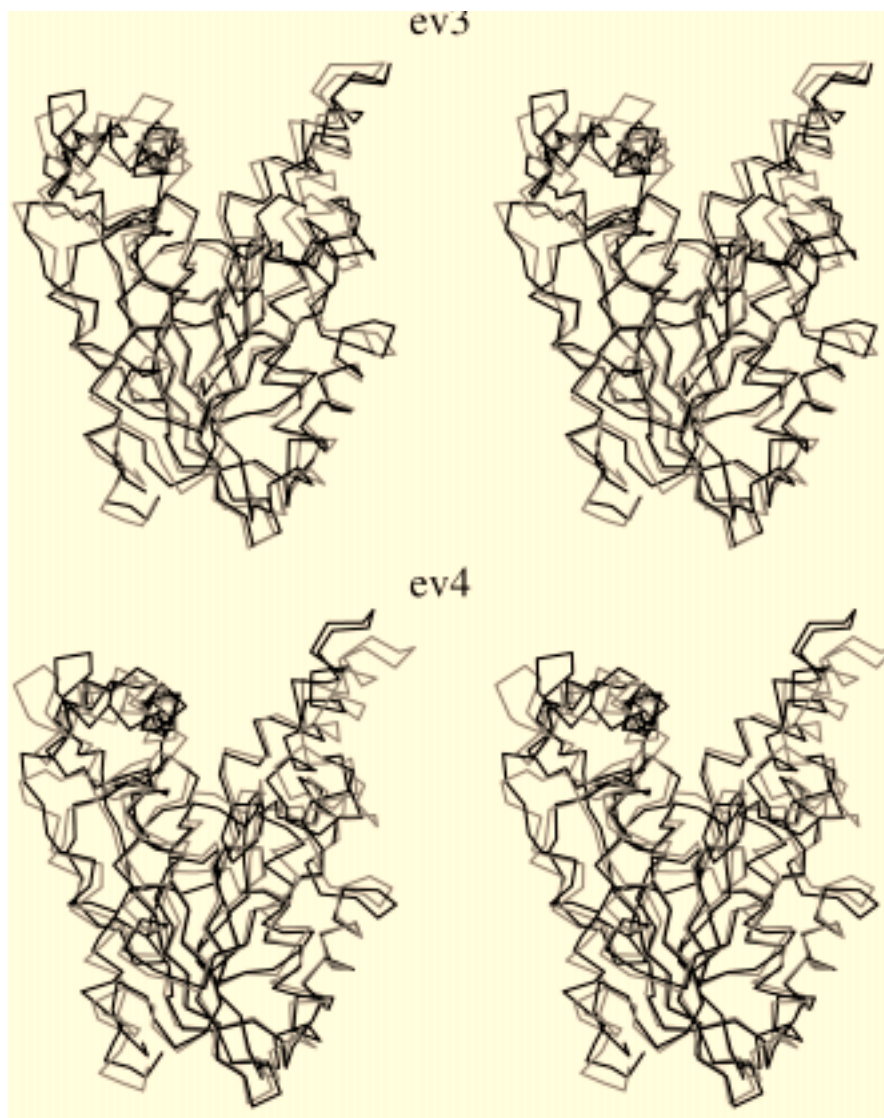
The above ED analysis is consistent with the X-ray results (12). By comparing the activated structure of PcL with the inactive structure of PgL, the following observations have been made: (i) the largest difference between PcL and PgL structures occurs for residues 130-166 (U1 domain); (ii) the C-terminal side of $\alpha 4$ and the N-terminal side of $\alpha 6$ (in PgL) are melted into loops in PcL; (iii) the strand $\beta 6$ in PcL is characterized by being relatively longer than its equivalent in PgL; (iv) an intermolecular interaction with the U1 domain of the symmetry-equivalent molecule induces a small conformational change in the β hairpin of residues 219-222.



6a

Figure 6: Representation (stereo pictures) of the motions found in the first six eigenvectors (EV1-EV6) obtained from the C_{α} coordinate covariance matrix of the concatenated trajectories of the two different simulations. For each eigenvector, the MD structures with the minimum and the maximum projection are shown. The regions around the U1 and U2 domains are indicated in the picture for eigenvector 6.

6b

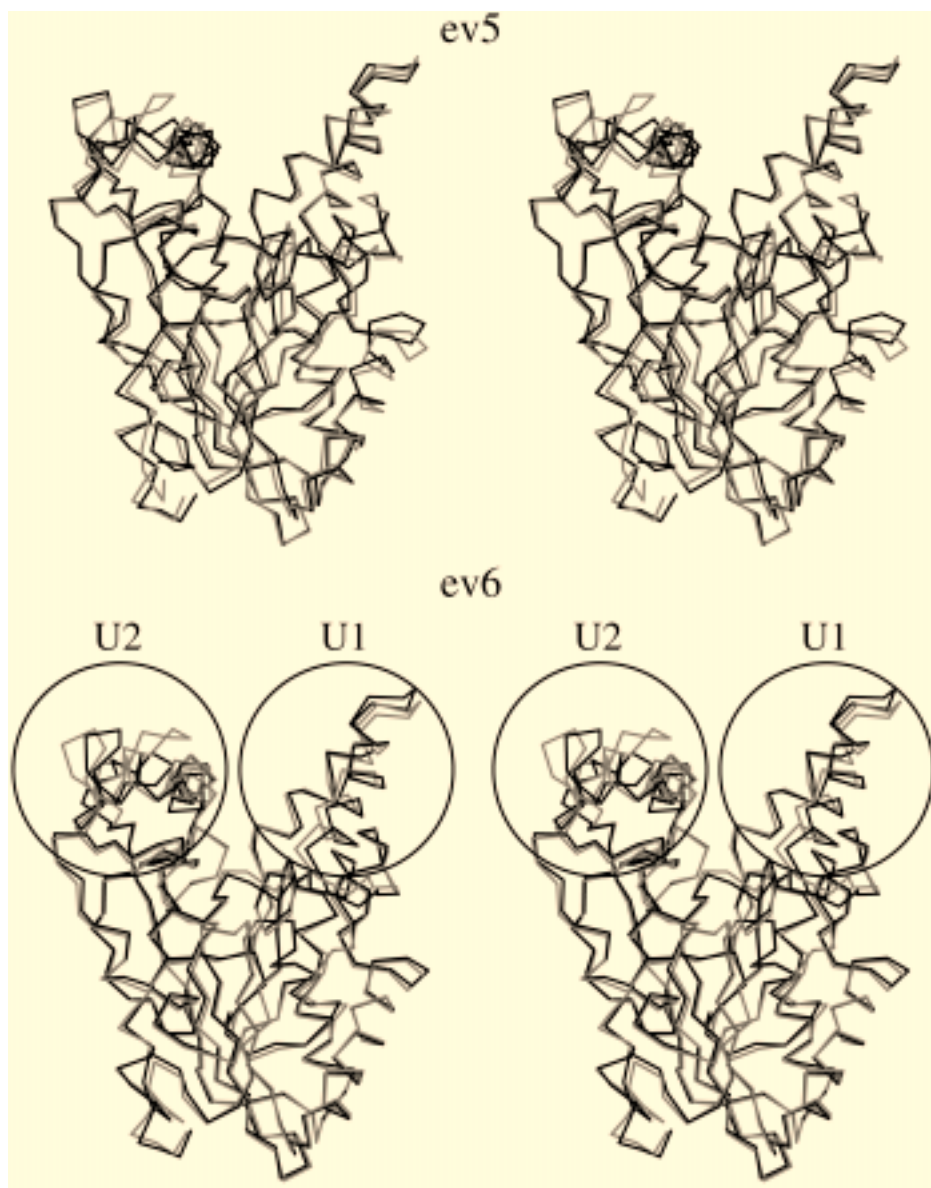


Combined Analysis

To investigate the differences in dynamics for the different solvent environments, we have performed a ‘combined’ ED analysis. The covariance matrix was constructed from the C_{α} coordinates for the concatenated trajectories of two 1 ns simulations for vacuum and water solvent. The components of the ‘combined’ eigenvectors as a function of residue number are shown in Figure 5. The overall features of the combined eigenvectors are consistent with the results obtained from the ED analysis of separate simulations. The essential motions described by the first six combined eigenvectors are illustrated in Figure 6. It is noticeable that large displacements of the U1 and U2 domains are observed for several eigenvectors.

Differences in the essential subspace can be studied by projecting the separate trajectories onto the combined eigenvectors. The averages and the mean square fluctuations of these fluctuations as a function of eigenvector indices are displayed in Figure 7. The average projection shows differences in equilibrium structures for different simulations, whereas the mean square fluctuation can be used to study differences in dynamics. A large difference is observed in the average value of projection of eigenvector 1 corresponding to the displacement of the whole protein. It can be suggested that the equilibrium structures of PcL are different in vacuum environments and water solvents. There are differences in the mean square fluctuations for the first few eigenvectors. Although there is a large shift in equilibrium structure along the first eigenvector as discussed above, the fluctuations along this

6c



direction in the two separate trajectories are similar. There is a significant difference in fluctuation in the direction described by the second and third eigenvectors in the combined analysis. Contributions from U1 and U2 domains, in particular the region around the helix $\alpha 5$, are clearly found to be dominant for these dynamically important eigenvectors (see Figure 5).

Comparison with PgL Simulations

For a comparison, MD simulations were done on the closed form of lipase from *Pseudomonas glumae* (PgL) (13). When the sequences of PcL and PgL are compared, there are fifty different residues between them in addition to one insertion. Despite similar assignments of secondary structures to both lipases, there are some large and significant differences in the tertiary structures of the two enzymes (12). The largest difference between PcL and PgL was observed for residues 130-166, which encompasses the two helices $\alpha 5$ and $\alpha 6$ of the U1 domain. In PgL, the U1 domain fully buries the active site, whereas in PcL the active site is highly open to the solvent by the opening of the helical lid.

Starting from the closed structure of PgL, MD simulations for different solvent environments and subsequent ED analysis were done as in the case of PcL. In Figures 8 and 9, the displacements as a function of residue number averaged over

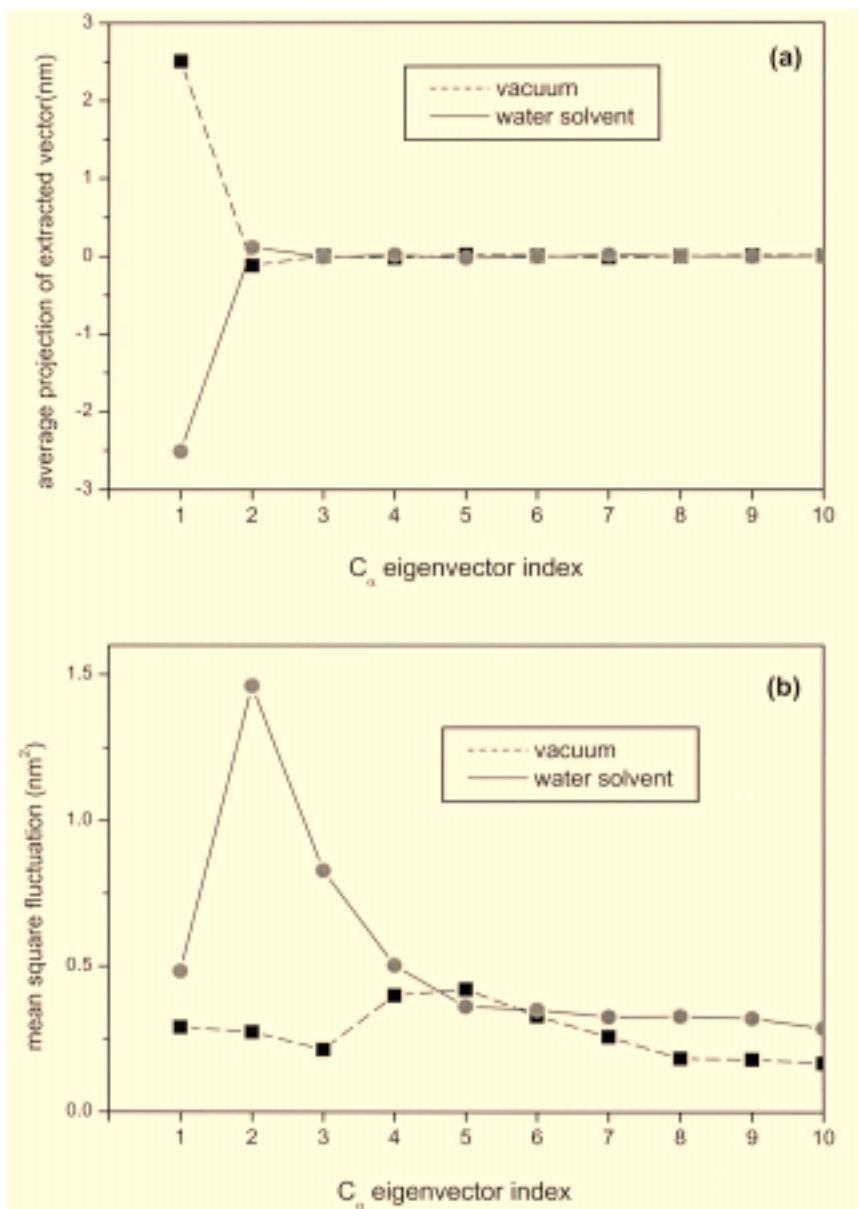


Figure 7: (a) Average values of the projections of the separate trajectories onto the combined eigenvectors obtained from the concatenated trajectories as a function of the eigenvector index. (b) Mean square fluctuations of the projections of the separate trajectories onto the combined eigenvectors obtained from the concatenated trajectories as a function of the eigenvector index.

the first six eigenvectors obtained from the simulations of PcL and PgL are compared for the vacuum and the water solvent simulations, respectively. The overall pattern of displacements for PgL is similar to that of PcL. In particular, the U1 and U2 domains are found to represent the major components of the essential motions of PgL. It is interesting to note that the relative flexibility for PcL and PgL structures show opposite behavior in the vacuum and the water solvent environments. The closed structure of PgL showed larger displacements in vacuum simulations (Figure 8), while the open structure of PcL exhibited larger displacements in water solvents (Figure 9). The distinct differences in flexibility for PcL and PgL can be noticed in the U1 domain, which is responsible for the opening/closing of the lid. Another notable difference is found in the region starting from the C-terminal side of U1 domain to the region just prior to the U2 domain, where the closed structure of PgL showed enhanced fluctuations. This result suggests that the structural properties in this region may play a role in the interfacial activation of the lipases.

Conclusions

We have performed an ED analysis to study the concerted motions in a triacylglycerol lipase from *P. cepacia* simulated in vacuum and water solvent. The main motivation was to obtain a detailed understanding of the activation mechanism for

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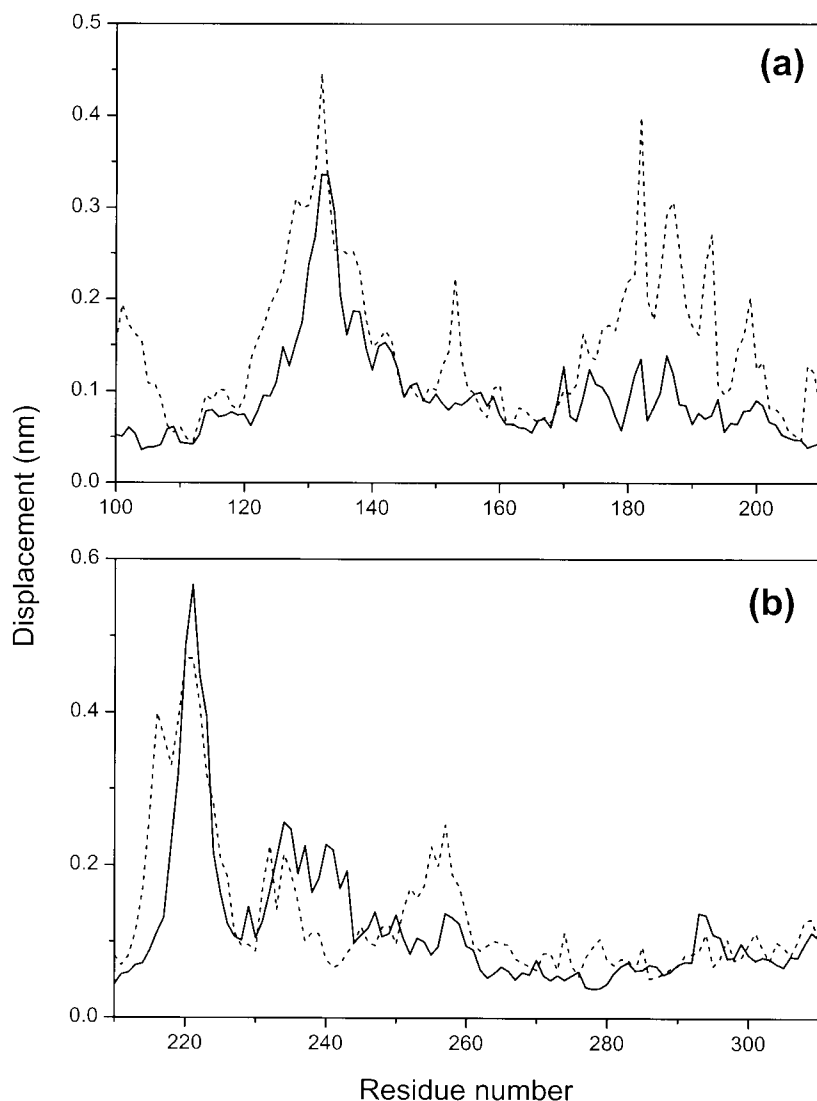


Figure 8: Absolute value of the components averaged over the first six eigenvectors as a function of residue number, obtained from the vacuum simulations for the PgL (dotted-line) and the PcL (solid line) structures. The regions around the U1 domain and U2 domain are shown separately: (a) residues 100-210; (b) residues 210-310.

the lipase. Starting from the active (open) structure of PcL, the ED analysis of the simulations in various solvent environments revealed large correlated motions likely to be responsible for such activation.

Our results indicate that fluctuations in the two regions, U1 domain (active-site lid) and U2 domain, are important in the activation of PcL. In particular, the loop between helices $\alpha 4$ and $\alpha 5$ in U1 domain and that between strands b3 and b4 in U2 domain showed extensive flexibility. In contrast, the catalytic triad exhibited very little displacement. These results are consistent with the observations from the X-ray structural determination of the same lipase.

A combined analysis of the trajectories showed some differences for the simulations in different solvent environments. Vacuum and explicit water solvent simulations showed similar behaviors concerning the flexibility of U1 and U2 domains. There are subtle differences in the detailed ED for the two simulations, which may provide information about ‘interfacial activation’ of the lipase. Most noticeable difference is a large displacement of the region around the helix $\alpha 5$ in the water simulation. It can be argued that the open structure of PcL becomes unstable in polar (water) solvents, leading to the closing of the so-called ‘lid’ region.

The simulations and ED analysis on the closed structure of PgL provided consistent results with the above discussions. It is also noted that PcL and PgL structures showed different flexibility in the U1 domain and the loop regions between the U1

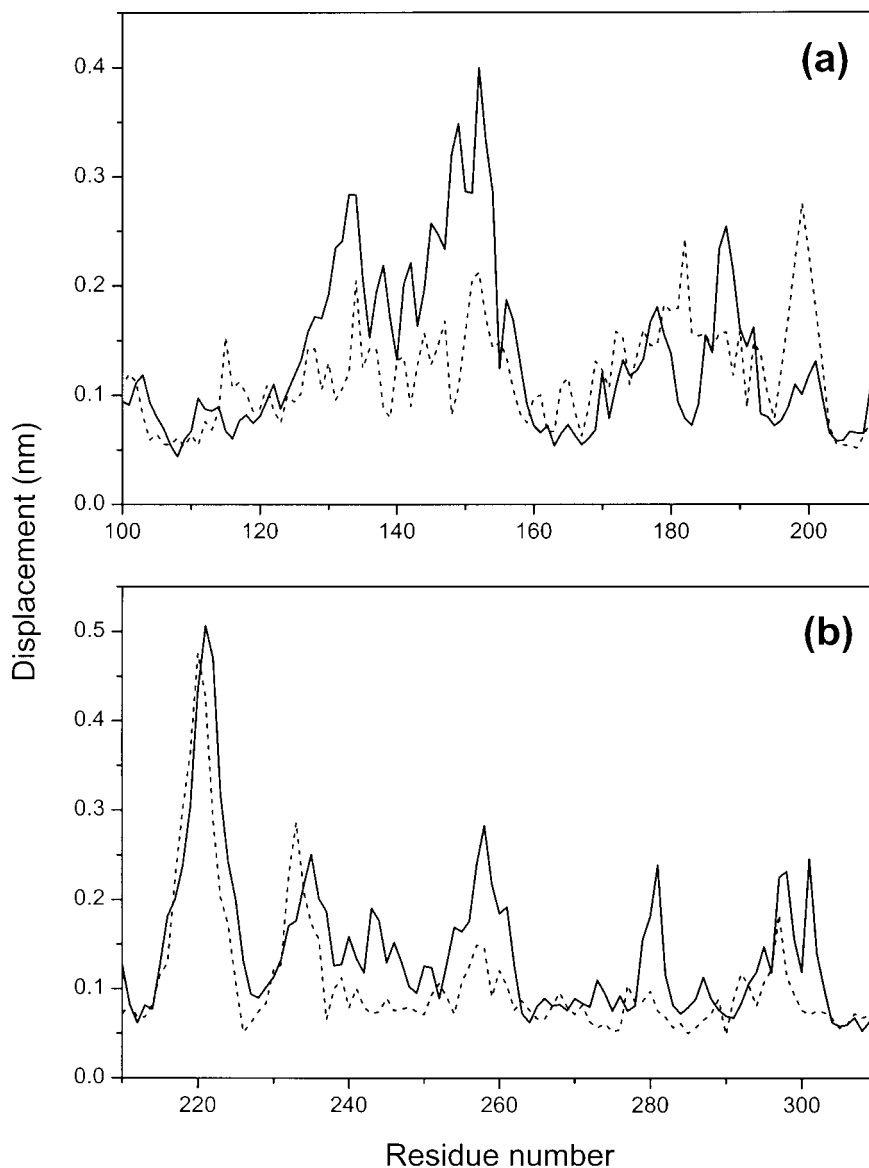


Figure 9: Absolute value of the components averaged over the first six eigenvectors as a function of residue number, obtained from the water solvent simulations for the PgL (dotted-line) and the PcL (solid line) structures. The regions around the U1 domain and U2 domain are shown separately: (a) residues 100-210; (b) residues 210-310.

and U2 domains. The relative flexibility of the two structures in these regions was found to be dependent on the surrounding solvents. In vacuum environments, PgL showed relatively large displacements in these regions (Figure 8(a)). It can be argued that the correlated movements of these regions are likely to be related to the opening of the lid and subsequent activation of the lipase. The behavior is reversed for the PcL structure (Figure 9(a)). It can be concluded that structural changes for PcL and PgL, which are responsible for the essential motions of the protein, are to be induced in the different solvent environments.

It may be argued that the analysis of the essential dynamics is not in itself a direct proof for the activation mechanism of the lipase. However, the present study provides useful information on the characteristics of flexible regions closely related with functionally relevant motions. Possible scenarios for the activation of the lipase can be inferred from the changes in the dynamical behavior of these regions for the active and inactive structures under different environments.

Acknowledgments

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