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Identifying key electrostatic interactions in *Rhizomucor miehei* lipase: the influence of solvent dielectric*

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Abstract. The conformational change associated with the interfacial activation of *Rhizomucor miehei* lipase involves the displacement of an α-helical lid (residues 82–96) away from the active site on moving from water (high dielectric) to lipid (low dielectric). The presence of two media of very different dielectric properties suggests that electrostatic interactions play an important role in this process. We have used linearized Poisson–Boltzmann calculations to examine the key electrostatic interactions which contribute to lid stability in the closed and open states. It is the two charged residues of the lid, Arg86 and Asp91, that form the strongest electrostatic interactions with the rest of the protein. We identify key residues whose interactions with the lid are significantly perturbed by the change in the dielectric of the medium: Asp61, Arg80, Lys109, Glu117 and the active-site residues Asp203 and Asp256, all of which lie within approximately 20 Å of the lid. We suggest that these residues are good candidates for site-specific mutation studies, which could help elucidate their role in the lipase activation mechanism.

Key words: *Rhizomucor miehei* lipase – Interfacial activation – Conformational change – Electrostatic interaction energy – Poisson–Boltzmann calculation

1 Introduction

Lipases (EC 3.1.1.3) are important industrial enzymes which catalyze the hydrolysis of fats and oils and can also play a role in the synthesis of various useful esters (e.g. cocoa butter, surfactants) [1]. Lipase activity increases significantly at the interface of lipid and water compared to a water solution [2]. This interfacial activation is associated with a conformational change in the lipase, where a lid region consisting of a single or multiple α-helices or loops rotates around its hinge regions to accommodate the substrate [3]. Crystal structures of *Rhizomucor miehei* lipase (RmL) in the closed and ligand-bound open conformations [4, 5] have revealed that the lid consists of an α-helix (residues 85–91) and two hinge regions (residues 83–84 and 91–95). This conformational change is unusual because unlike most enzymes where the active site becomes closed when a substrate is bound, in lipases the active site is opened when a ligand is bound [6]. Lipase activation generally occurs at the interface of water and lipid, media with very different dielectric properties [2].

Crystallographic studies on *Pseudomonas cepacia* lipase suggest that the lid motion is associated with changes in the dielectric of the medium [7]. Several computational studies have emphasized the importance of electrostatic interactions in the activation mechanism of lipases [8–11]. Other mechanisms have been proposed, including the ''substrate'' theory which postulates that the surface structure of the lipid determines the activation [12–15]. One strand of our investigations into the mechanism of the conformational change in RmL is to examine the electrostatic interactions of the enzyme. Of the residues comprising the lid, only two are charged, namely Arg86 and Asp91. In the closed state, both Arg86 and Asp91 are fully solvent-exposed and are probably well-hydrated around their side chain polar atoms. In the open state, both side chains are partially buried: the accessibilities of the side chain terminal atoms of these two residues to a solvent sphere of radius 1.4 Å decrease by 85–90% with respect to their respective accessibilities in the closed state. The Arg86 side chain polar atoms are stabilized by the backbone carbonyls of Leu58 and Ile59, while the side chain of Asp91 is involved in a hydrogen bond with the side chain oxygen of Ser82. This, coupled with the notion that the conformational rearrangement upon changes in the solvent dielectric primarily involves the motion of this lid, leads us to believe that the electrostatic interactions of the lid must be involved in the stability and/or the

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activation of this functionally important motion [16]. In this work we explore the effect of varying the solvent dielectric on inter-residue interactions between the lid and the rest of the lipase. This was performed by computing electrostatic interaction energies by solving the linearized Poisson–Boltzmann (PB) equation [17]. The effect of the dielectric on these interactions was investigated by comparing the energies obtained using dielectric constants of 4 and 78 for solvent to model lipid and water environments, respectively. Calculations were performed separately for the closed and open conformations in order to identify interactions which stabilize/destabilize either state upon changing the dielectric of the medium.

2 Model and calculations

Crystal structures of the closed conformation of RmL, Protein Data Bank (PDB) entry 3tgl [18], and the open conformation with an inhibitor bound, PDB entry 4tgl [19], were obtained from the Brookhaven PDB. The inhibitor was removed from the open RmL and only the buried crystallographic water molecules were retained (35 and 26 water molecules in the closed and open states, respectively). The structures were modelled using the CHARMM extended atom force field [20, 21], assuming neutral pH. A more detailed description of the preparation of the starting structures has been outlined elsewhere [11]. Electrostatic interaction energies were computed for both the closed and open conformations using a dielectric constant of 2 for the protein, and either 4 (“lipid”) or 78 (“water”) for the solvent. Charges and atomic radii were assigned from the QUANTA96 parameter sets [22]. The philosophy of the calculations was similar to that reported by Plou et al. [23]. In each calculation the charge of a residue(s) was set to zero, and the electrostatic potential due to the rest of the lipase was calculated at the site of each atom of the residue(s) by using the program UHBD (version 5.1) [24, 25] to solve the linearized PB equation by finite differences. The linear PB approach is appropriate under conditions of low charge density of solute and/or low salt concentrations [26]. The electrostatic interaction energies were obtained by multiplying the potential at the site of each atom by the charge of that atom and summing the interaction energies for that residue [26, 27]. In each calculation the temperature was set to 300 K and the ionic concentration to zero. Accuracy was improved by finite difference focussing [28, 29] and dielectric boundary smoothing [30]. Focussing was applied such that in the first calculation the grid spacing was set to 4 Å leading to a cubic grid of length 600 Å around the system. This was followed by two focussing runs, with the grid spacing decreased to 2.0 Å and then to 0.1 Å.

3 Results and discussion

Electrostatic interactions of the lid (residues 82–96) with the rest of the lipase were investigated by performing electrostatic interaction energy calculations for the closed and open conformations of RmL. We first computed the interaction of each residue of the lid with the rest of the lipase and then considered the lid as one entity and computed the electrostatic interaction energies between the whole lid and the rest of the lipase. The effect of the solvent medium was taken into account by using dielectric constants of 4 and 78 for the solvent to represent “lipid” and “water” environments, respectively. The difference in the electrostatic interaction energies between the two solvent models was computed. For the closed conformation, the energy difference was calculated for the transfer from “water” to “lipid” and for the open conformation from “lipid” to “water”. This is based on the assumption that the lid opens up when the lipase is adsorbed at a lipid interface and closes in an aqueous environment. Thus, for both the open and closed states, a positive energy difference indicates a destabilization of the interaction and a negative energy difference indicates a stabilization of the interaction with respect to the stated change of dielectric constant.

Among the electrostatic interactions of the individual residues of the lid with the rest of the lipase, it is clear that the interactions of Arg86 and Asp91 dominate (Fig. 1). These residues experience large changes in their electrostatic interaction energies when the solvent dielectric is changed from that of water to lipid. When the interactions of the entire lid with individual residues of the rest of the lipase are considered, our calculations reveal that both short- and long-range electrostatic interactions are perturbed upon a change of the dielectric (Fig. 2, Table 1). The largest dielectric-dependent changes ($\sim 5\, k_B T$) are observed for residues Asp203,

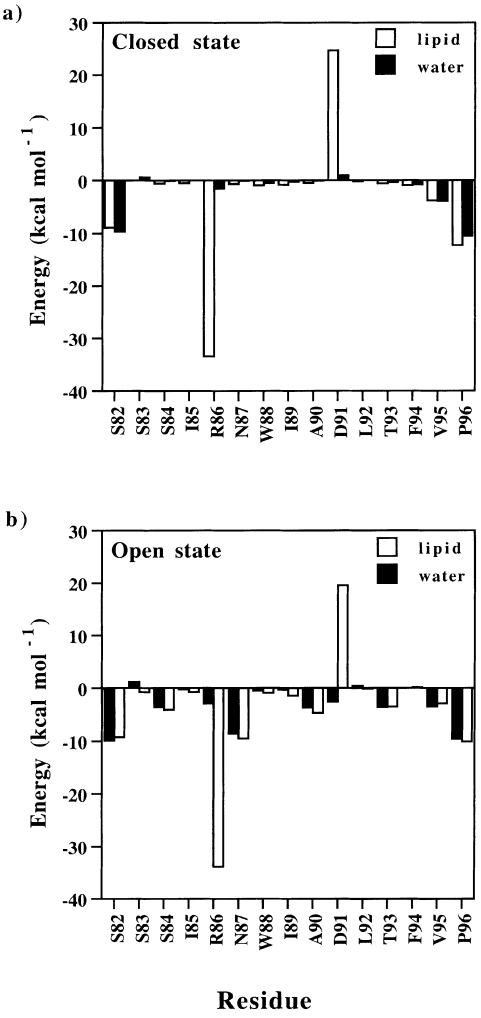

Fig. 1. Electrostatic interaction energies of individual residues of the lid with the rest of the *Rhizomucor miehei* lipase (RmL) for the **a** *closed* and **b** *open states* (the final grid size for focussing was 0.5 Å to accommodate the whole lid)

Asp256, Asp61, Lys109, Glu117 and Arg80, all of which are located in the proximity (~20 Å) of the lid region (Fig. 3). Asp203 belongs to the catalytic triad and Asp256 is located next to the catalytic histidine (His257); these residues are in the region which is in close proximity to the lid in the closed state, which we term region C (see Fig. 3). Residues Asp61, Lys109 and Glu117 are in a region which is in close proximity to the lid in the open state, which we term region O (see Fig. 3). Arg80 is located close to the first hinge (residues 83–84) of the lipase, around which the lid rotates during the conformational change. We now analyze the effect of the change in the dielectric medium by considering the changes in electrostatic interaction energies of these key residues and their location in region C or region O.

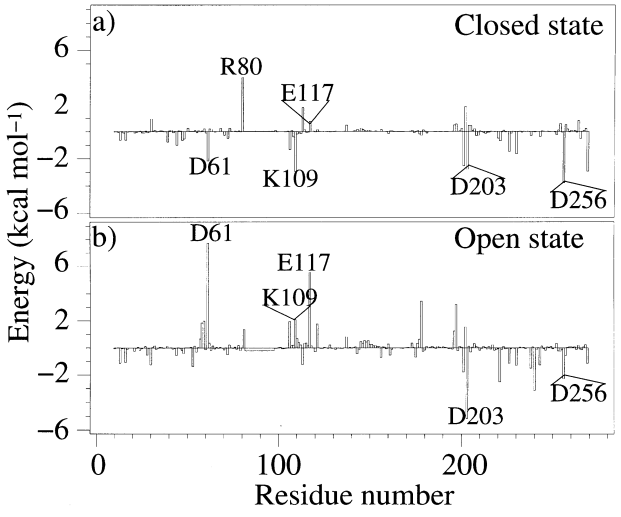

Fig. 2. Differences in electrostatic interaction energies between the lid and the rest of the RmL, when **a** the *closed state* is transferred from "water" to "lipid" and **b** the *open state* is transferred from "lipid" to "water"

For the two residues in region C, Asp203 and Asp256, the attractive electrostatic interactions with the lid in the closed state are enhanced when the medium is changed from "water" to "lipid" (Table 1), indicating an increase in stabilization of the closed conformation. Conversely, in the open state, the interactions between these two residues and the lid are strongly repulsive in "lipid", and become screened in "water", leading to destabilization of the open conformation. This conformation-dependent change from attractive to repulsive interactions can be explained in terms of the orientations of Arg86 and Asp91 in the closed and open states (Fig. 3). In the closed state, the side chain of Arg86 points towards that side of the lipase where Asp203 and Asp256 are located, whereas in the open state it points in the opposite direction (involving a ~20 Å displacement of Arg86 from the two aspartic acids). The side chain of Asp91 points towards solvent in the closed state and towards the active site in the open state (involving a ~4 Å displacement of Asp91 from the two aspartic acids). This, coupled with the partial burial of Asp91 in the open state, leads to increased repulsions with the two aspartic acids in the open state. Further detailed analysis revealed (data not shown) that the balance between attraction with Arg86 and repulsion with Asp91 largely determines the interactions between the lid and Asp203 and Asp256.

The three residues in region O, Asp61, Lys109 and Glu117, make significant contributions to the electrostatic stabilization of the open state in "lipid" (Table 1). Residues Asp61 and Lys109 form attractive electrostatic

Table 1. Electrostatic interaction energies of the lid (residues 82–96) with the non-lid residues computed for the closed and open conformations of *Rhizomucor miehei* lipase. For the closed conformation, the difference in electrostatic interaction energy is shown for the transfer from "water" ($\varepsilon = 78$) to "lipid" ($\varepsilon = 4$); for the open conformation the difference is from "lipid" to "water". Only interactions with changes greater than k_BT in magnitude in both conformations are listed. Key residues where one of the differences is approximately 5 k_BT are shown in *bold*. *D* is the distance in Å between the centroid of the lid and the centroid of the residue; E_W and E_L are the values of the interaction energies in "water" and "lipid", respectively; $E_L - E_W$ and $E_W - E_L$ are the differences in the interaction energies. All energies are in kcal/mol

	Closed state				Open state			
	D	E_L	E_W	$E_L - E_W$	*D*	E_W	E_L	$E_W - E_L$
Glu13	29.8	−0.7	0.0	−0.7	31.9	0.1	1.2	−1.1
Glu16	28.0	−0.7	0.0	−0.7	29.7	0.1	1.1	−1.0
Arg30	17.6	1.5	0.6	0.9	16.8	−0.5	0.7	−1.2
Asp61	**13.0**	**−2.8**	**−0.6**	**−2.2**	**9.3**	**−6.3**	**−14.0**	**7.7**
Arg80	**12.7**	**5.1**	**1.1**	**4.0**	**11.3**	**−3.6**	**−3.6**	**0.0**
Lys106	20.5	−0.4	0.9	−1.3	19.4	0.4	−1.6	2.0
Lys109	**14.2**	**−5.5**	**−2.6**	**−2.9**	**10.4**	**−3.6**	**−5.6**	**2.0**
Asp113	14.5	1.9	0.1	1.8	10.1	0.3	1.5	−1.2
Glu117	**17.9**	**0.8**	**0.0**	**0.8**	**13.1**	**−0.9**	**−6.5**	**5.6**
Glu201	20.0	−2.7	−0.2	−2.5	25.0	0.2	1.9	−1.7
Arg202	19.9	2.0	0.1	1.9	25.1	−0.1	−1.7	1.6
Asp203	**13.4**	**−3.6**	**−0.8**	**−2.8**	**18.3**	**1.5**	**6.6**	**−5.1**
Glu221	21.9	−0.9	−0.2	−0.7	24.5	0.4	2.9	−2.5
Asp226	26.6	−1.6	−0.1	−1.5	31.0	0.1	1.2	−1.1
Glu230	27.6	−1.8	−0.1	−1.7	31.2	0.1	1.4	−1.3
Asp256	**15.7**	**−4.1**	**−0.3**	**−3.8**	**21.0**	**0.2**	**2.5**	**−2.3**
Thr269	19.7	−3.1	−0.2	−2.9	21.9	0.1	1.2	−1.1

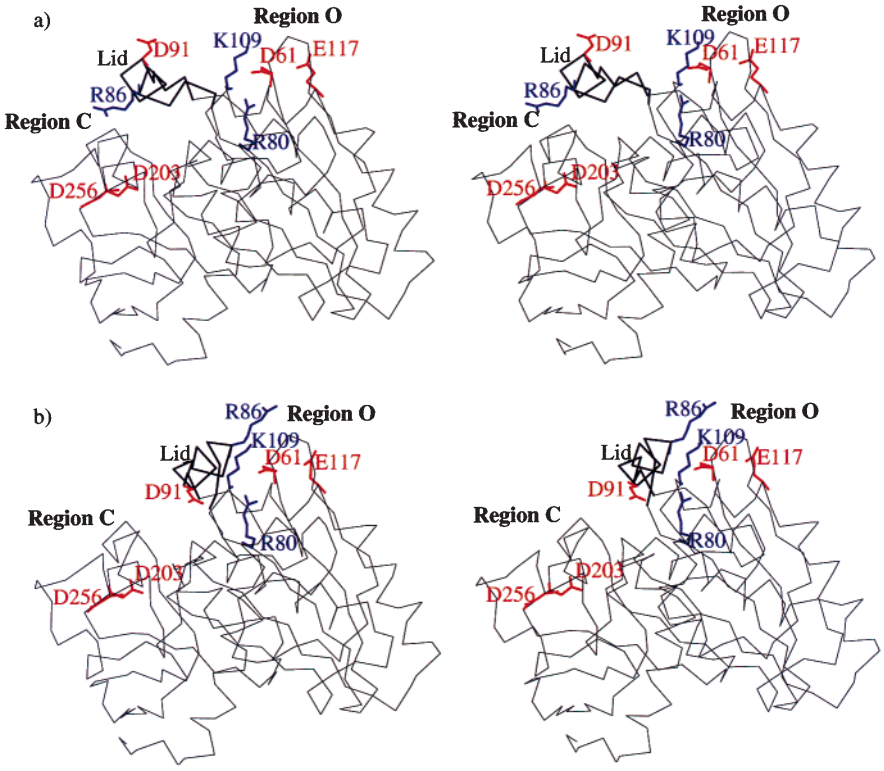

Fig. 3. Ca traces of the **a** closed and **b** open conformations of RmL showing the residues forming the key electrostatic interactions around the lid region. Acidic residues are coloured *red* and basic residues are *blue*

interactions with the lid in both conformations. These attractive interactions are enhanced in the closed state upon changing from "water" to "lipid", leading to destabilization of the closed conformation. In the open state these interactions are screened when the medium is changed from "lipid" to "water", indicating destabilization of the open conformation. Another residue in the vicinity of region O, Arg80, has repulsive interactions with the lid which are strongly enhanced in the closed state upon transfer from "water" to "lipid": in the open state Arg80 has equally strong attraction with the lid both in "lipid" and in "water". This change from repulsion in the closed state to attraction in the open state is due to the formation of strong attractive interactions between Arg80 and Asn87/Asp91 in the open state: Asn87 forms a hydrogen bond with Arg80 in the open state.

The lid has several long-range electrostatic interactions which are of significant magnitude in both states (see Table 1). For example, there are a number of negatively charged residues which are located in the vicinity of region C: Glu13, Glu16, Glu201, Glu221, Asp226 and Glu230. These residues show a similar type of interaction with the lid as Asp203 and Asp256 (attractive in the closed state and repulsive in the open state).

Our motivation for investigating the electrostatic interactions of the lid originates from our attempt to understand the mechanism underlying the conformational change associated with activation of RmL. Although this study is restricted to the consideration of electrostatic interactions and uses a continuum representation of the solvent, it gives us some ideas about the effect of changing the dielectric constant of the medium. Our results show that several interactions stabilizing the open state are screened when the dielectric constant is changed from that of lipid to water, which suggests that dielectric modulated electrostatic interactions could play a role in lid closing. In the closed state the situation is not as clear as the two key interactions stabilizing the closed conformation are actually enhanced in "lipid". Clearly, further studies are needed to go beyond this static description of the closed and open states of lipase and to explore the potential energy surface and the pathway of this conformational change.

4 Concluding remarks

In this study, we examined the electrostatic interactions which contribute to the stability of the lid in the closed and open conformations of RmL. Mapping of the electrostatic interactions of the lid region was used to identify specific non-lid residues which were sensitve to a change in the dielectric of the medium: Asp61, Arg80, Lys109, Glu117, Asp256 and Asp203, the latter being a catalytic residue. Of the two key electrostatic interaction sites on the lid itself, Asp91 and Arg86, only the latter, to our knowledge, has been modified. The sites identified in this study are potential candidates for mutation studies to further investigate their role in lipase stability and activation.

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