Conformer Models of TEM-1 β-lactamase

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1 Introduction

β-Lactamases are enzymes that play an important role in bacterial resistance to β-lactam antibiotics. Three from four classes (A, C and D) of these enzymes are serine proteases . The best studied are class A enzymes (to which belong *Escherichia coli* TEM-1 enzyme). They are monomeric two domain proteins with average molecular weights about 28 kDa, which contain nucleophilic S70 at the active site supported by a set of polar residues: K73, S130, E166, K234, S235, (R244) and etc. The 3D crystal structure of TEM-1 free- and acyl-enzymes are solved in atomic details. The intense studies on mutants and functional properties have been supported by many theoretical investigations on protein molecular mechanics and dynamics (MM/MD) simulation as well as electrostatic analysis (EA) for proving great variety of reaction mechanisms. Nevertheless the success is not full probably because lack of detail correlation between catalytic and conformational properties. The presented results are a sample step to fulfill this gap. They are based on: the presence of pH-dependent conformational forms in classes A and C β-lactamases and stable multiple unfolding intermediates related to set of conformers for class A enzymes; "abnormal" pH-dependent (k_{cat}/K_M) curve of TEM-1 β-lactamase and same "abnormal" unfolding in haotropic solvents. The detailed EA of TEM-1 b-lactamase reaction cycle lead us to conclusion for inability to converge protein structures of free and acyl-enzime structures (without acyl moiety) into a single same "solution structure" by standard MM/MD approaches. Each of structures has own local free energy minima with unknown barriers and path(s) between them. Thus as a **working hypothesis** we can assume that TEM-1 β-lactamase before acylation (free enzyme and Michaelis complex) is in conformational state "A" and after acylation (acyl-enzyme and next intermediates to enzyme-product aduct) is in other state "B", which have to keep their main features even in solution.

2 Methods

The atomic coordinates of crystal structures of free enzyme (FE) and acyl-enzyme (AE) were taken from Protein Data Bank. Programs for modeling and model management were: "CHARMm" (MSI), "Sybil" (Tripos), "MolMol" (ETH), "What_If" (EMBL). Programs for electrostatic calculations were: "DelPhi" (kindly donated by Prof. B. Honig) and "PHEI" (home created by Dr. V. Spassov). Each of FE and AE as "crystal" and "solution" models were compared using: (1) distance analysis (ΔR^2_{ii}) by full overlapping 70, 130 and 236 C^{α}-atoms; (2) static accessibility (ΔSA_{ii}) and surface solvent access (ΔSS_{ii}) differences; (3) difference of the residue averaged electrostatic potential (at pH 6.5 and ionic strength 0.1) ($\Delta\Phi_{ii}$) and (4) difference in electrostatic free energies ($\Delta G_{el,ii}$) for each pair.

3 Results

(1) Comparison of FE and AE protein structures by superposition of all main-chain atoms give RMS deviations 0.61- 0.65 Åand can be qualified all they to be highly similar. However using only 3-point C^{α} superimpose (at S70, S130 and A236 active site highly fixed distances) both A and B structures do not overlap and shows "periodical" changes in ΔR^2_{ii} (Fig. 1) - higher than 1.0 Å at 98, 114, 141, 227 and 252 residues. The "shifts" are bigger for start and end of β-domain and periodically repeated in 3-4 fragments of α-domain. (2) In average A structures is more solvent accessible (more of points at Fig. 2 are above zeroed line an only about 10 groups are less accessible (below zero, include. P62, P167 and P174) Usually there is compensatory effects from neighbor residues The total molecular surface is 11830.63 and 11091.81 $Å^2$ for "crystal" A and B structures respectively against 11181.03 and 10989.81 $Å^2$ for "solution" ones. Both A models have more exposed polar atoms than B and this difference is less for hydrophobic atoms. In general B-structures are more compact than A. (3) The differences of averaged electrostatic potentials at each i-th residue ($\Delta\Phi_{c,i}$) for "crystal strictures" is shown at Fig. 3 and is similar for "solution" models. Several general features are: mainly active site residues have different $\langle\Phi_i\rangle$; opposite $\Delta\Phi_i$ changes at S70 and S139; changes are very localized. (4) The $\Delta G_{el,ii}$ is bout -3.5 kcal/mol for "crystal pair" and only -0.5 kcal/mol for "solution pair" for B as more stable. However binding of substrate reverse stability when A-complexes are more stable up to 6 kcal/mol. pH-Dependent $\delta\Delta G_{el}$(B-A) follows sigmoidal curve with effective pKa = 8.3 - similar to that of alkaline limb of k_{cat}/K_M (pH) /the specificity curve/.

4 Proposed Model

All main α-helices (H1, H2-2, H-4-5-6, H8-9 and H11 and interfacial between domains β-sheet with five main β-strands are taken as rigid bodies as well as two loops (named *omega* and *twin*) are shown at Fig. 4. Helices H1 and H11 are at one ("bottom") side of β-sheet and all others - at another ("up") side. Between "central helix" H2, H4-5, H9 and "up" β-sheet side is located the substrate pocket area [24]. The model assume any change in position of these fragments as rigid-body like to be coupled: this means "pushing in" *omega loop* needs shifting "up" the H4-5-6 fragment which allows "in" for the twin loop and H8-9 corner to H2 end.. This can be taken as a mechanistic description of A $\rightarrow$ B transition.

5 Conclusions

(a) If β-lactam ring opening is the spring (source) for this transition, than obviously A-conformer is the structure of acylation and B-conformer - for deacylation parts of reaction cycle. (b) If the model is correct it is supported by Pro cis$\rightarrow$trans isomerization at *omega loop* and "linker chains" connecting two domains. (c) Within the *omega loop* each conformer has own salt bridge: E168R164 and R164D179. (d) The shift of *omega loop* not only change position and orientation of its E166 carboxylic group but also coupled relative positions of S70 (at start of H2) and S130 (at H4-5-6) as well as orientation of "oxyanion hole" dipoles (HN $<$ 236 in A-conformer and HN$<$70 - in B one, not both dipoles at once). Thus each cycle part has own activating dipole (two enzymes in one).

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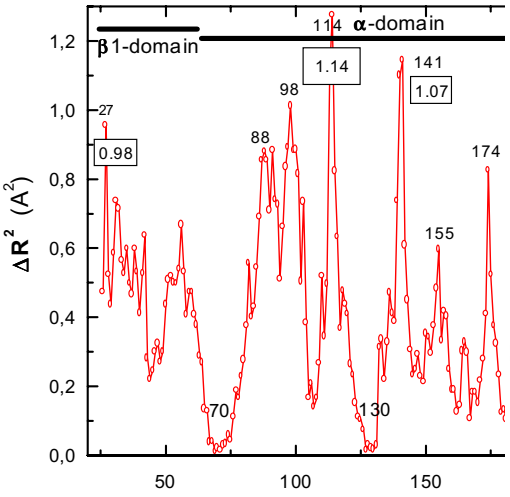

Fig.1

Fig.4

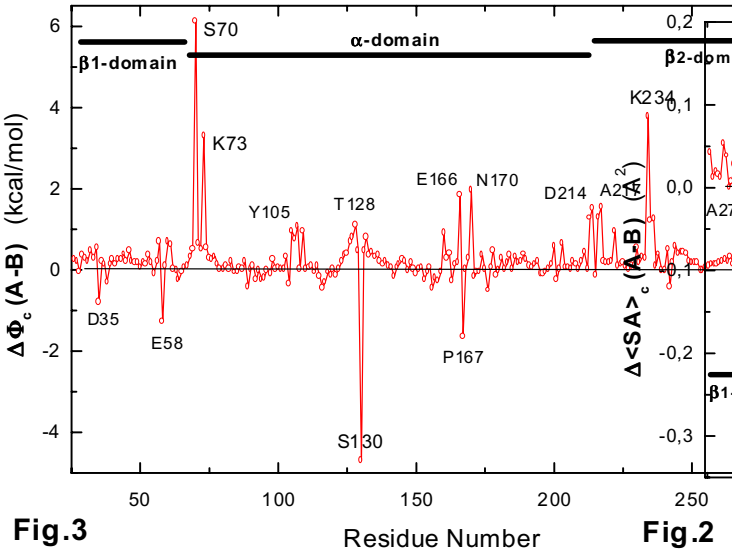

Fig.3

Fig.2