A model for the secondary structure of β-lactamases

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A 3-dimensional model, common for the secondary structures of four β-lactamases obtained from *Escherichia coli, Bacillus licheniformis, Bacillus cereus* and *Staphylococcus aureus*, is proposed. The predictions of the structures were made by the hydrophobicity profiles method complemented by the modified Chou and Fasman's method. The model proposed presents 56% constancy and can be described as a 2-domain structure, in agreement with low resolution X-ray data reported for the *E. coli* enzyme. The model would explain how a common function can be performed by enzymes of very different sizes, composition and sequence.

β-Lactamase *Secondary structure prediction* *Hydrophobicity profile* *Chou and Fasman's method*

1. INTRODUCTION

β-Lactamases are enzymes responsible for the bacterial resistance to β-lactam antibiotics, such as penicillins and cephalosporins; they inactivate the antibiotic action by disrupting the β-lactam ring. They constitute a group of enzymes that, even if they perform a common function, show a great diversity in their molecular masses, their amino acid composition and their activity to various antibiotics [1]. The primary structures of several β-lactamases have been determined [2] and they show that the invariant amino acid residues amount to ~20% of the total number.

Much effort has been spent in trying to solve the 3-dimensional structures of these enzymes and their active sites, as accurate information would allow the logical design of inhibitors and resistant antibiotics, and so be clinically very important.

Crystallographic data have been reported for β-lactamase I of *Bacillus cereus* to 3.5 Å resolution, and for R-TEM β-lactamase from *Escherichia coli*, to 5.5 Å resolution [3,4], but the tertiary structure of none of them have yet been completely solved.

The knowledge of the secondary structure of the β-lactamases could be of great help at this stage, since, in addition to facilitating the interpretation of the electron density maps, it could provide information about the configuration of the active site, and it could furnish a better pattern to compare the structures of the enzymes among them than the primary structure. One can think that a structural homology better than 20% should be found in the vicinity of the active site, since the same substrate must be accommodated by all these enzymes.

In this line of reasoning is a prediction of secondary structures of four β-lactamases and two lysozymes, as well as a comparison of the secondary structures of β-lactamases and two penicillin carboxypeptidases reported in [5,6]. The results point to the existence of a great structural homology among all these enzymes, with a clear predominance of helical structure, much larger than that expected from ORD measurements.

The aim of this work is to revise the prediction of the secondary structures of β-lactamases obtained from *E. coli, Bacillus licheniformis, B. cereus* and *Staphylococcus aureus*, with the method of the hydrophobicity profiles [7]. We intend to propose a location of the active site and to postulate spatial models for the secondary structures predicted which could account for all the information available.

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2. METHODS AND RESULTS

Denaturation–renaturation experiments on proteins have provided evidence that the nature and sequence of amino acids determine to a great extent the secondary and tertiary structures of the proteins. Of the numerous methods devised to attempt a prediction of the secondary structure with the information contained in the primary structure, two of them, reported to give 80% reliability, were used to predict the secondary structure of the four β-lactamases. These were the hydrophobicity profiles method [7] and the Chou and Fasman's method, as modified in [8,9].

The method of the hydrophobicity profiles postulates that the folding of the polypeptide chain occurs in a way that allows the location of the hydrophilic amino acids in the protein surface and the hydrophobic ones in the interior of the molecule. The 'bulk hydrophobic character' for each of the 20 natural amino acids, as defined in [10], is employed to draw the hydrophobicity profile of the protein. Four typical profiles are defined for an exposed helical structure, an exposed and a buried β-strand and for a β-turn. The prediction of the secondary structure by this method consists simply in the identification of these basic patterns in the hydrophobicity profile of the protein [7].

Chou and Fasman's method to predict secondary structures consists of the assignment of conformational parameters to each of the 20 natural amino acids, complemented with a series of empirical rules [8]. The conformational parameters P_α, P_β and P_t, represent the normalized frequency of occurrence of each amino acid in a particular type of secondary structure, as obtained from a data base of 29 proteins whose tertiary structures were fully known by X-ray diffraction methods. A probability average greater than 1.0, obtained for a group of amino acids taken in sequence (6 for a helix, 5 for a β-strand and 4 for a β-turn) is an indication that a certain type of structure is likely to occur in that region of the sequence. In order to improve the sensitivity of the method in the vicinity of the limit value 1.0, the probability average can be replaced by a product of the conformational parameters [9]. This and two other modifications to the method, one that considers 4 conformational parameters for each amino acid in a turn structure, and another that uses different parameters for a parallel or an antiparallel β-structure, were employed in the prediction reported here [11,12].

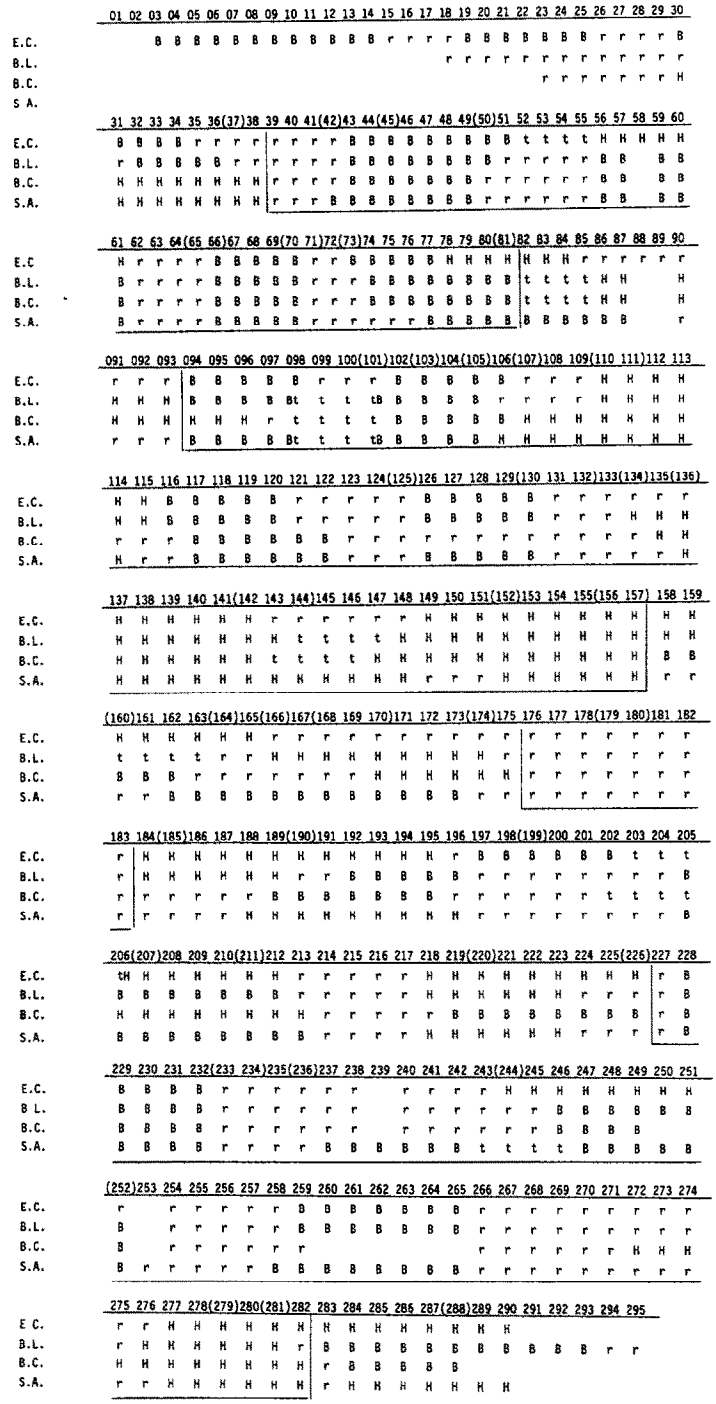
The secondary structures of each of the four β-lactamases were predicted independently by both methods. About 80% of the structure predicted by the hydrophobicity profiles' method was confirmed by the Chou and Fasman's method. When discrepancies were found, the results obtained by the first method were preferred only if the profile was a typical one. Otherwise, the Chou and Fasman's results were included in a final joined prediction. The primary structures used were those reported in [2], that gives the best alignment of the sequences.

The final prediction of the secondary structures of the four β-lactamases is given in table 1. The proposed structures show four main regions plus a minor zone that are structurally constant at least in three of the four enzymes. These are framed in table 1. On the average, 56% of all amino acid residues are involved in the structurally constant regions, as compared to only 20% invariance in the primary structure. The constant structures may differ in the lengths of the β-strands, helices or random coiled zones, but still they are easily recognized (fig.1).

Models of the predicted secondary structures were built using rigid arrows, cylinders and 'hair pins' to represent the β-strands, helical zones and β-turns respectively. These elements were joined by mobile connections and by flexible wire that represented the random coiled zones. The lengths of the building elements were scaled to the number of amino acids involved, and to the distances between α-carbons in that particular type of structure. First a 'Greek key' model was tried, and then, the following complementary information was considered: distinction between exposed and buried β-strands, as predicted by the hydrophobicity profiles [7]; preferences for β-strands to be part of a parallel or an antiparallel β-sheet, as given in [12]; stabilization of helical and β-structures in one of the structural groups defined in [13]; proximity of some amino acid residues according to chemical evidence [14,15]. All these constraints defined 3-dimensional models which described the general features of the structures predicted for the four enzymes analyzed. In fact, the distribution of the random coiled zones suggested two Greek keys to

Table 1

Secondary structures proposed for four beta-lactamases



E.C., *Escherichia coli* beta-lactamase; B.L., *Bacillus licheniformis* beta-lactamase; B.C., *Bacillus cereus* beta-lactamase; S.A., *Staphylococcus aureus* beta-lactamase; (n), amino acid residue invariant in the four enzymes; B, beta-strand; H, helical structure; t, beta turn; r, random coiled structure

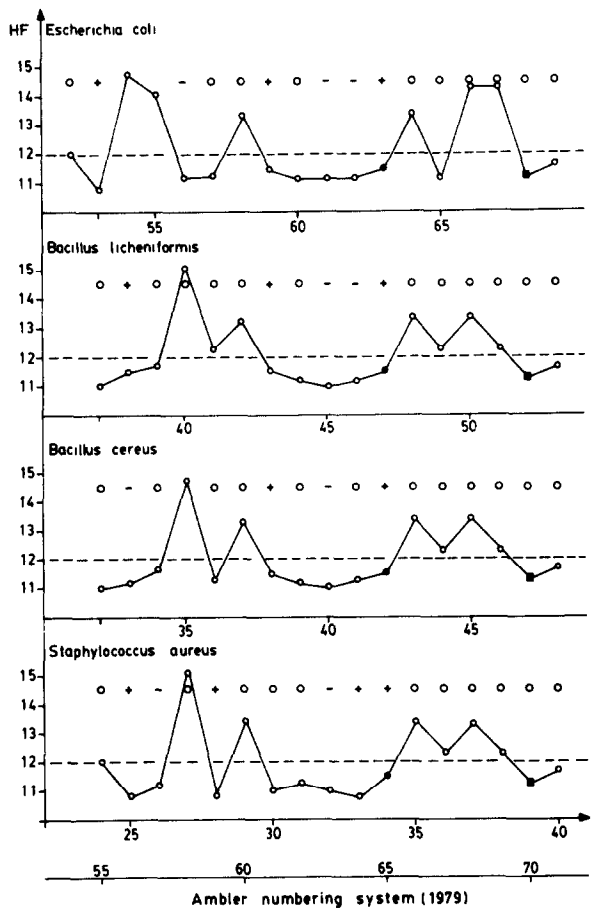


Fig.1. Sequence alignment by means of the hydrophobicity profiles: the 'charged loop' previous to Ser 70 is shown for the four β-lactamases. The vertical scale represents the bulk hydrophobic character in kcal. Values over the dotted line are considered as hydrophobic. The numbers in the horizontal scales give the position of the amino acid residues in the individual sequences. A scale with the common numbering system proposed as in [2] is also included. The charged residues are marked with + or − signs. The only invariable residues in the shown sequences are Arg 65, marked with a filled circle, and Ser 70, represented by a filled square.

describe each of the structures, thus defining a two-domain structure. The models, not completely packed for the sake of clarity, are shown in fig. 2, 3.

The amount of helical and β-structure predicted for each enzyme, as compared with those predicted in [5] is given in table 2.

3. DISCUSSION

Much has been written about the validity of the methods devised to predict secondary structures from sequences [16]. We think that, even though no predictive method can give 100% certainty, some of them can provide good clues for possible foldings of the polypeptide chain. Any proposed secondary structure ought to be able to account for the experimental data available, and it must suggest new experiments that could prove its validity.

The similarity between the predicted secondary structures of the four enzymes as compared to the modest invariability in the primary structures is remarkable. The last constant region, for example (residues 227–282), includes only 7 conserved amino acids. Moreover, the region containing residues 31–157 presents 84% of all amino acids involved in invariable secondary structure, with only 22% constancy in the sequence. The reproducibility of the hydrophobicity profiles in zones with few conserved amino acid residues is illustrated in fig.1 for a 'charged loop' in the vicinity of Ser 70, amino acids that have been reported to belong to the active site of β-lactamases [14]. Only two invariable residues are included in that zone, therefore, the constancy in the structure indicates that the replacements of the amino acids have been conserved in a new sense: a similar hydrophobic character has been maintained.

The hydrophobicity profiles turned out to be a good tool to align the sequences of the four β-lactamases in zones with a low constancy in the primary structure. Table 1 shows a sequence alignment based mainly on the shape of hydrophobicity profiles and the structural invariabilities, that coincides very well with that proposed in [2]. Only some changes in the location of the deletions were suggested by the profiles: deletion of 1 amino acid in location 58 instead of 57, and 2 amino acids in locations 88 and 89 instead of 96 and 97 for the *B. licheniformis, B. cereus* and *S. aureus* enzymes; a deletion of 2 amino acids in positions 250 and 251 instead of 247 and 248, and the deletion of 6 amino acids in the strand 260 to 265, instead of 258 to 263 for the *B. cereus* enzyme.

The secondary structures proposed have between 22% and 32% helical structure, in good agreement with the values 25% to 30% estimated by CD and ORD methods [17]. The amount of ex-

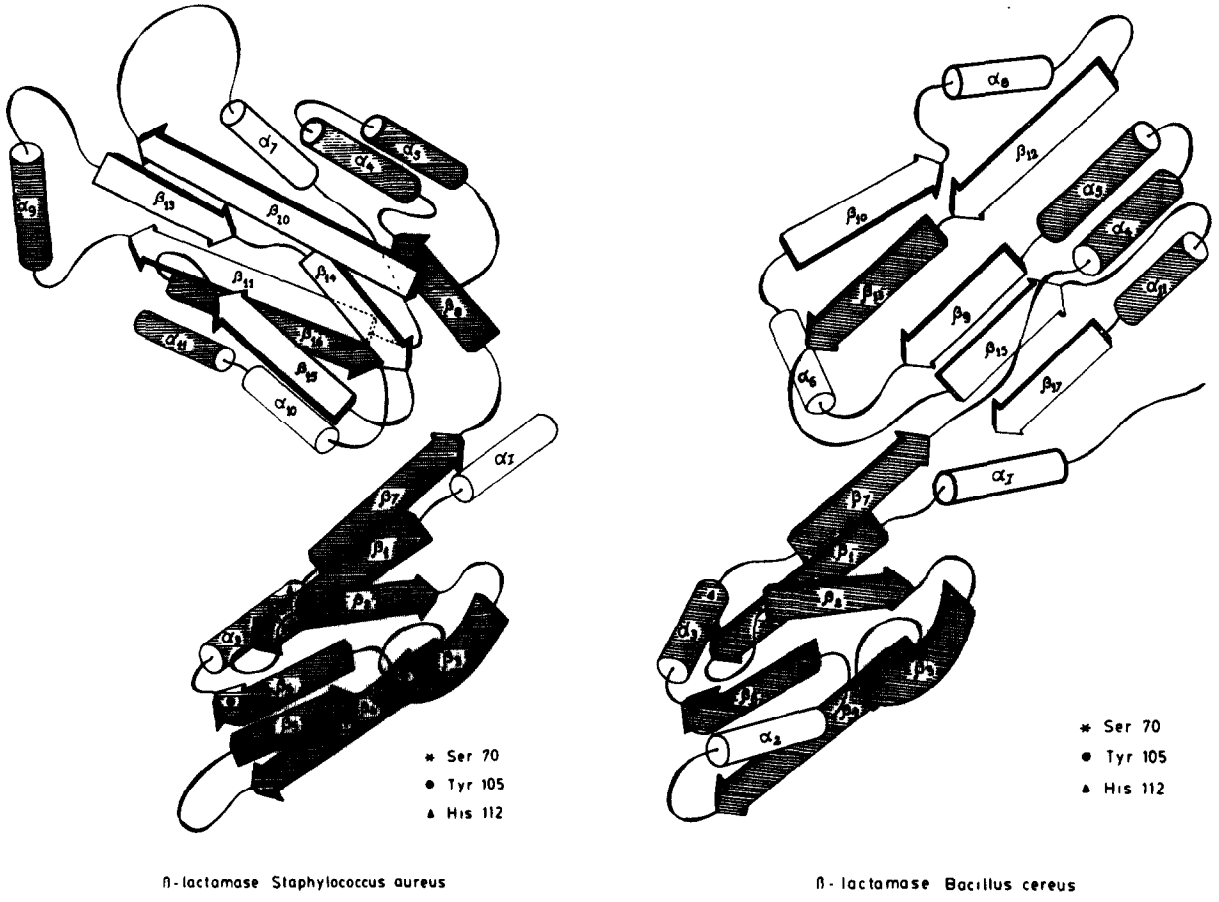


Fig.2. Three-dimensional models proposed for the secondary structures of β-lactamases from *Staphylococcus aureus* and *Bacillus cereus*. The shadowed structural elements are conserved in the four enzymes analyzed. The positions of some important amino acid residues are marked. The zones of random coiled structure between β_7 and α_4 allow different relative positions of the two domains.

tended structure is, however, much larger than the 10% expected from those methods. Table 2 allows a comparison of the results reported here with those predicted in [5], and gives the coincidences in the predictions in terms of the reliability parameters Q_α and Q_β [11]. The discrepancies came from overprediction of helical structure and underprediction of β-structure in [5] with respect to this work.

The 3-dimensional models for the proposed secondary structures of the four β-lactamases shown in fig.2, 3 represent, in our opinion, a good possibility for the folding of the polypeptide chain. As can be seen, all of them can be described as two domains joined by more or less flexible strands of the polypeptide chain. The first domain would include up to amino acid residue 122 (strand β_7) and it can be described as an antiparallel β-barrel. The predicted secondary structure of this domain is almost entirely conserved for the four enzymes. The second domain is more variable and contains a larger amount of random coiled structure. It can be described as a typical $\alpha + \beta$ structure, with a large antiparallel β-sheet surrounded by helices that are stabilized among themselves [13]. A structure with two domains agrees with the results reported from the 5.5 Å resolution structure of R-TEM β-lactamase from *E. coli* [4], where the electron density maps clearly showed two regions joined by two bridges of density. The author in [2] has also proposed a two domain structure for the β-lactamases, and also, ORD results [17] obtained

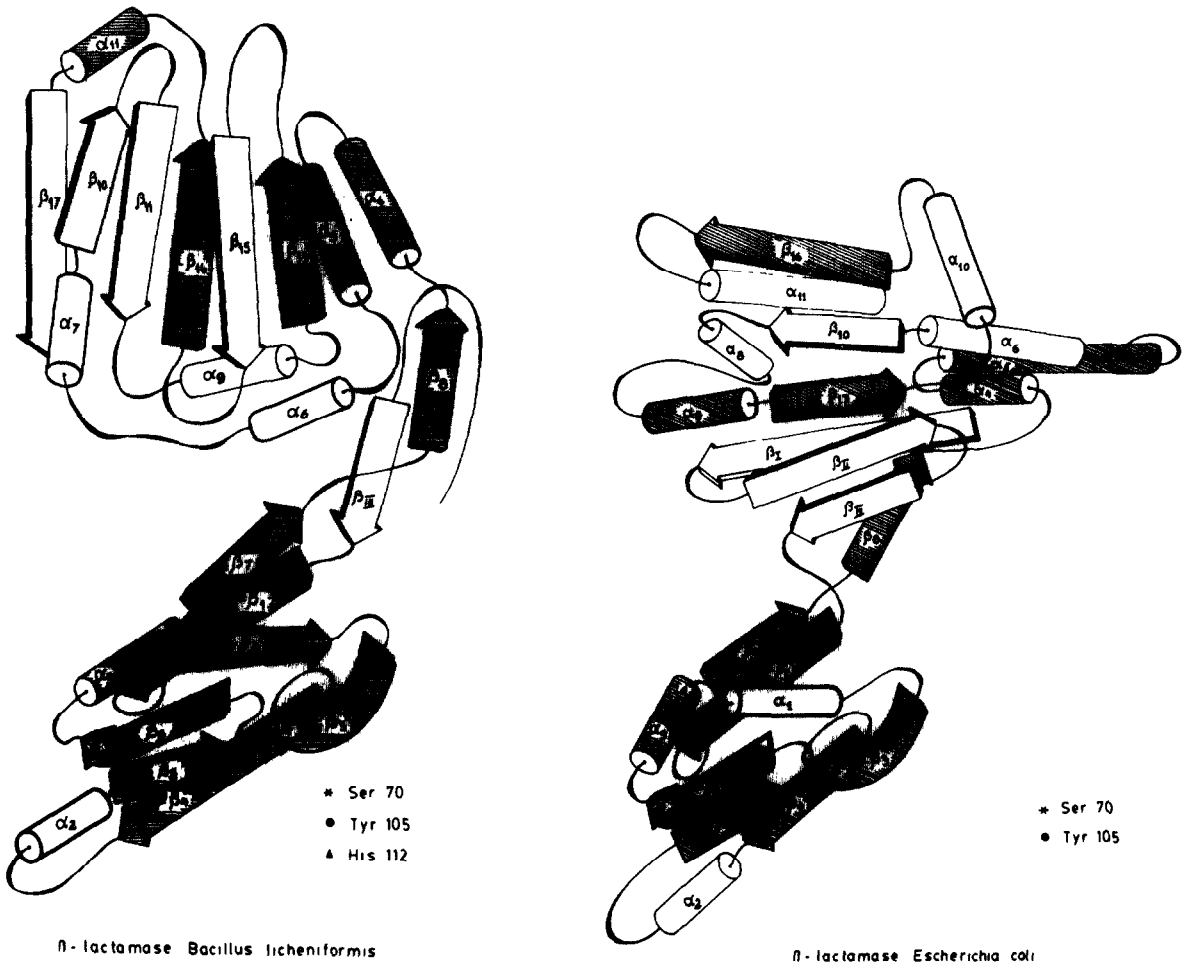


Fig.3. Three-dimensional models proposed for the secondary structures of β-lactamases from *Bacillus licheniformis* and *Escherichia coli*. In these structures the two domains are joined by two strands of the polypeptide chain. The three β-strands predicted for the first 35 residues of the *E. coli* enzyme have been included in domain II. See fig.2 for other specifications.

for denaturation–renaturation experiments made in the *S. aureus* enzyme, support the hypothesis of a multidomain structure.

The molecular masses for domain I ranged from 10 kDa to 13 kDa, the extreme values corresponding to the *S. aureus* and the *E. coli* enzymes respectively. These values are comparable to the molecular masses of 12 kDa found for β-lactamases encoded by *Pseudomonas aeruginosa* plasmid R 157 and Rms 149 [2,18], and the value of 15 kDa of β-lactamase from *Streptomyces* UCSM-104 [19]. One could therefore think that small β-lactamases could be molecules similar mostly to domain I of the structures proposed here, especially since the active site of β-lactamases seems also to be restricted to domain I. The high constancy in secondary structure of this domain, as well as the fact that it includes almost all the amino acid residues that seem to have something to do with the activity of the enzyme, points to it as a functionally important part of the protein. In addition to Ser 70, domain I includes Tyr 105, which, even if it is not part of the active site, produces a loss of activity when it is intermolecularly cross-linked by a reaction with nitromethane [15]. His 112 (present in 3 of the four enzymes here studied) whose oxidation produces a loss of enzymatic activity, is also located in domain I [15]. According

Table 2

Distribution of helical and β-structures in the predicted secondary structures of four β-lactamases

	Reference	Source of β-lactamase			
		E. coli (%)	*B. licheniformis* (%)	*B. cereus* (%)	*S. aureus* (%)
Helical structure	This work	32	22	27	25
	C–F [5]	36	37	38	39
	G [5]	49	50	47	51
Q_α	C–F [5]	64	77	68	76
	G [5]	45	52	66	66
β-Structure	This work	28	34	28	37
	C–F [5]	17	17	11	15
	G [5]	20	19	15	25
Q_β	C–F [5]	74	73	66	72
	G [5]	62	64	66	73

C–F, structure predicted by the Chou and Fasman's method, fig.1 [5]; G, structure predicted by the Garnier's method, fig.1 [5]; Q_j, (%j + %noj)/2, measures the coincidence of the structure type j predicted in [5] with that predicted in this work; %j, % amino acids 'correctly' predicted to be in structure type j; %noj, % amino acids 'correctly' predicted not to belong to structure type j

to the model proposed, the active site would be formed by parts of the following constant regions: $\beta_1, \beta_3, \beta_4$ and the charged loop previous to β_3. The presence of charges near Ser 70, coincides with the existence of carboxylic groups in the active site [15].

Even if one accepts that domain I controls the catalytic action, one could still think that domain II could cooperate with it, since the interdomain region is flexible enough to allow a close contact. However, the existence of active β-lactamases of smaller molecular mass argues against this possibility, and points to domain I as the place where the β-lactamase activity is restricted.

On the other hand, the existence of β-lactamases of much higher molecular mass, and the structural constancy that domain II exhibits, make us think that these enzymes were created to accept more than one substrate, or that they have evolved from molecules that performed other functions, carboxypeptidases, for instance [5]. Any of these possibilities requires further and careful investigation.

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