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Individual contributions of the aromatic chromophores to the near-UV Circular Dichroism in class A β-lactamases: A comparative computational analysis

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ABSTRACT

Class A β-lactamases are enzymes which are responsible for the bacterial resistance against antibiotics and therefore are of great importance in rational inhibitor design. In this paper we comparatively analyze all the individual contributions of the aromatic chromophores in three class A β-lactamases (from *Staphylococcus aureus*, *Streptomyces albus* and *Bacillus licheniformis*) to their near-UV Circular Dichroism. The analysis is performed using recently developed procedure based on established theoretical method. We found that in β-lactamase from *S. albus* the most significant contributions to the total near-UV CD intensity exhibit Y251 and Y229. In the tryptophan-containing β-lactamases from *B. licheniformis* and *S. albus*, W229 and W251 express the strongest individual contributions. A comparative analysis of the individual contributions of conservative chromophores in class A enzymes namely W165, W210, W229, W251, Y97 and Y105 is presented.

1. Introduction

β-Lactamases are of great interest to the pharmaceutical and biotechnology industries as they are responsible for bacterial resistance against antibiotics [1]. As a result intensive experimental and computational investigations have been reported [2–5]. ß-Lactamases are separated in four classes (A, B, C and D). The A class ß-Lactamases contain an active site serine residue which acts as a nucleophilic agent that attacks the lactam bond [6]. This class of ß-Lactamase is the most extensively investigated one. The enzymes from this class are approximately 30 kDa proteins, consisting of one polypeptide chain organized into two domains: a-helical and mixed aß [7].

Circular Dichroism (CD) [8] is a powerful tool for the elucidation of structural information for proteins. The far UV CD spectra are informative for secondary structure changes, whereas the near-UV method is sensitive to changes in protein tertiary structure resulting from ligand binding, mutation effects and catalysis [9,10].

A combination of CD measurements and computational approaches leads to a fast and accurate understanding of the absolute configuration of small molecules [11–13] In addition, deeper atomistic insights into specific interactions between protein chromophores and the influence of structural and electrostatic effects can be attained via this dual approach [4,14–21]. The CD spectrum of TEM-1 β-lactamase was calculated in good agreement with the experimental one [19]. TEM-1 β-lactamase as the enzymes which are object of this study belongs to class A which common structural feature is that the central section of the protein sequence folds into a globular unit which contains mainly of the α-helices and the N- and C-terminals form a five-stranded β-sheet which is surrounded by the the helices [22]. The Root Mean Square deviation superposing the α-carbon atoms in respect to TEM-1 structure is as follows: for the enzyme from *Staphylococcus aureus* − 2.47 Å, for the enzyme from *Streptomyces albus* − 2.21 Å and for *Bacillus licheniformis* 2.10 Å. On the basis of the agreement between the predicted and computed spectrum of TEM-1 enzyme, the theoretical spectra of the β-lactamases from *S. aureus*, *S. albus* and *B. licheniformis* were computed and analyzed in terms of mechanisms of generations of rotational strengths [21,23]. However, the CD method has low signal resolution, therefore it is difficult to analyze experimentally the individual contributions of protein chromophores. The problem is accentuated by the structural complexity, the large number of chromophores and conformational flexibility of proteins. The absence of such atomistic information leads to numerous limitations in its application and prevents the elucidation of the role of protein chromophores, especially in the near-UV spectra, whereby the aromatic residues are major contributors. CD experiments on mutated proteins may facilitate the understanding of chromophore contributions. However, mutagenesis leads to delicate structural

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effects which influence the orientations between the chromophores leading to inaccurate information. Aromatic residues play an important role ligand orientation, substrate activation, and reaction mechanisms [24,25] therefore detailed atomistic insight about their spectral contributions and mechanisms is important for deeper exploration of enzyme functions. In our recent paper we presented a novel computational strategy to calculate individual chromophore contributions and linked them to structural and environmental changes in TEM-1 β-lactamase [26]. This methodology allowed for systematic computational analysis of different but structurally similar β-lactamases from class A.

In this paper we apply our recently developed approach to investigate how β-lactamases from class A differ in respect to their chiroptical properties. We demonstrate the potential of computational methods to reveal this relationship from a unique and unusual point of view: namely how the contributions of the individual chromophores differ within three β-lactamases from class A (from *S. aureus* (containing no tryptophans), *S. albus* (containing both tryptophans and tyrosines) and *B. licheniformis* (containing tyrosines and tryptophans)). In particular, the following key findings will be presented:

i) The extraction of individual contributions of the thirteen tyrosines in β-lactamase from *S. aureus*
ii) Revealing the individual contributions of the four tryptophans and six tyrosines in β-lactamase from *S. albus*
iii) Differentiating between the individual contributions of the three tryptophans and six tyrosines in the β-lactamase from *B. licheniformis*
iv) Systematic comparative analysis of the evolution of the individual contributions of chromophores which are conservative in class A β-lactamases (W165, W210, W229, W251, Y97 and Y105)
v) Evolution of the overall mechanistic type of the modelled spectra between the above three enzymes and TEM-1 β-lactamase.

2. Methodology

The individual contributions of the aromatic chromophores will be characterized in the following directions:

i) Relative individual contribution of each chomophore in respect to the total CD generated by the one electron mechanism
ii) Relative individual contribution of each chromophore in respect to the total CD generated by coupled oscillators mechanism
iii) Relative individual contribution of each near-UV chromophore in respect to the total CD spectral intensity.

The methodology for calculating the above contributions is described in details in [26] and is presented briefly here:

The individual net rotational strength of a chromophore *Rn* was calculated as a sum of the absolute values of the rotational strengths for all transitions of a particular chromophore:

$$Rn,i = \sum_{j=1}^{n} |Ri,oj|. \quad (1)$$

The individual percentage contribution to the total one electron rotational strength Rroe was calculated as a percentage from the quotient between the individual absolute contribution of a chromophore transitions contributing to the one electron mechanism and the total one electron rotational strength:

$$Rroe,i = (|Roe,i| \div |Rtot,oe|) \times 100. \quad (2)$$

The individual percentage contribution to the total coupled oscillator rotational strength Rrco was calculated in the same manner.

The individual percentage contribution in respect to the total rotational strength was calculated as a percentage from the quotient between the individual net rotational strength and the total rotational strengths over all chromophores and transitions *Rtot,all*:

$$Rrtot,i = (|Rn,i| \div |Rtot,all|) \times 100. \quad (3)$$

The calculations used the crystal structures of the enzymes taken from Protein Data Bank [27] as initial coordinates. In the case of β-lactamase from *S. aureus* PC1 — the structure (PDB code: 3blm) [28]; for the enzyme from *B. licheniformis* 749/C — the structure with PDB code 4blm [29] and for the β-lactamase from *S. albus* G [30] the PDB structure 1bsg. The calculation details of TEM-1 β-lactamase which is used for comparisons are presented in [26].

2.1. Theoretical background

The basic molecular unit of the CD phenomena is the rotational strength (RS) which represents the imaginary part from the scalar product between the electric transition moment and magnetic transition moment of a particular transition [31]. In the proteins most of the choromophores (disulfide group and non-planar peptide group are the only exceptions) are achiral, however, become secondary chiral by the effects of the protein environment [32]. Three mechanisms may be followed in this (achiral to chiral) process: i) *one electron mechanism* or *static field effect* [33], which represents coupling between electrically and magnetically allowed transitions within the same chromophore; ii) *μ–μ mechanism* or the coupling between electrically allowed transitions in different chromophores [34,35] (this mechanism includes the so-called *exciton effect* [36]); iii) *μ–m mechanism*, which is realized through the coupling between magnetically and electrically allowed transitions in two distinct chromophores [37]. Mechanisms (ii) and (iii) are also called *coupled oscillators* type.

2.2. Computational method

Calculation of rotational strengths in complex systems as proteins which contain hundreds or thousands of chromophores cannot be cone directly even using powerful supercomputers, therefore we applied the well-established matrix method of Baylay, Nielsen and Schellman [38]. The method is implemented in the program MATMAC, which was kindly provided by Prof. Joerg Fleischhauer RWTH-Aachen, Germany [39]. It utilizes matrix diagonalization, which makes it easy to implement in computer programs and also includes a modification of Goux and Hooker which include the matrix elements of the momentum in order to avoid the origin dependence of the magnetic transition moment [40]. The method for calculations of monopoles is the same as described in [15] and [41] and utilizes a semi-empirical method (CNDO/S). Experimental excitation energies and electric transition dipole moments were used for the excited states of the model chromophores as described in [15–17]. The method uses the protein X-ray structures thus allowing the orientation and distances between the chromophores within the protein to be taken into account. The matrix method implemented in the program MATMAC was successfully implemented to investigate mechanisms of CD spectra in ribonuclease A and ribonuclease S. The programme predicts rotational strengths in close agreement to experiment and revealed the important role of the coupling between Y73 and Y115 to tyrosine Lb rotational strengths which was experimentally confirmed [15]. The method is also used to calculate CD spectra of myoglobin, lactate dehydrogenase and other proteins [14,42–46].

All rotational strength contributions of the chromophores were calculated in the interval 240–300 nm. The rotational strengths are presented in Debye–Bohr Magnetons (DBM). More details about the matrix method are presented in Ref. [6,15,18,19,32,38,47]. To generate a CD-spectrum, the calculated rotational strengths were combined with Gaussian band shape functions as was reported by

Kurapkat and co-workers [15]. The visualization of the enzyme structures was done with VMD 1.8.4 [48].

3. Results and discussion

3.1. Individual contributions of tyrosine chromophores in β-lactamase from S. aureus

This enzyme contains thirteen tyrosine chromophores (Fig. 1). The analysis of the individual chromophore roles is presented in Table 1. The most significant individual contribution is exhibited by Y251, which generates 21.4% from the total near-UV CD. It follows the one electron mechanism and generates also the most significant part (32%) from the total one electron rotational strength. This residue is located on the solvent exposed area of β-sheet. Y229 (analogous to W229 in TEM1, *B. licheniformis* and *S. albus*) has the second strongest relative contribution to the total RS (20%). However, it follows coupled oscillators mechanism (interacting with Y47 as described in [23]) and provides 67% of it. The tyrosine Y40 which is located on a short α-helix at the top of the αβ domain provides 12% from the total RS and 17% from the total one electron (generated by the static field effect) RS. Y105 is a conserved tyrosine which occurs also in β-lactamases from *E. coli* (TEM-1) and *B. licheniformis*. It is located in a loop structure in all-α domain and generates around 10% from the total CD intensity and 14% from the total one electron RS. Y68 contributes 8% to the total near UV intensity. It is also "one electron chromophore" and provides 12% of the total near-UV CD which is generated by the one-electron mechanism. Y165 (analogous to W165 in TEM-1 and *S. albus* β-lactamases) is located in a solvent exposed loop of the all-α domain. It contributes 7% from the total CD and 10% from the one electron intensity. Y129 which is situated in a solvent exposed part of an α-hellix in all α-domain generates 5.3% from the total RS and 8% from the one electron RS, respectively. Y172 is located in a loop in the αβ-domain. It generates 3% from the total coupled oscillators RS and 5% from the total RS. Y88 is positioned within a 3–10 helix in an all α-domain. It is a one electron chromophore which provides 6.6% from the total one electron and 4.5% from the total near-UV RS. The "coupled oscillators" Y171 and Y47 provide lower than 1% than the total RS and Y112 seems not to contribute. The CD spectrum of this enzyme is predominantly generated by the one electron mechanism, which provides 67% from the total intensity. Coupled oscillators mechanisms still make a considerable contribution, providing around 32% from the total RS.

Table 1
Individual contributions of all tyrosine chromophores in β-lactamase from *S. aureus*.

Chromophore	Relative % contribution to the total one electron RS	Relative % contribution to the total coupled oscillators RS	Relative % contribution to the total RS
Y40	16.9	0	11.5
Y47	0	1.8	0.7
Y68	11.5	0	7.9
Y88	6.6	0	4.5
Y105	14.0	0	9.6
Y112	0	0	
Y129	7.7	0	5.3
Y165	10.1	0	7.0
Y171	0	2.9	0.9
Y172	0	16.4	5.1
Y229	0	62.7	19.8
Y241	0	16.8	5.3
Y251	31.5	0	21.4

3.2. Tryptophan and tyrosine chromophores individual roles in β-lactamase from S. albus

This enzyme contains four tryptophans and six tyrosine chromophores (Fig. 2). The most contributive are two tryptophans W229 and W251 which both are "coupled oscillator" chromophores and interact each other (Table 2). They are closely spaced to each other and located at the end of β-sheet within the αβ domain. W229 and W251

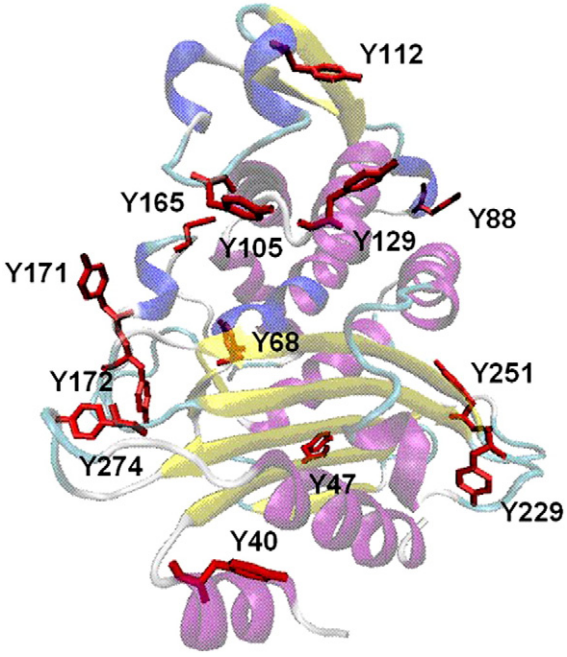

Fig. 1. Structure of β-lactamase from *S. aureus*. Tyrosines are shown in red.

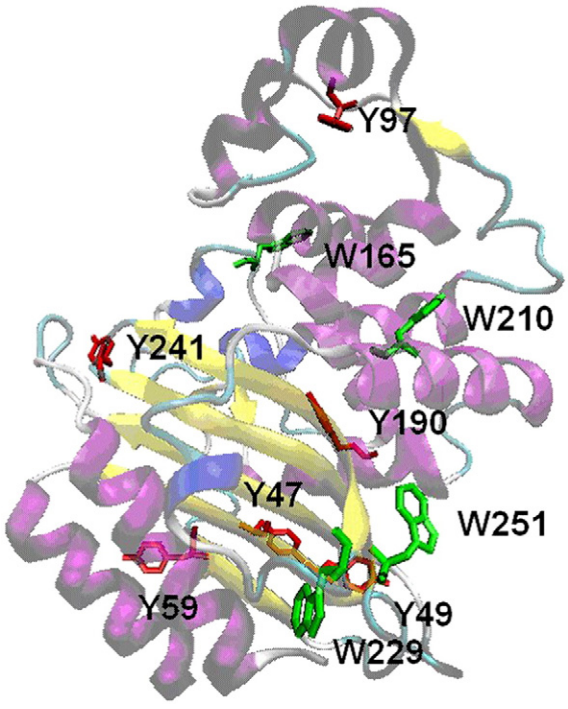

Fig. 2. Structure of β-lactamase from *S. albus*. Tryptophans are shown in green and tyrosines in red.

Table 2
Individual contributions of all tyrosine chromophores in β-lactamase from *S. albus*.

Chromophore	Relative % contribution to the total one electron RS	Relative % contribution to the total coupled oscillators RS	Relative % contribution to the total RS
W165	19.8	0	1.54
W210	45.1	0	3.66
W229	7.41	47.91	44.62
W251	7.49	50.31	46.31
Y47	0	0.16	0.15
Y49	0	0.60	0.55
Y59	0.62	0	0.05
Y97	1.00	0	0.08
Y190	14.20	0	1.15
Y241	9.26	0	0.75

contribute 45% and 46% respectively from the total rotational strength and 48% and 50% from the total coupled oscillators RS. The other two tryptophans which are also conserved in other class A β-lactamase, namely W165 and W210 act as "one electron" chromophores which have minor contributions to the total RS −1.5% and 3.7%. The strongest contributor within the tyrosines was Y190 which is a "one electron" residue providing 1.2% from the total and 14% from the total one "electron" RS. The other three "one electron" tyrosines have minor roles as follows: Y59 — 0.05%, Y97 (also conserved in some class A β-lactamases), — 0.75% and Y190 — 1.2%. Two tyrosine chromophores which are "coupled oscillators" — Y47 and Y49 — have small contributions to the total intensity 0.2% and 0.6% respectively. This spectrum is a strongly "coupled oscillators" type at 92% and 96% dominated by tryptophans. It is interesting to note that in the case of tryptophans stronger influences have "coupled oscillator" chromophores. However, from the tyrosine chromophores the "one electron" ones have a stronger more pronounced effect.

3.3. Individual roles of the chromophores and mechanistic type of the spectrum in B. licheniformis

The protein contains three tryptophans and five tyrosines (Fig. 3). The strongest individual contributions were W229 and W251 which were "coupled oscillator" type chromophores and generated 47% from the total RS and 48% from the total "coupled oscillators" RS (Table 3). The third tryptophan, W210 provides a relatively insignificant contribution from the total RS — only 2%. However it has the major contribution to the total "one electron" RS (88%). It's worth mentioning that both W229 and W251 also make very slight "one electron" contributions in addition to their major "coupled oscillators" one. The tyrosine chromophores here also can be divided to "one electron" (Y105, Y129, and Y241) and "coupled oscillators": Y60 and Y97. The strongest contribution to the total RS here as a "couple oscillator" was Y129 (2.3%) which contributes very weakly to the total "coupled oscillator" spectrum. The other "coupled oscillators" tyrosines Y60, Y97 and Y274 have a minor influence on the total RS resp. 0.2%, 0.5% and 0.02%, respectively. The one electron tyrosines Y241 and the Y105 also contribute weakly to the total RS (0.8% and 0.2%). This spectrum is almost entirely (97.3%) "coupled oscillators" type. Again the "coupled oscillators" mechanism is the favourable one for the tryptophans and the "one electron" mechanism is the more effective mechanism in the case of the tyrosines.

3.4. Comparative systematic analysis of the individual contributions of conservative chromophores in class-A enzymes

There are conserved residues which are also near-UV CD chromophores which are encountered in several class A β-lactamases. The analysis of their individual contributions provides the opportunity to monitor how these contributions evaluate between the different enzymes. All individual contributions in different class A enzymes analysed below can be seen in Fig. 4.

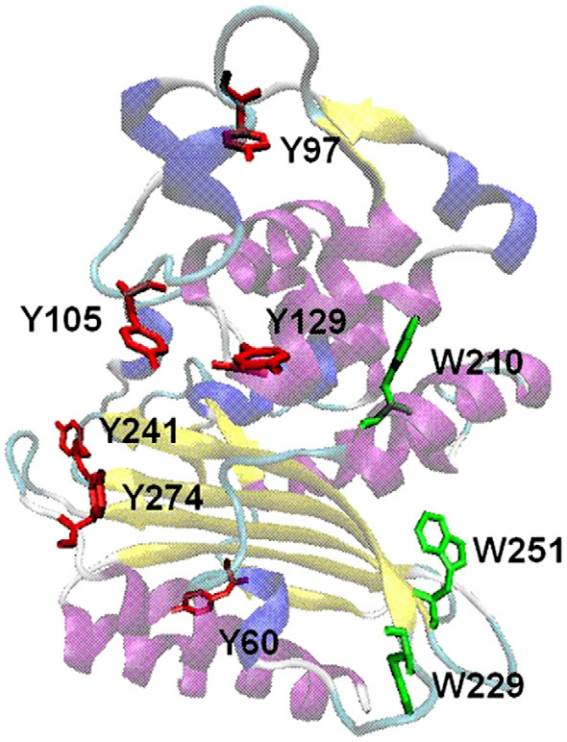

Fig. 3. Structure of β-lactamase from *B. licheniformis*. Tryptophans are shown in green and tyrosines in red.

3.4.1. W165

W165 is encountered in TEM1-β-lactamase (Fig. 5) as well in β-lactamase from *S. albus*. It provides relatively small contributions to the total RSs in both enzymes, however it's contribution in TEM-1 is at least twice as large (3.7%) [26] than in the *S. albus* (1.5%). In both cases it acts as "one electron" chromophore, however in the case of TEM-1 it generates 4% from the total one electron RS whether in the β-lactamase from *S. albus* it generates almost 20% from the total one electron RS. The influence of catalytically important structural changes and electrostatic effects on the contributions of W165 were investigated in detail [26]. It is important to underline that the structural changes of the same enzyme (TEM-1) β-lactamase can provoke stronger changes in its W165 contribution than the structural differences between free enzymes from TEM-1 and *S. albus*.

Table 3
Individual contributions of all tyrosine chromophores in β-lactamase from *B. licheniformis*.

Chromophore	Relative % contribution to the total one electron RS	Relative % contribution to the total coupled oscillators RS	Relative % contribution to the total RS
W210	88.1	0	2.1
W229	1.00	48.6	47.30
W251	1.00	48.8	47.51
Y60	0	0.17	0.16
Y97	0	0.54	0.53
Y105	6.25	0	0.17
Y129	0	0.01	2.28
Y241	2.46	0	0.75
Y274	0.1	0.02	0.02

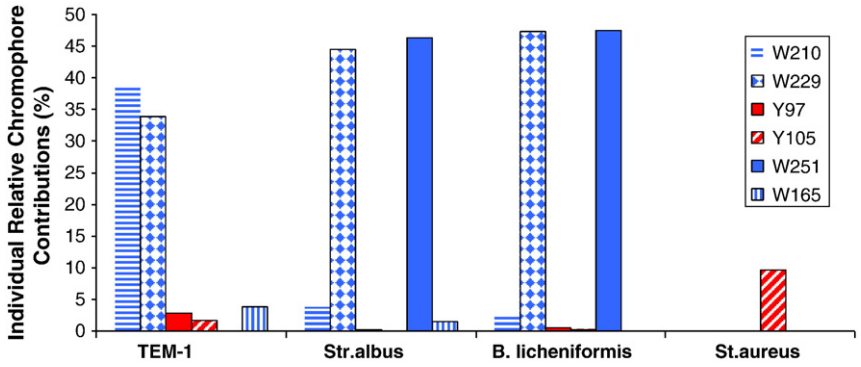

Fig. 4. Individual contributions of the conservative chromophores in four class A β-lactamases.

3.4.2. W210

This chromophore occurs in TEM-1 as well as in the β-lactamases from *S. albus* and *B. licheniformis*. Whereas in the enzymes from *S. albus* and *B. licheniformis*. it provides the lowest contribution to the total RS (3.7% and 2.3%, respectively) in TEM-1 it is the most significant chromophore and generates 39% from the total RS [26]. In all enzymes it participates in the one electron chromophore, however its contribution in respect to the total one electron RS is 88% in *B. licheniformis*, 44% in TEM-1 and 20% in *S. albus* enzymes.

3.4.3. W229

TEM-1, *S. albus* and *B. licheniformis* contain this chromophore. In the last two enzymes it behaves in quite a similar manner and provides around 45–47% from the total RS. In these enzymes it is also a strong "coupled oscillators" type chromophore which couples strongly with W251. It also accounts for approximately 50% from the total "coupled oscillators" RS in both enzymes. In the free enzyme from TEM-1 β-lactamase it acts as a one electron chromophore and provides 34% from the total RS and 39% from the total "one electron" RS [26]. As a result of important structural changes due to interaction with the transition state analogous inhibitor it (W229) transforms into a strong "coupled oscillators" chromophore.

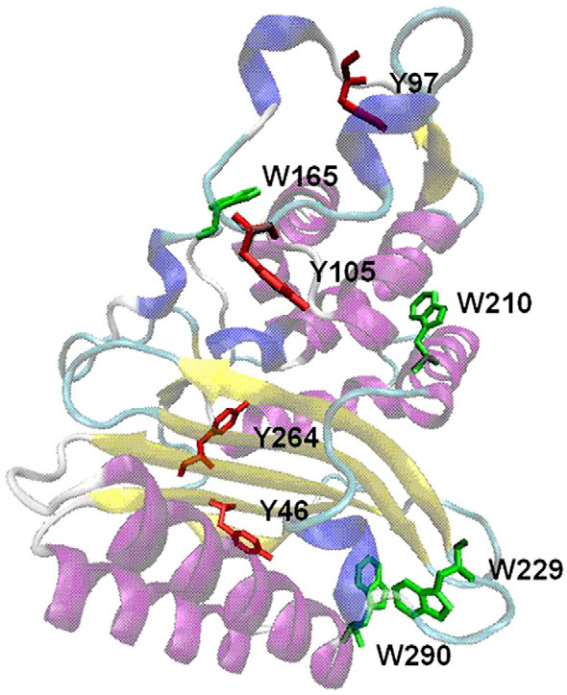

Fig. 5. Structure of β-lactamase from *E. coli* (TEM-1). Tryptophans are shown in green and tyrosines in red.

3.4.4. W251

B-lactamases from *S. albus* and *B. licheniformis* contain this chromophore. In both enzymes it acts as a "coupled oscillator" type chromophore interacting with W229 and providing a matching contribution to the W229 chromophore.

3.4.5. Y97

This chromophore is found in β-lactamases isolated from *S. albus*, *B. licheniformis* and TEM-1. In *S. albus* it generates only 0.1% from the total RS, in *B. licheniformis* it provides 0.5% whether in TEM-1 it exhibits the most significant contribution of 2.8% [26] in what??? In *S. albus* it realizes "one electron" mechanism whereas in *B. licheniformis* and the free enzyme from TEM-1 it behaves as "coupled oscillators" type chromophore. It is important to note that the conformational changes in TEM-1 enzyme induced by interactions with ligands may alter more considerably with the contribution of Y97 [26] than the structural differences between different enzymes from class A.

3.4.6. Y105

This tyrosine residue is conserved in TEM-1 β-lactamase as well in β-lactamases from *S. aureus* and *B. licheniformis*. In the last enzyme it has a very small contribution to the total RS of 0.2%. In TEM-1 it provides 1.5% [26] and in β-lactamase from *S. albus* it raises up to 10%. In the enzyme from *S. aureus* it realizes the "one electron" mechanism whereas in *B. licheniformis* and TEM-1 β-lactamase it follows "coupled oscillators" mechanism. In this case again the conformational changes induced by ligands in TEM-1 β-lactamase induces stronger perturbations of the relative individual contribution of Y105 to the total RS than the structural differences between different enzymes from class A (*B. licheniformis*, *S. aureus*, and TEM-1).

3.5. Overall mechanistic nature of the modelled spectrum

Finally we are going to compare the total relative contributions of the two types of mechanisms: one electron and coupled oscillators in the four enzymes from class A.

In the case of TEM-1 the spectrum is generated 88% by the one electron mechanism and 11% by the coupled oscillator mechanism [26]. In the case of β-lactamase from *S. aureus* (which contains only tyrosines) the spectrum is again mostly dominated by the one electron effect with 67%, however the relative part of the coupled oscillators is increased to 32%. It is worth mentioning that in TEM-1 structural changes perturbate this proportion within 10% [26]. However this ratio dramatically increases in β-lactamases from *S. albus* and *B. licheniformis* where the contribution of the coupled clusters mechanism is above 90% (92% and 98% respectively). From

this point of view the four enzymes can be divided in two subgroups based on their "mechanistic type" of spectrum: "one electron" enzymes — TEM-1 and *S. aureus* and "coupled oscillator" enzymes — β-lactamases from *S. albus* and *B. licheniformis*.

The analysis improves our understanding of structure–spectra relationships in proteins. In particular the role of individual chromophores is revealed from both quantitative and mechanistic aspect which is gained without structural perturbations to the proteins. The application of the method to structurally similar proteins (like β-lactamases from class A) provides not only information about the evolution of the conservative chromophores contributions but also uncovers the structural similarity between proteins from other unusual sides which could be linked to experimental science and industrial applications. The potential to differentiate contributions between chromophores from the same type (e.g. tryptophans or tyrosines) without structural perturbations would be a useful computational tool complementing experimental techniques in biophysical chemistry and protein science but also in biotechnological and pharmaceutical technologies.

4. Conclusions

In this paper we described the novel application of methodology for assignment of the individual chromophore contributions in complex heterogeneous multichromophore protein systems, namely class-A beta-lactamases. We found that in β-lactamase from *S. albus* the most significant contributions to the total near-UV CD intensity are exhibited by Y251 and Y229. In the tryptophan-containing β-lactamases from *B. licheniformis* and *S. albus*, W229 and W251 express the strongest individual contributions. We performed systematic analysis of the individual contributions of conservative chromophores in class A enzymes, namely W165, W210, W229, W251, Y97 and Y105.

The analysis provides a unique opportunity to investigate protein structural similarity and evolution from another perspective. A better understanding of the trends in the evolution of class A β-lactamases from a unique mechanistic point of view and an insight into how the individual chromophore contributions are altered between proteins with similar structure was presented. Finally, this study demonstrated the power of computational methods to provide insight which cannot be attained experimentally but nevertheless successfully complemented experimental studies. This study provided atomistic dimension to the CD phenomena which may have important applications in biomedicine and the biotechnology and pharmaceutical sectors.

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