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REGULAR ARTICLE

Computational insight into protein circular dichroism: detailed analysis of contributions of individual chromophores in TEM-1 β-lactamase

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Abstract Circular dichroism (CD) is an extremely powerful method in dynamically developed areas such as proteomics and drug design. However, it is characterized with a low signal resolution, and therefore it is difficult to assign the signals to specific chromophores. In this study, we demonstrate a systematic computational strategy for revealing the contributions of all individual chromophores to complex near-UV CD spectra of proteins. The methodology not only reveals the individual chromophores contributions without any structural perturbation, but also makes mechanistic insight into physical mechanisms possible. We have applied our strategy to a TEM-1 β-lactamase from *E. coli*—an enzyme of crucial importance to bacterial resistance to β-lactam antibiotics. We analyzed the free enzyme structure, two acyl-enzyme structures and the structure of the transition state analog, thus simulating delicate but very important conformational changes that could take place during enzyme catalysis and binding. Such analysis also accounts for the important effects of the electrostatic environment that could be altered during experiments. We revealed in silico (without structural manipulations) that the strongest contribution in the near-UV CD is due to W210. The individual contribution of each aromatic chromophore was very dynamic with respect to structural changes and electrostatic effects. In contrast, the disulfide group contribution is relatively resistant to such structural dynamics but was dramatically influenced by alterations in the electrostatic environment.

Keywords Circular dichroism · Beta-lactamase · Matrix method · Aromatic and disulfide chromophores

1 Introduction

Chirality is one of the most fundamental properties of molecular and bimolecular systems and circular dichroism (CD) is an extremely powerful method in the dynamically developed areas of proteomics and drug design [1]. CD is an excellent choice for fast structural elucidation of proteins [2]. In the far-UV region it is extensively used for characterizing the secondary structure of proteins [3, 4], while in the near-UV the method is applied for understanding changes in protein tertiary structure resulting from interactions with ligands (substrates, agonists, inhibitors, allosteric modulators), mutations effects and environmental changes [5, 6]. Most recent instrumental developments made possible the utilization of synchrotron radiation for CD measurements which greatly expanded the abilities of the method and its applications in structural genomics [7]. Integrated application of CD measurements and calculations is found to be a very powerful method and is often the only choice for determination of the absolute configuration of small molecules (when X-ray crystallography is not possible) [1, 8, 9].

However, the CD method suffers from low signal resolution, and therefore it is difficult to analyze even contributions of individual chromophores in organic molecules

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with moderate size and relatively rigid conformations [1]. In proteins, due to the structural complexity, large number of chromophores and conformational flexibility, an analysis of individual chromophore contributions becomes a significantly more complicated task. In turn, the lack of this atomistic information leads to numerous limitations in its applications and to difficulties in relating the spectral data to atomistic structure and interactions. CD experiments with protein mutants may provide qualitative insight into the chromophore contributions; however, mutagenesis leads to subtle structural changes that alternate the orientations and interactions between the chromophores and thus can lead to misleading conclusions.

A key fundamental point toward completing this knowledge is to find an accurate and efficient approach in revealing the role of each individual chromophore, which meets following criteria:

i) It does not make a perturbation, even in smallest details in the structure.
ii) It is based on physically accurate theory, representing the electronic structural complexity of the rotational strength (molecular unit of the CD).
iii) It is fast enough, but reasonably accurate, to be applied to multiple structures.
iv) It is sensitive enough to detect and explain the finest structural changes related to catalysis, interactions with ligands and environmental changes.

Such a strategy will provide quantitative atomistic insight into the relationship between protein structure and the measured spectra. A second step would be to elucidate how the individual contributions respond to very fast structural changes related to catalysis and binding, which could be a valuable complementation to the time-resolved spectral experiments.

In this paper, we demonstrate for the first time a novel strategy, based on an established theoretical method, for obtaining missing crucial information about the contribution of each individual chromophore to the CD spectrum, which cannot be achieved by CD experiment, but successfully complements it. We performed this analysis on the near UV spectrum (240–300 nm) of a TEM-1 β-lactamase from *E. coli*—an enzyme that plays a central role in bacterial resistance to β-lactam antibiotics [10]. The free enzyme structure, two acyl-enzymes and the structure of the transition state analog were analyzed thus simulating the fine and delicate structural changes that could take place during time-resolved measurements. Many experimental procedures and measurements perturbate the electrostatic environment around chromophores (e.g. varying the ionic strength and pH); therefore, quantitative insight into the sensitivity of the individual chromophore contributions to electrostatic effects is important, and such an analysis is presented in the paper.

TEM-1 β-lactamase is a 29-kDa protein. It consists of a polypeptide chain, which exhibit an interesting folding. If the central section of the sequence folds into a globular unit containing most of the α-helices, the N- and C-terminals form a five-stranded β-sheet which is surrounded by the the helical unit and another group of small helices [11.]. The protein contains four tryptophans (165, 210 229 and 290), four tyrosines (46, 97, 105 and 264), five phenylalanines (60, 66, 72, 151 and 230) and one disulfide bond (formed between Cys77 and Cys123), which determine its chiroptical properties in the near-UV (Fig. 1). The CD spectrum of TEM-1 β-lactamase was calculated in very good agreement with the experimental one [12–14]. The influence of the structural changes and electrostatic interactions were analyzed in terms of mechanisms of rotational strengths [14–17].

The individual contribution of each aromatic and the disulfide chromophore (in total 14 chromophores) is revealed in the following ways:

i) The individual net rotational strength generated by each chromophore over all its transitions.
ii) The relative contribution of each chromophore with respect to the total CD, generated by the one-electron mechanism.

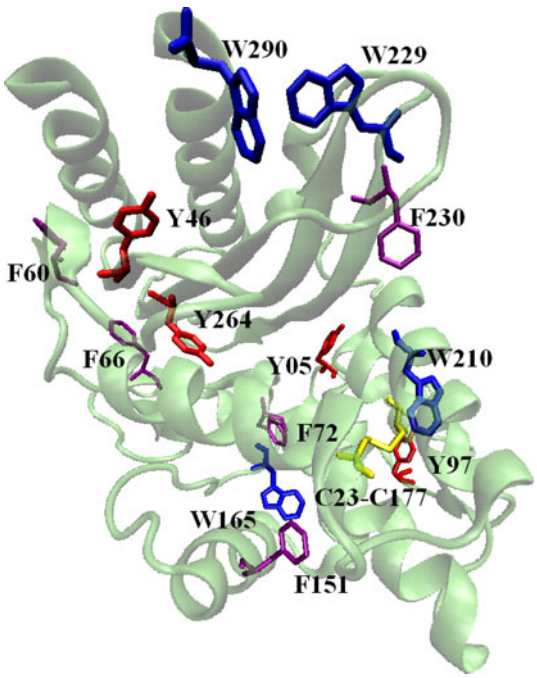

Fig. 1 TEM-1 β-lactamase: All near-UV chromophores are shown in liquorice. Tryptophans are shown in *blue*, tyrosines in *red*, phenylalanines in *purple* and the disulfide group in *yellow*

iii) The relative contribution of each chromophore with respect to the total CD, generated by coupled oscillator mechanism.
iv) The relative percentage contribution of each near-UV chromophore with respect to the total CD intensity.
v) Analysis of the influence of the electrostatic changes on the contributions.
vi) Analysis of the total percentage contributions of the aromatic and the disulfide chromophores.

2 Methodology

2.1 Initial structures

All calculations were done using the corresponding X-ray structures, taken from the Protein Data Bank (PDB) [18]. For the free enzyme, the crystal structure with pdb code 1btl was used [19]. In order to understand the sensitivity of the individual chromophore contributions to the fast, functionally important structural changes, an analysis was performed on two crystal structures (pdb codes 1tem and 1bt5), representative of the acyl-enzyme intermediate of the acylation reaction [20, 21] and a structure of the transition state analog of that reaction (pdb code 1axb) [22].

2.2 Theoretical background

Rotational strength is the most important molecular unit of the CD. It is defined as the imaginary part of the scalar product between the electric transition moment and the magnetic transition moment of a particular transition [23].

$$R = \mathrm{Im}\{\mu_{0i} \cdot m_{io}\} \quad (1)$$

Most protein chromophores (with the exception of disulfide groups and non-planar peptide groups) are achiral and become optically active under the influence of the protein environment [24]. This happens via three mechanisms: (i) the one-electron mechanism or static field effect [25], where electrically and magnetically allowed transitions in one chromophore interact with each other; (ii) coupling between electrically allowed transitions in two different groups (μ–μ mechanism) [26, 27] (the exciton effect is a degenerated case of this mechanism [28]); (iii) coupling between magnetically allowed and electrically allowed transitions in two separated groups (μ–m mechanism) [29]. Mechanisms (ii) and (iii) are also called coupled oscillators.

2.3 Computational method

Protein rotational strengths were calculated by applying the so-called matrix method, developed by Baylay, Nielsen and Schellman [30]. This method is derived from the first-order perturbation theory of Tinoco and utilizes matrix diagonalization, which makes it easy to implement in computer programs. The method is implemented in the program MATMAC, which was kindly provided by Prof. Joerg Fleischhauer (RWTH-Aachen, Germany) [31]. It also includes a modification by Goux and Hooker for the incorporation of the matrix elements of the momentum, in order to overcome the origin dependence of the magnetic transition moment [32]. The electric and magnetic dipole moments are chosen as in [33] and [13]. More details about the matrix method are presented in the supporting information and also can be find in [24, 30, 33, 34]. The monopoles are calculated as in [33, 35]. Experimental excitation energies were utilized for the excited states of the model chromophores as described in detail in [12, 13, 33].The visualization of the TEM-1 β-lactamase structure was done with VMD 1.8.4 [36].

2.4 Analysis of the individual chromophore contributions

Computational analysis of the individual chromophore contributions was performed using several criteria in order to systematically analyze their nature from different sides.

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

$$Rn, i = \sum_{j=1}^{n} |Ri, oj| \quad (2)$$

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

$$Rroe, i = (|Roe, i| \div |Rtot, oe|) \times 100 \quad (3)$$

The individual relative contribution to the total coupled oscillators rotational strength *Rrco* was calculated in the same manner.

The individual relative 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*:

$$Rrtot, i = (|Rn, i| \div |Rtot, all|) \times 100 \quad (4)$$

With the terms "coupled oscillator spectrum" or "one-electron spectrum" we define the part of the total rotational

strength (or CD intensity, CD spectrum) which is generated by the particular mechanism (i.e. the coupled oscillators one or one-electron one).

All rotational strength contributions from the chromophores were calculated in the interval 240–300 nm. The rotational strengths are presented in Debye-Bohr Magnetons (DBM).

3 Results and discussion

Our analysis is based on the calculated CD spectrum of TEM-1 β-lactamase, which is in reasonably good agreement with the experimental spectrum [12–14]. A comparison between the calculated and experimental CD spectra in the near-UV (in the interval 240–260 nm), where the disulfide group and all phenylalanines absorb, is presented in Fig. 2. A comparison between the calculated and experimental spectra in the interval 260–300 nm, where the tyrosine and tryptophan residues absorb, is presented in Fig. 3.

3.1 Aromatic chromophores

3.1.1 Tryptophans

Tryptophan (Trp) is an aromatic chromophore that not only contributes most intensively to protein absorption and CD spectra, but is also the most complex and the least symmetric. Tryptophan residues often participate in the binding sites of enzymes and receptors; therefore, their spectral contributions can provide reasonable information and structural insight into ligand binding. The indole group of Trp has two transitions, namely Lb and La, that overlap at around 280 nm and contribute to the near-UV spectra. Tryptophan is very sensitive to environmental changes—a possible reason is that the La transition is characterized by a large change in the electric dipole moment [37]. The experimental transition moment directions have been determined for the Trp transitions, which provide a good base for accurate calculations of tryptophan CD spectra [38]. In TEM-1 beta-lactamase, there are four tryptophan residues—165, 210, 229 and 290.

3.1.1.1 W165 This residue is situated in a solvent accessible area in the all-α domain (Fig. 1). W165 forms a contact with N136, I142, T140, P145, L162 and the phenylalanine chromophore F72 (Fig. 4). Its contributions to the CD spectra of the free enzyme, acyl-enzyme and transition state structures are shown in Table 1. The net rotational strength of the free enzyme is 0.081. It generates 4.24% from the total one-electron rotational strength and 3.74% from the total rotational strength in the near-UV of the free enzyme. The structural change leading to 1tem acyl- enzyme structure changes its contributions to the CD spectrum considerably. All types of contributions (the individual net, the contribution to the total one-electron RS and to the total CD) decreased almost four times in respect to the free enzyme. The structural change related to the formation of the second structure, that is representative for the acyl-enzyme—1bt5, leads to an increase in the individual contribution of W165 with respect to the free enzyme. Its relative participation in the total electron rotational strength is increased from 4.24% in 1btl to 7.13% 1bt5. Consequently, the relative contribution of that chromophore with respect to the total rotational strength is increased from 3.74 to 6.10%. In the structure 1axb, which is representative of the transition state, the increase in all kinds of contributions is considerably higher than in the 1bt5 structure, and the contribution with respect to the total rotational strength is 11%. The electrostatic interactions in the free enzyme structure lead to an increase in the

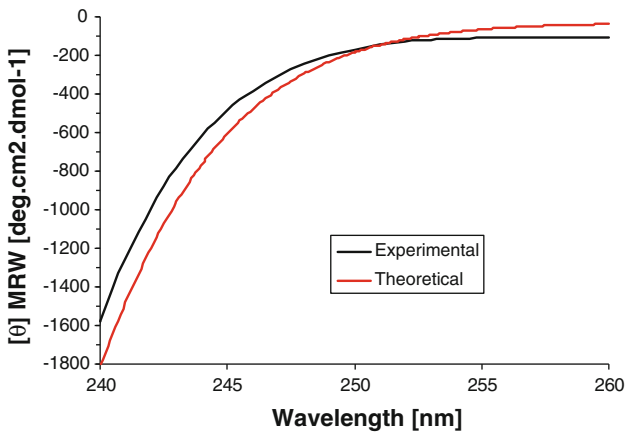

Fig. 2 Comparison between experimental and computed CD spectrum of TEM-1 in the interval 240–260 nm

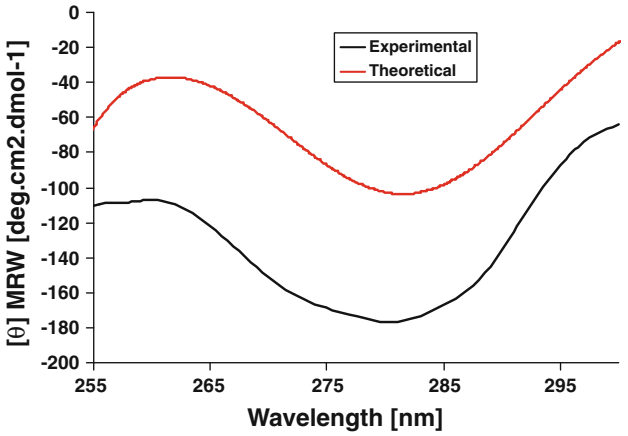

Fig. 3 Comparison between experimental and computed CD spectrum of TEM-1 in the interval 255–300 nm

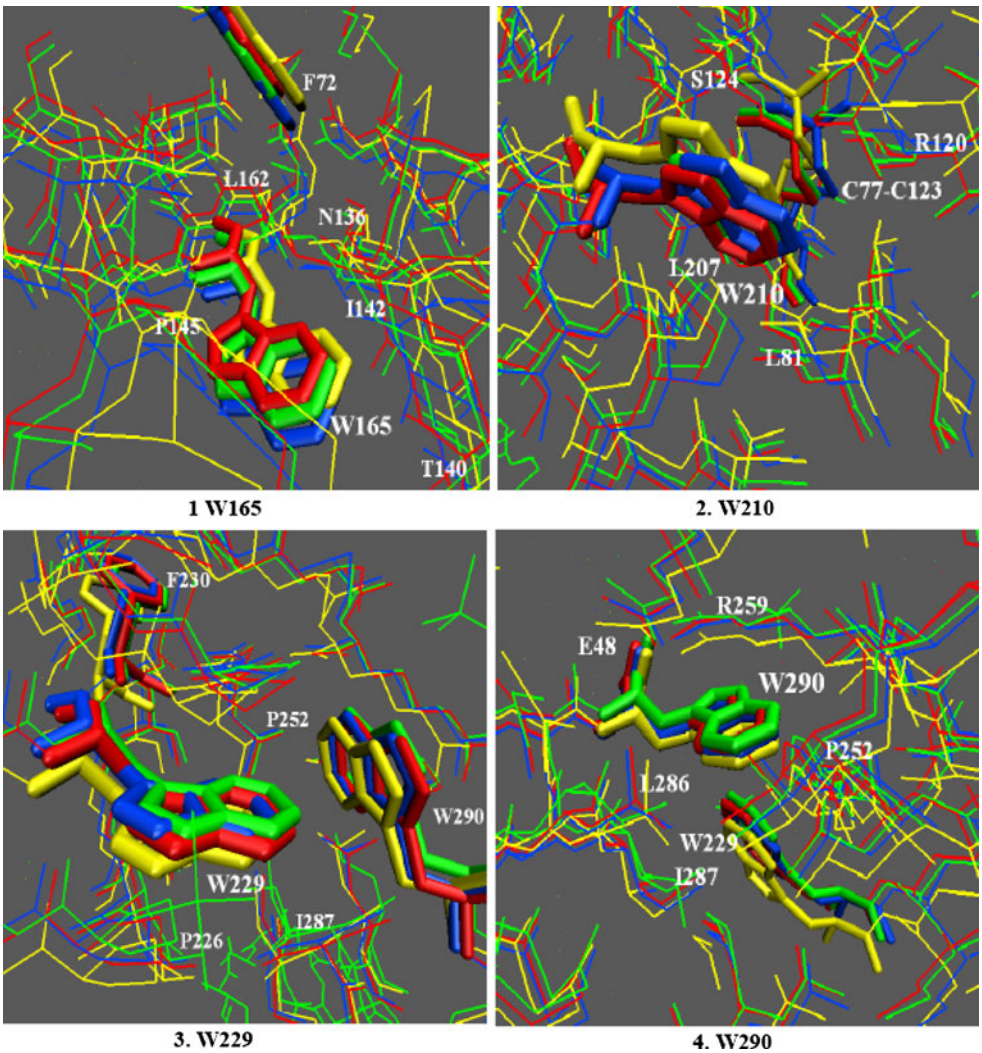

Fig. 4 Superimpositions of the structures around the tryptophan chromophores between the free enzyme 1btl (in *green*), acyl-enzyme structure 1tem (in *red*), acyl-enzyme structure 1bt5 (in *blue*) and transition state analogous structure 1axb (in *yellow*): *1* W165, *2* W210, *3* W229, *4* W290

individual net contribution and in the individual relative contribution with respect to the total rotational strength. This effect is comparable to that due to the structural change to the 1bt5 acyl-enzyme structure; however, the contribution to the total one-electron mechanism is considerably higher.

3.1.1.2 W210 W210 is located in a solvent accessible area, almost opposite W165 within the all-α domain (Fig. 1). It is situated close to the disulfide chromophore and to the S124, R120, L207 and L81 side chains (Fig. 4). The individual net contribution ins the free enzyme structure is high (0.838 DBM), and its contributions to the one-electron and total rotational strength are almost ten times higher than those of W165 (i.e. 44 and 39 vs. 4.2 and 3.7%, respectively; Table 2). In the acyl-enzyme structure, 1tem, the individual net contribution, as well as the contributions to the total "one-electron" and the overall total rotational strength are even higher than those in the free enzyme. This trend is also preserved in the acyl-enzyme structure, 1bt5, and the transition state structure, 1axb. In the latter structure, W210 exhibits the strongest contributions (1.142-DBM net contribution, 45% from the total rotational strength and more than half from the one-electron rotational strength). The accounting for the electrostatic interactions, however, leads to a strong decrease in the net contribution (0.398 and change in its sign), also to a dramatic lowering of the contribution to the total one-electron rotational strength (0.18%) and a lowering of the percentage contribution to the total CD (to 18.25%). It is important to mention that when the local Coulomb interactions are taken into account, a considerable coupled oscillator contribution arises.

Table 1 Contributions of W165 in the free enzyme structure (1btl), acyl-enzyme structure (1tem), acyl-enzyme structure (1bt5), transition state analogous structure (1axb) and under effects of electrostatic interactions (EI)

W165	Individual net contribution	Relative % contribution to the total one electron RS	Relative % contribution to the total coupled oscillators RS	Relative % contribution to the total RS
1btl	0.081	4.24	0.00	3.74
1tem	0.020	0.97	0.00	0.92
1bt5	0.129	7.13	0.00	6.10
1axb	0.279	13.60	0.00	11.04
EI	0.123	22.40	0.00	5.63

Table 2 Contributions of W210 in the free enzyme structure (1btl), acyl-enzyme structure (1tem), acyl-enzyme structure (1bt5), transition state analogous structure (1axb) and under effects of electrostatic interactions (EI)

W210	Individual net contribution	Relative % contribution to the total one electron RS	Relative % contribution to the total coupled oscillators RS	Relative % contribution to the total RS
1btl	0.838	43.85	0.00	38.71
1tem	0.933	45.45	0.00	43.38
1bt5	0.695	38.42	0.00	31.17
1axb	1.142	55.58	0.00	45.17
EI	0.398	0.18	26.78	18.25

3.1.1.3 W229 The residue is located in the $\alpha\beta$-domain in a solvent accessible area just at the boundary between a β-sheet and an α-hellix (Fig. 1). Superimposed picture of all four protein structures is presented in Fig. 4. It interacts with chromophore W290 and with the R252, S258, R259, C221 and P226 residues. W210 has a considerable role in all kinds of contributions (Table 3). In the free enzyme, it has a 0.734-DBM net individual contribution, which provides 38.41% from the "one-electron" rotational strength and almost 34% from the total rotational strengths in the near-UV. In the acyl-enzyme structures, 1tem and 1bt5, the net individual contribution is a little lower 0.668 and 0.538 DBM, respectively. The same trend is also found in the percentage contribution of W229 to the "one-electron" rotational strength (32.59 and 29.74%) and the total rotational strengths (31.10 and 25.45%). In the 1tem structure, W229 generates weak contributions to the coupled oscillator rotational strength (through coupling with W290). The transition state analog structure, 1axb, is interesting with respect to the chiroptical contribution of that residue. It is almost two times lower than in the previous two structures, it generates only 0.63% from the total one-electron rotational strength, and the biggest part from the "coupled oscillator" rotational strength—70.95%. Its contribution to the total rotational strength is 13.84%, which is the lowest in all of the structures for this chromophore.

This chromophore is also sensitive to the local electrostatic environment, and when it is accounted for the W210 net contribution decreases from 0.734 to 0.484 DBM. The relative contribution to the total one-electron rotational strength is dramatically reduced (from 38.41 to 2.37%). In contrast the percentage contribution to the "coupled oscillators," the rotational strength is dramatically increased from 0 to 29%. The contribution to the total rotational strength is decreased from 34 to 22%.

3.1.1.4 W290 W290 is located in the $\alpha\beta$-domain in a solvent accessible area in front of W229 and at the boundary between an α-hellix and a β-sheet (Fig. 1). Within its vicinity, chromophore W229 and R259, E48, L286, I287 and P252 (Fig. 4) are located. W290 plays smaller role in the near-UV CD spectrum of all TEM-1 structures (Table 4). In the free enzyme, it has the following contributions: 0.041 DBM individual net, 2.14% with respect to the total one-electron rotational strength, 7.87% with respect to the total "coupled oscillators" type and less than 2% from the total near-UV rotational strength of the free enzyme. The conformational change induced by conversion of this structure to the acyl-enzyme structure, 1tem, increases and changes the sign of the individual net contribution to 0.113 DBM. However, it decreases the relative participation of that chromophore in the total one-electron rotational strength and dramatically increases its relative contribution to the total couple cluster rotational strength to 98%. The proportion from the total rotational strength due to W290 is increased to 5.25%. In the 1bt5 acyl-enzyme structure, the net individual role of W290 is

Table 3 Contributions of W229 in the free enzyme structure (1btl), acyl-enzyme structure (1tem), acyl-enzyme structure (1bt5), transition state analogous structure (1axb) and under effects of electrostatic interactions (EI)

W229	Individual net contribution	Relative % contribution to the total one electron RS	Relative % contribution to the total coupled oscillators RS	Relative % contribution to the total RS
1btl	0.734	38.41	0.00	33.90
1te	0.669	32.59	2.0	31.10
1bt5	0.538	29.74	0.00	25.45
1axb	0.350	0.63	70.95	13.84
EI	0.484	2.37	28.87	22.19

Table 4 Contributions of W290 in the free enzyme structure (1btl), acyl-enzyme structure (1tem), acyl-enzyme structure (1bt5), transition state analogous structure (1axb) and under effects of electrostatic interactions (EI)

W290	Individual net contribution	Relative % contribution to the total one electron RS	Relative % contribution to the total coupled oscillators RS	Relative % contribution to the total RS
1btl	0.041	2.14	7.87	1.89
1tem	0.113	0.83	98.0	5.25
1bt5	0.160	7.30	0.00	6.24
1axb	0.165	1.56	28.00	6.53
EI	0.366	5.10	20.71	16.78

increased with respect to the free enzyme (0.160 DBM). Part of the total one-electron rotational strength is increased to 7.30%; however, there is no contribution to the coupled oscillator rotational strength. In this structure residue W290 generates 6.24% from the total rotational strength. In the transition state-like structure 1axb, the individual net effect is comparable to that in 1bt5 structure. However, the contribution to the total one-electron rotational strength is lower than that in the free enzyme and in 1bt5 the acyl-enzyme. It takes a considerable part of the total coupled oscillators circular dichroism—28%. The relative part from the total rotational strength is similar to that in the 1bt5—6.53%.

This chromophore is dramatically sensitive to electrostatic changes. Its individual contribution in the free enzyme is increased under such effects from 0.041 to 0.288 DBM. The relative part from the total "one-electron" spectrum is increased to 5% and the contribution to the total "coupled oscillators" CD is increased to almost 21%. It is important to notice that the percentage contribution of W290 to the total CD is increased under electrostatic effects from 1.89 to 16.78%.

3.1.2 Tyrosines

The tyrosine side chain chromophore contains an alkylated phenol in which the phenolic oxygen perturbs the benzene much more strongly than the alkyl substituent. Relative to benzene, it has its electronic transitions red-shifted and intensified (e.g. Lb transition); however, they are polarized in the same directions, like the transitions in benzene [6]. It is the only aromatic chromophore that undergoes ionizations at a pKa near to neutrality (pK $\sim$ 9.5). TEM-1 enzyme contains four tyrosines—46, 97, 105 and 264.

3.1.2.1 Y46 Y46 located in the $\alpha\beta$-domain, at the β-sheet to α-helical part of the domain, in a relatively solvent accessible area (Fig. 1). In its surroundings, chromophores W290, Y264, F60, F66 and residues E58 and L250 (Fig. 5) are located. In the free enzyme, its net individual rotational strength is 0.128. Y46 does not take part in the one-electron CD but provides half from the total coupled oscillator spectrum (Table 5). Overall, in the free enzyme, it contributes 6% to the total near-UV CD spectrum. Structural changes induced by ligand binding in the acyl-enzyme and transition state structures lead to a decrease in its net individual and percentage contribution with respect to the total near-UV CD intensity. In particular, in the 1tem and 1bt5 structures, the net individual rotational strength of Y46 is similar (0.022 DBM in 1tem and 0.022 DBM in 1bt5). There is a slight contribution in both structures to the total one-electron spectrum (around 1%), and in contrast to the free enzyme, there is no participation in the coupled oscillators spectrum. The relative proportion from the total CD is very slight in both structures—around 1%. The situation in the transition state analog structure, 1axb, is similar to both acyl-enzymes, but with slightly higher contributions. The net individual, total one-electron CD

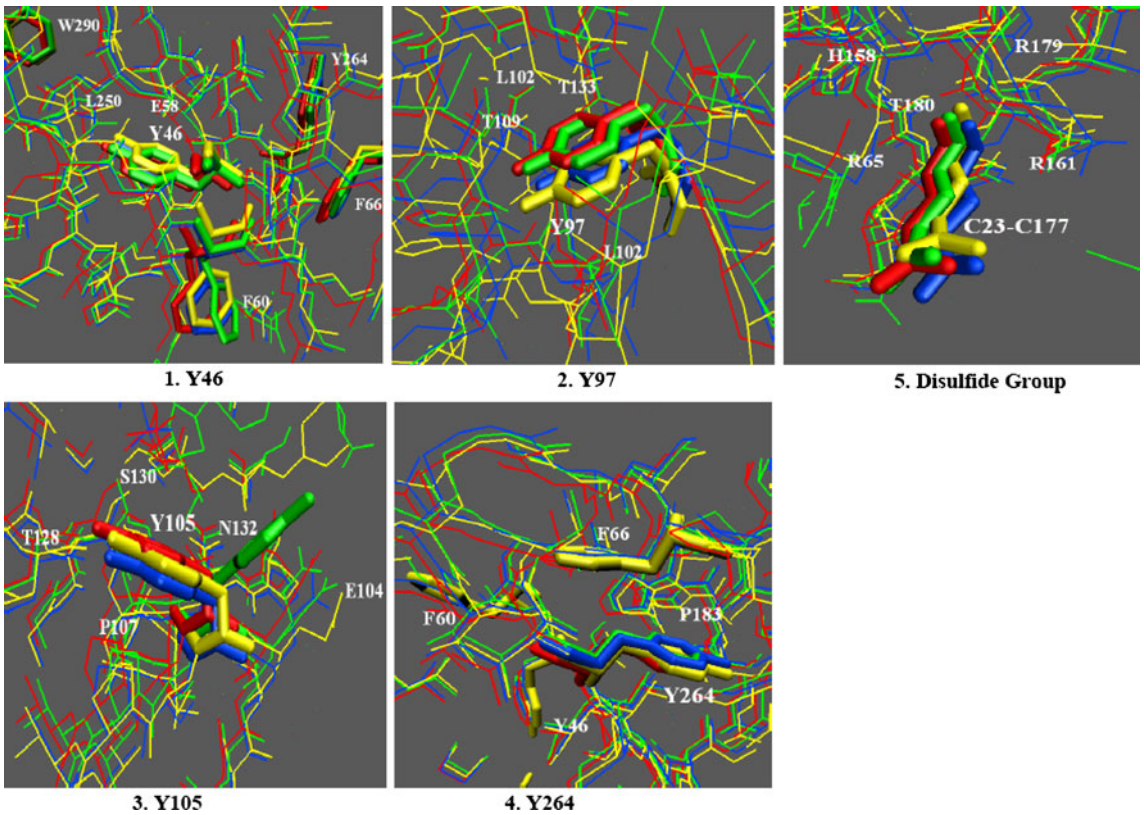

Fig. 5 Superimpositions of the structures around the tyrosine and the disulfide chromophores between the free enzyme 1btl (in *green*), acyl-enzyme structure 1tem (in *red*), acyl-enzyme structure 1bt5 (in *blue*) and transition state analogous structure 1axb (in *yellow*): *1* Y46, *2* Y97, *3* Y105, *4* Y264 and *5* the disulfide group

and total spectrum contributions are approximately three times higher than in the acyl-enzymes, but are still lower than in the free enzyme.

The electrostatic effects decrease the absolute value of the net individual rotational strength and change its sign. They also decrease the contribution of Y46 with respect to the total rotational strength from 5.91 to 3.71%. Y46 alters its tendency to participate in one-electron and coupled oscillator mechanisms under the electrostatic influence. While it generates 15% from the total one-electron CD here, it does not participate in coupled oscillators CD.

3.1.2.2 Y97 Y97 tyrosine chromophore is located at the top part of the all-α domain (in the coiled motif that links a 3_10 helix to an extended β-structure) shown in Fig. 1. It is surrounded by the side chains of L102, T133, T109, L102 and the Y105 chromophore (Fig. 5). Y97's CD contributions are presented in Table 6. For the free enzyme structure 1btl, Y97's individual net contribution is 0.062 DBM. It has no one-electron CD contribution and provides 24.41% from the coupled oscillator spectrum. The relative participation of Y97 with respect to the total near-UV CD is 2.86%. The individual contribution of Y97 in both of the acyl-enzyme structures is quite different—in the 1tem structure, it has minor individual, one-electron and total contributions. In the 1bt5 structure, its individual net and percentage contribution from the total CD spectrum are more significant and almost two times larger than in the free enzyme (respectively, 0.120 DBM and 5.67%).

Electrostatic effects can play a considerable effect on the CD contributions of Y97, decreasing almost all types of its contribution dramatically in the free enzyme. It generates a very slight contribution to the one-electron CD, but the contribution to the total spectrum can be neglected if the electrostatic environment is accounted for.

3.1.2.3 Y105 Y105 is located within the all-α domain at the turn structure that links two 3_10 helices. In its near vicinity, L102, T133, T109, L102 and chromophore Y105 are located (Fig. 5). An analysis of the Y105 CD contributions is provided in Table 7. In the free enzyme, Y105 contributes with 13.78% from the total coupled oscillators spectrum, does not contribute to the total one-electron spectrum and gives 1.61% from the total near-UV CD. In the 1tem acyl-enzyme, Y105 provides a small 4% contribution to the one-electron spectrum and gives no contribution to the coupled oscillators total rotational strength, in contrast to its behavior in the free enzyme. The relative part

Table 5 Contributions of Y46 in the free enzyme structure (1btl), acyl-enzyme structure (1tem), acyl-enzyme structure (1bt5), transition state analogous structure (1axb) and under effects of electrostatic interactions (EI)

Y46	Individual net contribution	Relative % contribution to the total one electron RS	Relative % contribution to the total coupled oscillators RS	Relative % contribution to the total RS
1btl	0.128	0.00	50.39	5.91
1tem	0.022	1.07	0.00	1.02
1bt5	0.024	1.33	0.00	1.14
1axb	0.076	3.70	0.00	3.01
EI	0.081	14.75	0.00	3.71

Table 6 Contributions of Y97 in the free enzyme structure (1btl), acyl-enzyme structure (1tem), acyl-enzyme structure (1bt5), transition state analogous structure (1axb) and under effects of electrostatic interactions (EI)

Y97	Individual net contribution	Relative % contribution to the total one electron RS	Relative % contribution to the total coupled oscillators RS	Relative % contribution to the total RS
1btl	0.062	0.00	24.41	2.86
1tem	0.004	0.20	0.00	0.19
1bt5	0.12	0.00	39.21	5.67
1axb	0.006	0.29	0.00	0.24
EI	0.002	0.36	0.00	0.1

Table 7 Contributions of Y105 in the free enzyme structure (1btl), acyl-enzyme structure (1tem), acyl-enzyme structure (1bt5), transition state analogous structure (1axb) and under effects of electrostatic interactions (EI)

Y105	Individual net contribution	Relative % contribution to the total one electron RS	Relative % contribution to the total coupled oscillators RS	Relative % contribution to the total RS
1btl	0.035	0.00	13.78	1.61
1tem	0.082	4.00	0.00	3.81
1bt5	0.181	0.00	59.15	8.56
1axb	0.214	10.42	0.00	8.47
EI	0.017	3.10	0.00	0.78

from the total CD due to Y105 residue is 3.81%. In the 1bt5 and 1ab structures, the role of Y105 is considerably significant. Its net individual contributions are higher 0.181 DBM in the 1bt5 structure and 0.214 DBM in the 1axb structure. In 1bt5, it provides almost 60% of the total coupled oscillator spectrum and has no one-electron contribution. However, in 1axb, its behavior is mirrored and provides 10% from the total one-electron rotational strength and has no coupled oscillator activity. The percentage participation from the total rotational strength in both structures is almost the same—around 8.5%.

The incorporation of electrostatic interactions not only leads to decrease the individual net contribution of Y105 and its relative contribution of the near-UV CD by 50%, but also alters its mechanism from coupled clusters to one electron.

3.1.2.4 Y264 Y264 shown in Fig. 1 is located in the $\alpha\beta$-domain of the β-sheet, and its side chain is in the opposite direction to that of Y46. F60, F66, P183 and Y46 are closely located to Y264. Its orientation in the superimposed modeled structures is presented in Fig. 5. This residue has very low contributions to the CD, comparable to those of some of the phenylalanine chromophores (Table 8). It contributes very slightly only to the one-electron CD and has no coupled oscillator contributions. Electrostatic interactions cancel its influence. In the free enzyme, 1btl and the 1tem acyl-enzyme structures it contributes between 0.5 and 0.8% to the total near-UV CD, and in the 1bt5 and the 1xab structures its contribution is even lower.

3.1.3 Phenylalanine contributions

The phenylalanine chromophores have small contributions to the near-UV CD. Their analysis is presented in the Supporting information (p. 2 and Table S1).

Table 8 Contributions of Y264 in the free enzyme structure (1btl), acyl-enzyme structure (1tem), acyl-enzyme structure (1bt5), transition state analogous structure (1axb) and under effects of electrostatic interactions (EI)

Y264	Individual net contribution	Relative % contribution to the total one electron RS	Relative % contribution to the total coupled oscillators RS	Relative % contribution to the total RS
1btl	0.011	0.58	0.00	0.51
1tem	0.017	0.83	0.00	0.79
1bt5	0.001	0.055	0.00	0.05
1axb	0.001	0.05	0.00	0.04
EI	0.000	0.00	0.00	0.00

Table 9 Contributions of the disulfide chromophore in the free enzyme structure (1btl), acyl-enzyme structure (1tem), acyl-enzyme structure (1bt5), transition state analogous structure (1axb) and under effects of electrostatic interactions (EI)

Disulfide	Individual net contribution	Relative % contribution to the total one electron RS	Relative % contribution to the total coupled oscillators RS	Relative % contribution to the total RS
1btl	0.199	10.41	0.00	9.20
1tem	0.259	12.62	0.00	12.04
1bt5	0.244	13.49	0.00	11.54
1axb	0.279	13.59	0.00	11.04
EI	0.732	31.88	23.11	33.56

3.2 Disulfide chromophore

A disulfide bond formed by the oxidation of the thiol groups of two nearby located cysteine residues plays a crucial role in the protein structure formation and stabilization [39, 40]. Delicate conformational changes and the isomerization of the disulfide group are vitally important for the accurate folding of the globular proteins [41, 42]. The disulfide group takes a special place in the protein chiroptical system. In fact, it is the only intrinsically chiral protein chromophore. It is well known that the total rotational strength of the free disulfide chromophore in solution is zero because of the free rotation around the single bond. In the protein environment, it exhibits a non-zero rotational strength because of restraint of its particular conformation within the protein environment [6].

The disulfide group located in the all-α domain links two nearby α helices shown in Fig. 1. It is closely positioned to residues H158, R179, T180, R65, P161 and chromophore W210 (Fig. 5). The net individual contribution of the disulfide chromophore in the free enzyme structure is 0.199 (Table 9). The relative part of the total one-electron CD due to the disulfide chromophore is 10.41%. With respect to the total near-UV rotational strength, it generates 9.20%. The interactions of the free enzyme with small ligands leading to analogs of the acyl-enzyme and transition state increase the individual net contribution of the disulfide group (0.259 DBM in 1tem, 0.244 DBM in 1bt5, and the strongest is the contribution in the 1axb transition state analog structure). The disulfide group saves the tendency to contribute to the total one-electron rotational strength (from 10.41 to 13.59%) in all structures. The strongest value has the one-electron disulfide contribution in 1axb and 1bt5 structures. It is important to mention that in all structures, of the free enzyme and the complexes, the disulfide group contributes only to the one-electron spectrum but not to the coupled oscillator type spectrum. The disulfide part from the total near-UV CD varies between 9.20 and 12.04%. In general, the acyl-enzyme and transition state structures exhibit a slightly higher disulfide contribution than those of the free enzyme. A relationship between the values of the disulfide torsion angle (CB-SG-SG-CB) in all structures is presented in Fig. S1 in the supporting information. The optimal value of the angle which corresponds to highest individual contribution of the disulfide group in respect to the total CD is -110.1° in the 1tem acyl-enzyme structure.

The accounting for electrostatic interactions strongly alters the qualitative and quantitative picture of the disulfide CD. In the free enzyme structure, the net individual contribution is increased more than three times (from 0.199 to 0.732 DBM). The relative part from the total one-electron spectrum due to the disulfide groups is increased three times (from 10.41 to 31.88%). Now, it also contributes to the total coupled oscillator spectrum and provides 23.11%. Under electrostatic effects, the disulfide part from the total near-UV CD is increased from 9.20 to 33.56%.

We can conclude that the CD contribution of the disulfide chromophore is relatively resistant to structural changes induced by interactions with ligands, at the same time it is considerably sensitive to a change in the electrostatic environment.

Table 10 Total contributions of the aromatic and the disulfide chromophores in the free enzyme structure (1btl), acyl-enzyme structure (1tem), acyl-enzyme structure (1bt5), transition state analogous structure (1axb) and under effects of electrostatic interactions (EI)

Contributions	1btl	1tem	1bt5	1axb	EI
All tryptophans	78.24	80.65	68.96	76.58	62.85
All tyrosines	10.90	5.81	15.42	11.76	4.59
All phenylalanines	1.57	1.23	0.39	0.32	0.39
Total aromatics	90.71	87.69	84.77	88.66	67.83
Total disulfide	9.20	12.04	11.54	11.04	33.56
Total one-electron spectrum	88.27	95.44	85.57	81.21	74.87
Total coupled oscillators spectrum	11.32	4.56	14.43	18.78	25.13

3.3 Analysis of the total contributions

The total contributions of the aromatic and the disulfide chromophores are presented in Table 10. All contributions are given with respect to the total rotational strength. In the free enzyme, 1btl, all tryptophan residues provide the main part of the near-UV CD spectrum, 78.24%. The conformational changes related to the formation of the 1tem acyl-enzyme structure and 1axb structure slightly influence the total tryptophan percentage contributions. The structural change related to the formation of 1bt5 has a more sensitive effect (decreases the contribution to 69%). All tyrosine contributions are more sensitive to conformational changes. The effect is highest in the 1bt5 structure (15.42) and lowest in the 1tem structure (5.81%). In the free enzyme and in 1axb, it has intermediate values of 11–12%. The phenylalanine chromophores exhibit very weak total contributions—between 0.3 and 1.5%. In total, the aromatic residues provide 91% from the total CD in the free enzyme, which slightly varies under structural changes—88% in 1tem, 85% in 1bt5 and 89% in 1axb. The disulfide group generates between 9.205 and 12.04% of the total CD spectrum.

The electrostatic interactions decrease all tryptophan's contribution from 78 to 63%, the tyrosine contributions by half, from 11 to 5%, and the phenylalanine contributions from 1.6 to 0.4%. The contributions of all aromatic chromophores decrease from 91 to 68% when electrostatic interactions are included. However, the effect of electrostatic interactions on the disulfide contribution is opposite to and more sensitive than that of the aromatics—total disulfide contribution is increased three times (from 9.20 to 33.56%).

From a mechanistic point of view, it is also interesting to estimate the contributions of both the one-electron and the coupled oscillator mechanisms to the total near-UV CD spectrum. In all structures, with or without electrostatics included, it is a common trend that the one-electron mechanism dominates the CD spectrum. However, its relative contribution is influenced by conformational and electrostatic changes. In the free enzyme, the one-electron rotational strength is 88% of the total spectrum, and in 1bt5 and 1axb its contribution is slightly different; however, in the 1tem structure, the one-electron mechanism is responsible for more than 95% from the spectral intensities. The electrostatic effects decrease the one-electron character of the spectrum from 88 to 75%. The coupled oscillator mechanism type ($\mu \rightarrow \mu$ and $\mu \rightarrow$ m mechanisms taken together) provides 11% of the CD intensity in the free enzyme which is increased to 14% in 1bt5 and up to 19% in the 1axb structure, where in the 1tem structure it decreases to 5%. Electrostatic interactions lead to a considerable increase in the coupled oscillator contributions from 11 to 25%.

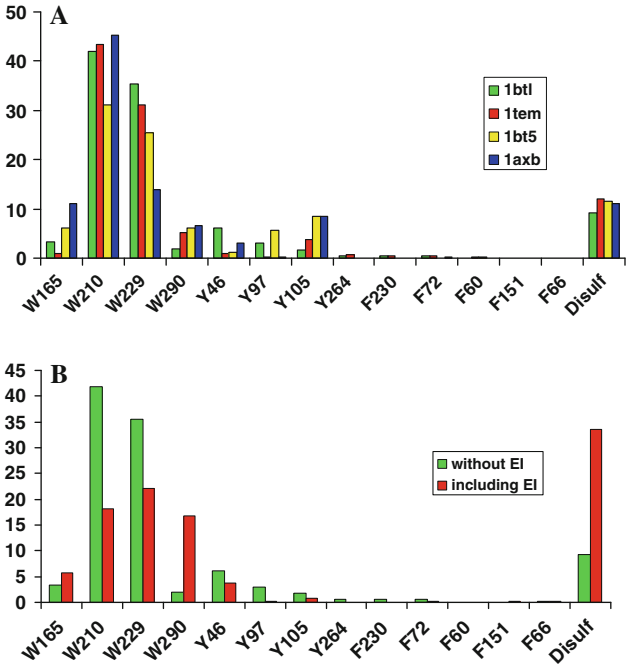

Fig. 6 Dynamics of the individual chromophore contributions: **a** as function of structural changes; **b** as function of the electrostatic changes

The dynamics of the individual contributions of all chromophores as a function of the structural changes are presented in Fig. 6a. It can be seen that where the aromatic chromophores are quite sensitive in total to fast small structural changes related to catalysis, the disulfide group is relatively resistant to them. W210 expresses the strongest contribution to the near-UV spectrum in all structures, which varies between 30 and 45%, followed by W229 which is even more sensitive to structural flexibility (varies between 14 and 34%) and the disulfide group (small changes between 9 and 11%). The electrostatic environment (Fig. 6b) leads to a strong decrease in the contributions of W210 and W229.

It increases the disulfide contribution by more than three times, making the disulfide group the most contributive chromophore to the total CD intensity.

4 Conclusion

The analysis made in this paper demonstrates the potential of computational chemistry to provide insights into the contributions of individual chromophores to protein CD spectra, which cannot be received with existing experimental techniques. Such a computationally aided atomistic chromophore analysis successfully complements the CD experiment, e.g. in mechanistic and time-resolved studies. The method, in combination with other established methods for fast structural changes, for example enzyme QM/MM modeling, can provide strong atomistic background and interpretation of the observed spectroscopic phenomena.

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