Calculation of the CD Spectrum of Class A β-Lactamase from *Escherichia coli* (TEM-1)

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Z. Naturforsch. **56 a,** 757–760 (2001); received September 12, 2001

The Circular Dichroism (CD) spectrum of β-lactamase from *Escherichia coli* (**TEM-1**) has been calculated with the matrix method on the basis of the x-ray diffraction structure. All known transitions in the peptide and side-chain groups, especially the aromatic and disulfide groups have been included. The calculations were performed with and without the tryptophan (Trp) residues. Rotational strengths calculated with the matrix method were combined with Gaussians to generate the CD spectrum. The calculated spectrum reproduces the signs and approximate magnitudes of the CD bands rather well only when the trytophan side chains are included. However, the experimental negative double band at 208 and 222 nm, which is characteristic for α-helices, is absent in the calculated spectrum.

Key words: β-Lactamase; CD-spectrum; Matrix Method.

1. Introduction

β-lactamases are enzymes which are produced by pathogenic microorganisms which catalyze the hydrolysis of β-lactam antibiotics and are the major reason for bacterial resistance. A large number of this type of enzyme has been studied, and four classes (A, B, C and D) are recognized on the basis of their primary structures and substrate specificities [1, 2]. The classes A, C, and D are unique serine proteases.

We wanted to calculate the CD-spectrum of a class A β-lactamase (isolated from *E.-coli*, TEM-1, EC 3.5.2.6, relative molecular mass: 28907 Da). For this enzyme the crystal structure [3] and the CD-spectrum [4] are known. For the calculation we used the so-called matrix method [5] with modifications by Goux and Hooker [6]. This method is implemented in our program MATMAC [7] and had been successfully applied to the calculation of the CD-spectra of other proteins [8].

2. Materials and Methods

For the calculations we took the coordinates of the x-ray structure (see Fig. 1) of the β-lactamase **TEM-1** at 1.8 Å resolution from the Protein Data Bank (File: 1btl.pdb) [9]. For this protein 1047 basis functions were incorporated. Each of these functions is a product of 353 group wave functions, of which 352 are ground states and one is an excited state. Functions with two or more excited groups were not taken into account. In order to set up the matrix of the hamiltonian, the positions and charges of the monopoles for the different groups are needed. For all the groups besides Trp we used the parameters from [8]. For the Trp side chain we took the indole molecule as the model chromophore and performed an INDO/2S-CI calculation [11] with only singly excited configurations with the programs DZDO and MCD3SP [12]. We took into account transitions from the 13 highest occupied into the 13 lowest unoccupied molecular orbitals. From the transition and permanent electric densities the monopoles for indole were then calculated with the numerical procedure described in [13, 8]. The parameters used for indole are collected in Table 1. The orientation of the calculated electric transition moments (see Fig. 2) are in qualitative agreement with the experimentally determined moments [14]. For the transition energies experimental values were used [14].

0932–0784 / 01 / 1100–0757 $ 06.00

Fig. 1. Structure of the TEM-1 β-lactamase. Helical regions are drawn as cylinders and beta-sheets as arrows. Random coil regions are symbolized as tubes. The side chains which were used in the calculation are drawn as sticks and balls. The figure was created with the program VMD [10].

Table 1. Experimental transition energies [14], calculated electric transition moments and oscillator strengths, and experimental oscillator strength [15] of indole.

Transition	Energy [cm^{-1}]	μ_x [D]	μ_y [D]	$f_{calc.}$	$f_{exp.}$ [15]
L_b	34423	–0.496	–0.599	0.01	0.01
L_a	35842	3.151	–1.310	0.19	0.12
B_b	45045	4.560	3.300	0.60	0.68
B_a	50000	3.890	–1.746	0.38	–

To generate the CD-spectrum, the calculated rotational strengths were combined with Gaussian band shape functions. Except for the disulfide transitions, the half bandwidths used were 16.8 nm in the spectral range between 300 and 250 nm, 14.1 nm between 250 and 157 nm, and 9.2 nm below 157 nm. For the disulfide we used a halfbandwidth of 30 nm for the long-wavelength transition and 15 nm for the short-wavelength component [8].

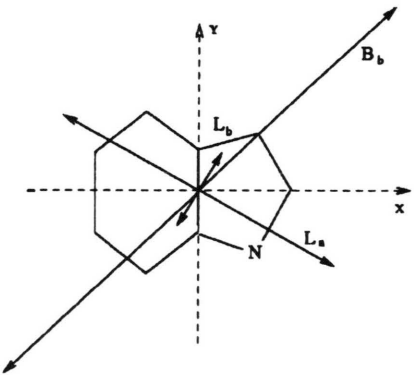

Fig. 2. Calculated electric transition moments.

3. Results and Discussion

3.1. The Near UV CD-spectra

The experimental and calculated CD-spectra of β-lactamase in the near UV are shown in Figure 3. The experimental CD-spectrum exhibits a negative band in the region between 240 - 300 nm. The calculated CD-spectrum without the Trp residues shows a positive band in this region, whereas the calculation with the tryptophans is in better agreement with the experiment. From the analysis of the matrix method results in Table 2 it can be seen that the main contribution to this negative band originates from an L_a transition in the Trp(210) residue.

3.2. The Far UV CD-spectrum

The CD in the far UV (see Fig. 4) is a reflection mainly of the secondary structure of the protein [16]. The spectrum can be decomposed into at least three bands: two negative bands at 208 and 222 nm and a positive one near 190 nm. This set of three bands is characteristic of the α-helix. The 222 nm band is theoretically assigned to the $n\pi^*$ transition of the peptide chromophores, while the other two bands result from exciton splitting of the $\pi\pi^*$ transition of peptide

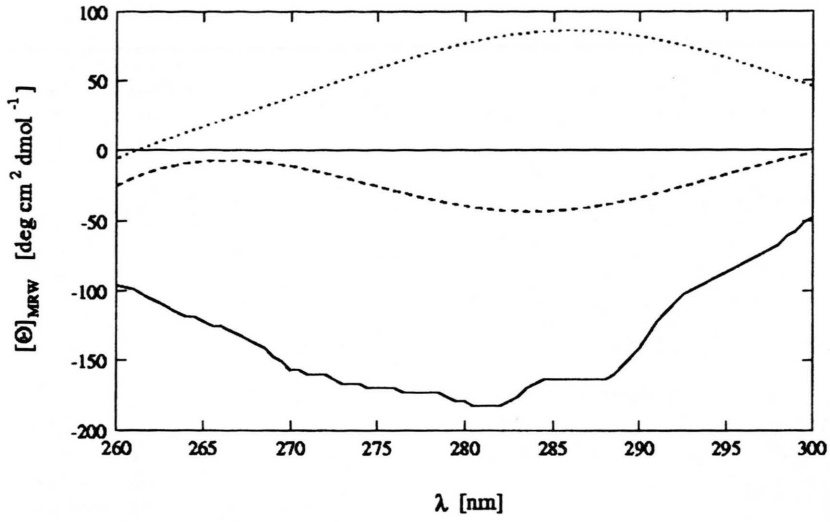

Fig. 3. Experimental and calculated near UV CD spectra of β-Lactamase; . . . : calculated without Trp, - - -: calculated with Trp, —: experimental.

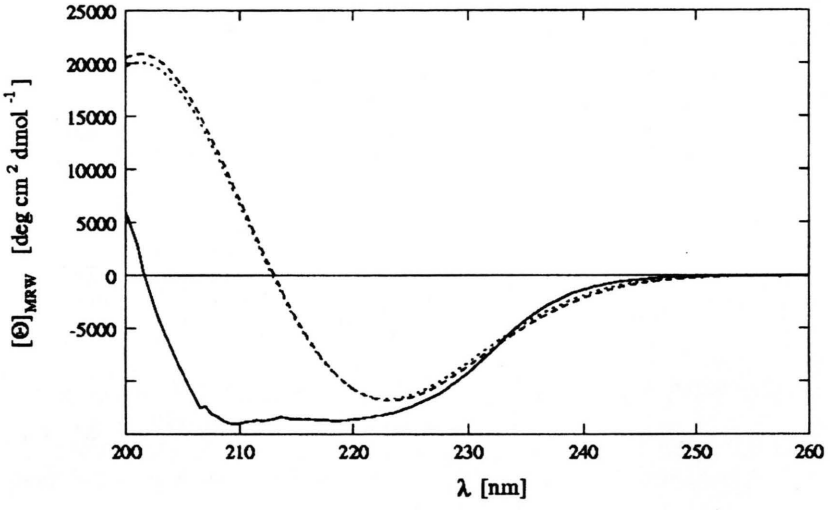

Fig. 4. Experimental and calculated far UV CD spectra of β-Lactamase; . . . : calculated without Trp, - - -: calculated with Trp, —: experimental.

groups in helices, the 208 nm band with transitions parallel to the helix axis and the 190 nm band with transition moments perpendicular to the axis. This splitting is not shown in the calculated spectrum. The absence of the negative 208 nm band from the calculated far-UV CD spectrum was also observed in the calculation for RNase A by Kurapkat et al. [8]. They attributed the failure to reproduce this feature of the α-helix CD spectrum to the short length of the helices in RNase A. The average helix length in β-lactamase is substantially larger than in RNase, so this explanation is not likely. A more plausible explanation is that the transition moment direction for the amide NV1 transition used in [8] and in the present calculation is smaller than the experimental value [17, 18]. The negative band between 220 and 230 nm mainly stems from B_b transitions in the residues TRP(229), TRP(290) and TRP(210) (see Table 2) and from $n\,\pi^*$ transitions in the peptide backbone.

Acknowledgements

The authors wish to thank Prof. R. W. Woody for helpfull discussions and Dr. M. Vanhove, who kindly provided us with the numerical CD-data of **TEM- 1**. This work was supported by Grant Fl 142/3-3 from the Deutsche Forschungsgemeinschaft to J. F., DAAD scholarship support to C. C., and Bulgarian Science Foundation projects X-823 to B. A., MU-X-1001 to C. C.

Table 2. Analysis of the Matrix Method Result for TEM-1 β-Lactamase. The numbering of the residues is the same as in the pdb file 1btl.pdb [9].

Transition		Local Group Character	%	Transition Wavelength (nm)	Rotional Strength (DBM)
1	L_b	TRP(210)	95.17	295.6	+0.001
	B_b	TRP(210)	4.43		
2	L_b	TRP(290)	87.66	295.0	+0.010
	L_a	TRP(290)	9.31		
	B_b	TRP(290)	2.88		
3	L_b	TRP(165)	95.40	292.0	+0.012
	L_a	TRP(165)	3.78		
4	L_b	TRP(229)	99.83	290.6	+0.001
5	$n_1\sigma^*$	Cys(77)-Cys(123)	74.46	288.1	+0.064
	$n_4\sigma^*$	Cys(77)-Cys(123)	24.88		
6	L_a	TRP(210)	97.82	280.4	–0.376
7	L_a	TRP(229)	84.40	279.5	+0.055
	L_b	TRP(290)	2.14		
	L_a	TRP(290)	13.14		
8	L_b	TRP(165)	3.61	278.9	–0.123
	L_a	TRP(165)	95.90		
9	L_b	TRP(229)	15.13	277.7	+0.131
	L_a	TRP(290)	7.22		
	L_b	TRP(290)	76.64		
10	L_b	TYR(264)	99.17	277.4	–0.005
11	L_b	TYR(97)	4.10	277.3	+0.021
	L_b	TYR(105)	95.09		
12	L_b	TYR(97)	95.66	277.2	+0.087
	L_b	TYR(105)	4.10		
13	L_b	TYR(46)	99.06	277.2	+0.090
14	L_b	PHE(230)	99.30	260.7	+0.010
15	L_b	PHE(72)	99.31	260.7	–0.010
16	L_b	PHE(60)	99.51	260.5	+0.001
17	L_b	PHE(151)	99.89	260.1	–0.0003
18	L_b	PHE(66)	99.93	260.1	+0.002
19	$n_1\sigma^*$	Cys(77)-Cys(123)	25.07	243.8	+0.204
	$n_4\sigma^*$	Cys(77)-Cys(123)	74.74		
20	$n_1\pi^*$	ASP(157)	0.50	230.9	–0.002
	$n_2\pi^*$	ASP(157)	0.50		
21	$n\pi^*$	Pep(LEU(102)-VAL(103))	39.65	228.2	0.044
	NV_2	Pep(LEU(102)-VAL(103))	2.45		
	L_a	TYR(105)	57.27		
22	L_a	TYR(97)	98.48	227.4	0.233
23	L_a	TYR(46)	76.81	227.3	1.780
	L_a	TYR(264)	21.82		
24	L_a	TYR(46)	21.56	227.1	–1.400
	L_a	TYR(264)	77.62		
25	$n\pi^*$	Pep(LEU(102)-VAL(103))	55.05	226.1	0.269
	NV_2	Pep(LEU(102)-VAL(103))	2.49		
	L_a	TYR(105)	41.83		
26	$n\pi^*$	PRO(252)	1.20	225.9	–2.512
	B_b	TRP(229)	54.04		
	L_b	TRP(290)	1.16		
	B_b	TRP(290)	39.16		
	B_a	TRP(290)	2.08		
27	$n\pi^*$	GLN(206)	1.90	224.6	–0.826
	L_b	TRP(210)	2.79		
	B_b	TRP(210)	76.51		

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