OPTICAL ROTATORY DISPERSION AND CIRCULAR DICHROISM OF BAKER'S YEAST TRANSKETOLASE IN THE FAR ULTRAVIOLET REGION OF THE SPECTRUM

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1. Introduction

Analysis of smooth optical rotatory dispersion curves of apotransketolase after Shechter and Blout [1] reveals marked differences in the degree of α-spiralization, determined by the A_{193} and A_{225} [2]. Study of the pH dependence of optical rotation [2] has shown that these differences could be due both to the apolar environment of the α-helical segments and to the contribution of the β-forms.

Optical activity in the far UV region of the spectrum furnishes valuable information about the structure of the protein molecule.

The present paper describes the data on optical rotatory dispersion and circular dichroism of transketolase in the absorption region of the peptide chromophore.

2. Methods

Transketolase (EC 2.2.1.1.) was prepared from baker's yeast essentially as described in [3]. The specific activity of TK* preparations was 10 U/mg of protein. The enzyme produced a single symmetrical peak in the ultracentrifuge and was proved homogenous in polyacrylamide disc electrophoresis. The preparation of apoTK is described in [4]. Before starting ORD and CD measurements the enzyme preparation was passed through a G-50 Sephadex column equilibrated with 0.002 M tris-buffer, pH 7.6. Measurements were carried out with a Jasco ORD/CD/UV-5 spectropolarimeter equipped with a device for CD measurements, with a cell light path length of 1 mm. CD data are expressed in terms of mean residue ellipticity, $[\theta]$, in units of degrees cm^2/dmole. The molecular ellipticity values were obtained from the equation:

$$[\theta] = 3300\ (\epsilon_L - \epsilon_R)$$

where $(\epsilon_L - \epsilon_R)$ is the difference between molecular extinction coefficients for left- and right-circularly polarized light. ORD data are expressed in terms of mean residue rotation, $[m]$, in units of degrees cm^2/dmole. An average amino acid residue weight of 115 was used in the calculations. The error in rotation and ellipticity measurements was 3% at 220 nm and 10% at 200 nm.

* *Abbreviations*
TK : transketolase
OA : optical activity
ORD: optical rotatory dispersion
CD : circular dichroism

3. Results and discussion

Fig. 1. shows the ORD spectrum of apotransketolase in the 195–250 nm range. The curve has a trough at 232–233 nm, a shoulder at 215–218 nm, crosses the abscissa at 223 nm and has a peak at 204 nm. The position of the peak is typical of β-structure rather than of α-helix. Polypeptides and proteins containing considerable amounts of β-structures are known to produce a peak at 205–208 nm [5], and

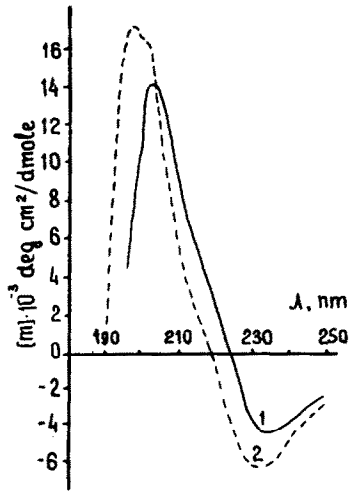

Fig. 1. ORD spectrum of apotransketolase in 0.002 M tris-buffer at pH 7.6. (1) Experimental curve. The protein concentration 0.16 mg/ml. (2) Calculated curve (20% of α-helix, 40% of β-form, 40% of coil).

helical polypeptides and proteins at 198 nm. The position of the trough in the ORD curve may be due to the double effect of the α-helix and β-structure (the α-helix has the trough at 233 nm and the β-structure at 230 nm).

CD measurements in the 200–250 nm range show that the ordered conformation of the enzyme is neither entirely α-helix, nor β-structure. Instead of two negative maxima (222 and 206–209 nm) characteristic of α-helical proteins or one maximum at 218 nm, typical of β-structure, TK has one negative maximum at 220–220 nm and a shoulder at 212–214 nm (see fig. 2). Thus, the complex character of the ORD and CD spectra, different from those typical of α-helix and β-structures, suggest that the OA exhibited by transketolase in the peptide chromophore absorption region may be due to the summation of contributions from the α-helix, β-structure and the coil.

Analysis of the ORD and CD spectra of proteins containing the three types of conformations indicated above is rather complicated. Greenfield et al. [6, 7] suggested an approach to solve this question. Using their principle we attempted to estimate quantitatively the three types of conformations, by comparing the ORD spectra of transketolase with those of poly-L-lysine calculated for different ratios of α-helical

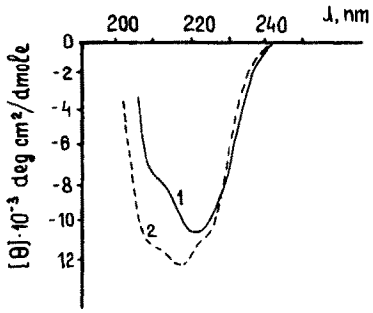

Fig. 2. CD spectrum of apotransketolase in 0.002 M tris-buffer at pH 7.6. (1) Experimental curve. The protein concentration 0.62 mg/ml. (2) Calculated curve (20% of α-helix, 40% of β-form, 40% of coil).

structure, β-structure and the coil. Combining these three tabular values (contribution of α-helix, β-structure and the coil) we were able to draw the curves which, when compared with the experimental one, resulted in a final curve which differed the least from the latter. Fig. 1 shows the calculated ORD curve (curve 2) which represents the best qualitative resemblance between the experimental and calculated ORD spectra. It corresponds to the following relative content of the above structures: 20% of α-helix, 40% of β-structures and 40% of the coil.

Fig. 2 (curve 2) shows the CD spectrum of poly-L-lysine calculated by the method of Greenfield and Fasman [7] for the structure containing 20% of α-helix, 40% of β-forms and 40% of the coil. This spectrum, like the calculated ORD spectrum, shows the best qualitative resemblance to the experimental CD spectrum of apoTK.

Comparing the experimental and calculated curves, it can be seen that the latter implies much higher intensity of electron transitions responsible for the character of the spectra. It is clear that Greenfield's principle does not take into account all the factors affecting optical activity in the molecule of the real protein.

The Cotton effect (associated with aromatic chromophore) observed in the CD spectrum of apoTK [8] may be accompanied by another Cotton effect near 220 nm. The exact position and the sign of this effect cannot be predicted. No marked changes in the CD spectrum in the far UV region was observed in interaction of thiamine pyrophosphate, the coenzyme of

TK, with the apoenzyme, though a more than two-fold increase in the Cotton effect was recorded in the aromatic amino acid absorption range [8]. This fact means that the influence of the 'aromatic' Cotton effects on the ORD and CD spectra of the apoenzyme is not responsible for the discrepancy between the calculated and experimental curves.

One of the main factors decreasing the rotatory power of peptide chromophore could be low dielectric constant of the apolar microenvironment of some sites of the peptide chain, for example that of the helical segments. The recent investigation of Straus et al. [9] shows that OA of proteins in the absorption range of peptide chromophore differs from that of model systems in that they have low rotatory force of electronic transitions and their helical π-π^* bands are shifted to the longer wavelength region. These differences are primarily due to the effect of the local microenvironment of the peptide bond or to the presence of short helical segments. In the case of TK the effect of apolar environment of the helical structure is especially pronounced. This is confirmed by the A_{193} and A_{225} dependence typical of polypeptides dissolved in an organic solvent [2].

It is a common practice to estimate the percentage of α-helical structure using the value of negative optical rotation at 233 nm, i.e. in the range where the α-helical proteins usually have an anomalous ORD trough. However, when dealing with proteins of complex structure it is essential that optical rotation in the whole accessible range of the spectrum should be taken into account. This gives a more consistent description of protein conformation.

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