THIAMINEPYROPHOSPHATE INDUCED CHANGES IN THE OPTICAL ACTIVITY OF BAKER'S YEAST TRANSKETOLASE

G.A. KOCHETOV, R.A. USMANOV and V.P. MERZLOV
Laboratory of Bioorganic Chemistry, Moscow State University, Moscow, USSR

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Induced optical activity results from a type of asymmetry arising from the spatial orientation of some limited sites of the protein molecule and is revealed by the presence of chromophores*.

Different types of substances act as chromophores in inducing OA** on interaction with protein: the haem prosthetic group; FAD; NAD; pyridoxalphosphate and their derivatives; metallic ions, e.g. Fe^{3+}, Mn^{3+}, Cu^{2+}, Cd^{2+}; specific substrates, inhibitors and some dyes [1,2]. It is interesting to study optically active side-chain chromophores which are part of the protein molecule [3]..

The stereospecificity of OA-inducing chromophores and the fact that induced OA does not depend on OA of the peptide chromophore makes the phenomenon of induced OA a valuable eye witness of events occurring at the enzyme active centre.

No data exist about the OA of enzymes with TPP as coenzymes. We have previously studied the OA of one of the thiamine enzymes, baker's yeast transketolase, by means of ORD [4]. This paper reports the results of changes in OA of transketolase arising on TPP interaction with apoenzyme.

Transketolase (EC 2.2.1.1) was prepared from baker's yeast as described [5]. The specific activity of TK preparations was 10 U/mg. The enzyme produced a single symmetrical peak in the ultracentrifuge and was proved homogeneous in polyacrylamide disc electrophoresis. Preparation of apoTK is described in [6]. To obtain the holoenzyme, Ca^{2+} † (1×10^{-3}M) and TPP (3×10^{-5} M) were added to the apoTK solution (1×10^{-5} M); the mixture was kept for 15 min at room temperature in 0.01 M tris-buffer, pH 6.5. All the CD and ORD measurements were made under the same conditions.

ORD measurements were carried out with a Jasco spectropolarimeter model ORD/UV-5, with a cell light path length of 10 mm. ORD data are expressed in terms of mean residue rotation, $Im'I_\lambda$, in units of degrees cm^2/dmole. Circular dichroic spectra were measured with a Roussel-Jouan dichrograph, and were checked with a Jasco ORD/CD/UV-5 spectropolarimeter equipped with a CD-attachment. CD data are presented in terms of molar dichroism, $[\Delta\epsilon']_\lambda$. An average residue weight of 115 was used in calculating $Im'I_\lambda$ and $[\Delta\epsilon']_\lambda$. All ORD and CD measurements were made at several protein concentrations, determined by the Lowry method.

Fig. 1 (curve 1) shows the apoTK ORD spectrum in the 270–400 mμ range. The apoenzyme ORD curve is smooth. No deviation from the linearity was observed in the Moffit or Shechter and Blout plots. However, a small ORD anomaly is difficult to observe below 300 mμ; the CD method only enables OA induced by a weak chromophore to be distinguished from the background of the peptide group OA. CD measurements showed that the apoTK reveals weak positive dichroism with a maximum at 280 mμ (see fig. 2,

* The other two types of asymmetry responsible for OA of the protein molecule are: the random coil and the secondary structure of the protein (α-helix and β-structure).

** *Abbreviations*: OA: optical activity; ORD: optical rotatory dispersion; CD: circular dichroism; TK: transketolase; TPP: thiaminepyrophosphate.

† Ca^{2+} is a cofactor of the baker's yeast TK [7].

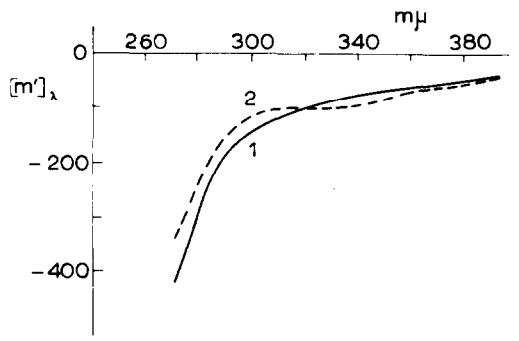

Fig. 1. ORD spectrum of transketolase in 0.01 M tris-buffer, pH 6.5. 1) apoenzyme; 2) holoenzyme.

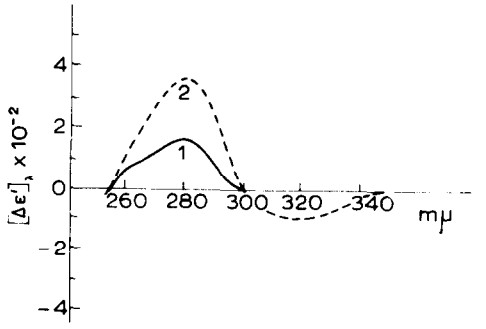

Fig. 2. CD spectrum of transketolase in 0.01 M tris-buffer, pH 6.5. 1) apoenzyme; 2) holoenzyme.

curve I). This Cotton effect may be the sum of the contribution of several electron transitions, as indicated by the asymmetric peak in the CD spectrum. Cotton effects in the near UV region have been reported for at least 20 proteins; they have been established to be due to optically active electron transitions of such chromophores as tyrosine, tryptophane, or the disulphide bond.

TPP-apoenzyme interactions induce some change in the ORD spectra in the 270-400 mμ range (see fig. 1, curve 2).* CD changes in this spectrum range have shown that the TPP-apoenzyme binding enhanced, more than 2-fold, the positive Cotton effect, with a maximum at 280 mμ, and caused the appearance of a negative Cotton effect with a maximum at 320 mμ (see fig. 2, curve 2). It should be noted that, when only one of the cofactors, Ca^{2+}, was added to the apoTK, no alterations in the ORD and CD spectra were observed.

The increase in the positive Cotton effect at 280 mμ may be due to the following: 1) direct interaction of one of the TPP groups with the side chain of the aromatic amino acid or with the disulphide group which are asymmetry centers; 2) changes in the conformation or in environment of specific residues localized in the proximity of the active centre.

The negative Cotton effect, with a peak at 320 mμ, i.e. far from the absorption range of the coenzyme**, is probably due to formation of a new bond – TPP binding to the protein molecule.

* TPP itself is optically inactive.

** TPP has an absorption spectrum with maxima at 235 and 265 mμ.

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