A CIRCULAR DICHROISM STUDY OF TRANSKETOLASE FROM BAKER'S YEAST

C.P. Heinrich, K. Noack, and O. Wiss

Research Department of F. Hoffmann-La Roche & Co. Limited Company, Basle, Switzerland.

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SUMMARY

The circular dichroism of transketolase from baker's yeast was examined in the 400 to 200 nm region in the presence and absence of its coenzyme and substrates. Coenzyme binding caused an extrinsic Cotton effect at 325 nm which is probably due to a charge transfer complex of thiamine pyrophosphate and a tryptophan moiety of the enzyme protein. Addition of donor substrates as D-fructose-6-phosphate and hydroxy-pyruvate abolished the ellipticity band at 325 nm. Steric hindrance might be responsible for the fact that 2-(1.2-dihydroxyethyl) thiamine pyrophosphate ("active glycolaldehyde") can no longer form a charge transfer complex. On the other hand, acceptor substrates in the transketolase reaction, such as ribose-5-phosphate, did not influence the broad negative dichroism at 325 nm.

INTRODUCTION

For a number of years, optical rotatory dispersion (ORD) and circular dichroism (CD) have been used as a specific tool in the study of active site conformation in enzymes. By observing changes in the ORD and CD spectra of enzymes upon addition of cofactors and substrates, one can obtain information on the binding of the latter on the enzyme surface (1). Thiamine pyrophosphate (TPP) dependent enzymes constitute an interesting group in this respect, because of the variety of situations encountered. Previous work of Kochetov et al. (2, 3) has shown that TPP, when bound to the active site of apotransketolase from baker's yeast, causes a weak negative Cotton effect around 325 nm, which is believed to be due to a charge transfer complex between TPP and a tryptophan moiety in the active site. The present communication provides further information on how the TPP-tryptophan complex is influenced by the presence of donor and acceptor substrates of transketolase.

MATERIALS AND METHODS

Crystalline apotransketolase was obtained from Sigma Chemical Co. (St. Louis, U.S.A.). The specific activity was 16 units/mg. The pentose-5-phosphate equilibrium mixture, which was used as substrate in the

transketolase assay, was prepared according to Ashwell and Hickman (4). The transketolase activity was assayed spectrophotometrically by the rate of oxidation of NADH in the coupled reaction with triosephosphate isomerase and glycerophosphate dehydrogenase (5). Protein was determined by the method of Lowry et al. (6). Hydroxypyruvate-lithium salt and ribose-5-phosphate-disodium salt were purchased from Sigma Chemical Co. Fructose-6-phosphate was a product of Boehringer (Mannheim, Germany).

Circular dichroism spectra were measured with a Roussel-Jouan dichrograph, in which the original light source was replaced by a 450 Watt xenon arc in order to gain more energy in the short wave-length region. The enzyme solutions were placed in cylindrical quartz cells with path lengths of 1 cm for measurements in the 400 to 240 nm region and of 0.1 cm for the 250 to 200 nm region. The measurements were made at room temperature ($\sim$+ 25°C). The concentration of the solutions and the path length were chosen so that the absorbance did not exceed 2.5. The specific activity of the enzyme solutions was found to be identical before and after the measurements, indicating that no denaturation had occurred.

RESULTS

We have measured the CD spectra of apotransketolase, of apotransketolase in the presence of Mg^{++}-ions, of the reactivated transketolase (addition of TPP), and of the transketolase plus either of the two donor substrates hydroxy-pyruvate and fructose-6-phosphate. Finally, the CD spectrum of holotransketolase in the presence of the acceptor substrate, ribose-5-phosphate, was obtained. The spectra are shown in figures 1 - 3, experimental details are given with the figures.

DISCUSSION

The CD spectrum of apotransketolase from baker's yeast in the 250 - 400 nm region is depicted in figure 1. A very strong negative circular dichroism band with a minimum at 220 nm (not shown in figure 1) and a weak positive multiple circular dichroism with prominent maxima at 282 and 287 nm and weaker maxima at 258, 263, and 272 nm characterize the spectrum. Generally, bands in a CD spectrum occur at the same wave-lengths as absorption bands. Therefore, the observed maxima might be correlated to phenylalanine (258, 263 nm), tyrosine (282 nm) and tryptophan (273, 280, 288 nm) absorption bands. The addition of Mg^{++}-ions to the resolved enzyme did not change the spectrum. When thiamine pyrophosphate was added to apotransketolase and Mg^{++}, the enzyme was reactivated by 93 % as found in the

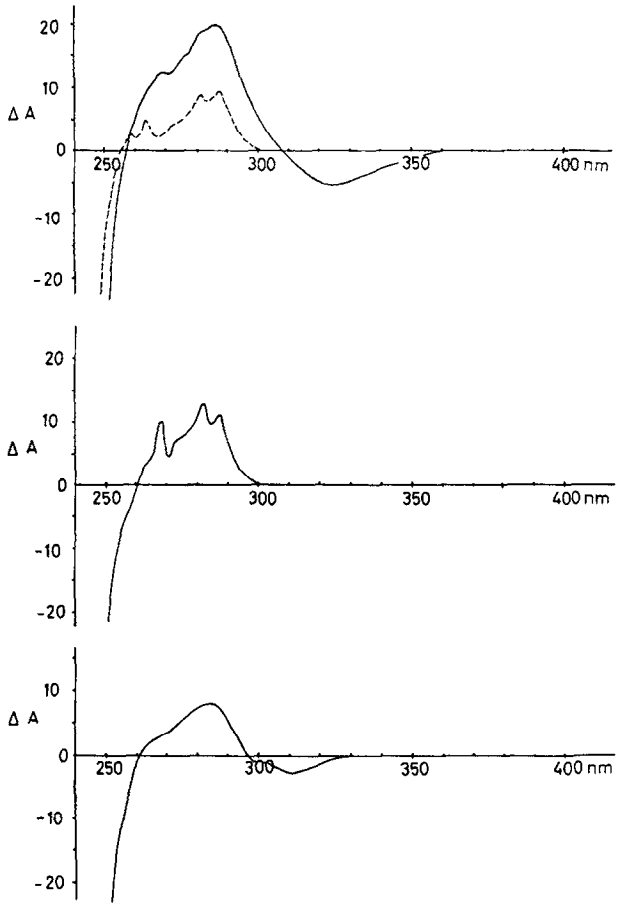

Figure 1

Circular dichroism spectra of transketolase in 10 mM Tris/HCl, pH 6.5. Identical curves were obtained for 0.57×10^{-5} M apotransketolase (0.8 mg/ml) and 0.57×10^{-5} M apotransketolase plus 6×10^{-3} M $MgCl_2$ (----). 0.57×10^{-5} M apotransketolase, 6×10^{-3} M $MgCl_2$, and 3×10^{-4} M TPP (——).

Figure 2

Circular dichroism spectrum of transketolase in the presence of hydroxypyruvate. The concentrations were 0.57×10^{-5} M apotransketolase, 6×10^{-3} M $MgCl_2$, 3×10^{-4} thiamine pyrophosphate, 4×10^{-3} M lithium hydroxypyruvate.

Figure 3

Circular dichroism spectrum of transketolase in the presence of ribose-5-phosphate. Holotransketolase plus 2.5×10^{-3} M ribose-5-phosphate disodium salt. The CD spectrum of the enzyme in the presence of ribose-5-phosphate was corrected for a small negative Cotton effect given by the free substrate. The enzyme preparation was different from that of figures 1 and 2.

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activity test. The CD spectrum of the active holoenzyme was different from that of apotransketolase (figure 1). The strong minimum at 220 nm was unchanged, whereas the positive multiple dichroism in the 255 - 300 nm re-

gion had increased. The maxima at 282 and 287 nm were doubled in intensity and a shoulder at 270 nm became apparent, so that the two weak maxima at 258 and 263 nm could not be distinguished any more. Finally, between 310 and 340 nm a new very broad minimum was observed. The new maximum at 270 nm in holotransketolase appeared at about the wave-length, where the thiazole ring system of TPP has its pH-independent absorption maximum (269 nm) (9). Since TPP itself is optically inactive, the appearance of an extrinsic Cotton effect (1) is attributed to asymmetry upon binding to the sterically organized constellation of groups on the protein surface. Besides the thiazole absorption maximum at 269 nm, a pH-dependent absorption band is found in TPP at 233 - 247 nm due to the aminopyrimidine ring system. A Cotton effect at this wave-length could also be expected because of asymmetrical binding of the aminopyrimidine ring to the protein. Unfortunately, such a Cotton effect, if existent, was hidden under the very strong peptide Cotton effect beginning at 255 nm. The increase of the circular dichroism band at 287 nm reflects the participation of tryptophan in the binding of thiamine pyrophosphate. Further evidence for this comes from the broad negative Cotton effect around 325 nm, which is very likely due to a charge transfer complex between TPP and a tryptophan moiety of the active site (2, 3, 10, 11). At pH 6.5, the thiazolium ring with the positively charged quaternary nitrogen atom is the electron acceptor, whereas tryptophan functions as the electron donor. The most favourable orientation for the two aromatic molecules in the TPP tryptophan complex is found when the ring systems are parallel. It is well known that charge transfer is very sensitive to distance (12). Conformational changes as well as bulky side chains could therefore disturb the close proximity between TPP and tryptophan. This is reflected in the change of the CD spectrum (figure 2) when the active holoenzyme was incubated with a donor substrate, such as hydroxypyruvate. The broad negative maximum at about 325 nm had again disappeared. The positive CD at 282 and 287 nm were reduced in intensity to the values of the apotransketolase. The disappearance of the charge transfer band around 325 nm in the CD spectrum was also observed, when fructose-6-phosphate, another donor substrate, was added to holotransketolase. Hydroxypyruvate as well as fructose-6-phosphate gave rise to the formation of "active glycolaldehyde" (13) and to liberation of carbon dioxide or erythrose-4-phosphate, respectively. Steric hindrance might be responsible for the fact that 2-(1.2-dihydroxyethyl)-thiamine pyrophosphate can no longer form a charge transfer complex with the tryptophan residue.

Acceptor substrates, such as ribose-5-phosphate, on the other hand, which do not form any TPP intermediate, should be bound at a different site on the enzyme surface in the active centre. This binding process should not disturb the charge transfer complex between TPP and tryptophan. This idea was experimentally confirmed: Figure 3 shows a broad minimum around 315 nm, somewhat shifted with respect to the minimum at 325 nm of holotransketolase.

No change of the CD spectrum was found upon addition of phosphate or sulfate to holotransketolase. Therefore, the binding of TPP to the enzyme remains unaffected. Both inhibitors of the enzyme are probably bound to the acceptor binding site. Unfortunately, the possible asymmetry of substrate binding cannot be examined in a manner comparable to that employed in coenzyme binding, because the substrates of transketolase do not exhibit chromophoric properties in a spectropolarometrically accessible wave-length range.

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