# POLARIZED LIGHT ABSORPTION SPECTRA OF SINGLE CRYSTALS OF ASPARTATE TRANSAMINASE FROM CHICKEN HEART CYTOSOL

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## 1. Introduction

Changes in orientation of the coenzyme, PLP, associated with intermediate steps of the catalytic cycle of Asp-transaminase have long been postulated and discussed [1–3]. New evidence has been obtained indicating such changes, using polarized light spectrophotometry of single crystals of cytosolic pig heart Asp-transaminase [4]. Here we report spectral studies on crystals of cytosolic chicken heart Asp-transaminase and its complexes with substrate analogues. Conformational changes in the active site of transaminase, occurring in the course of the catalytic reaction, are examined.

## 2. Methods

Asp-transaminase was isolated from chicken hearts as in [5]. Crystals of the enzyme, grown as in [6], were stored in the stabilizing solution (0.6 saturated $(NH_4)_2SO_4$ in 0.3 M potassium phosphate, pH 7.5). The crystals belong to space group $P2_12_12_1$ and contain 4 dimeric enzyme molecules/ unit cell [6]. Direction of the axes in the unit cell are shown in fig.1. Crystals 0.5–1.0 mm long, 0.3–0.5 mm wide and ~0.15 mm thick were mounted in a special microcell, which was placed into a Cary-118 spectrophotometer equipped with a polarizer. Absorption spectra were recorded with two orientations of the polarizer. In one orientation the plane of polarization of the incident light beam was parallel to the crystallographic *c* axis; in the other it was perpendicular to that axis, but not coincident with the *a* or *b* axis (fig.1).

*Abbreviations*: PLP, pyridoxal 5′-phosphate; Asp-transaminase, L-aspartate:2-oxoglutarate aminotransferase (EC 2.6.1.1); MeAsp, 2-methylaspartate

* Crystals were grown in the presence of 0.03 M MeAsp and then thoroughly washed free from it

## 3. Results

Absorption spectra of polarized light transmitted through a single crystal of Asp-transaminase are shown in fig.2A*. The positions of absorption

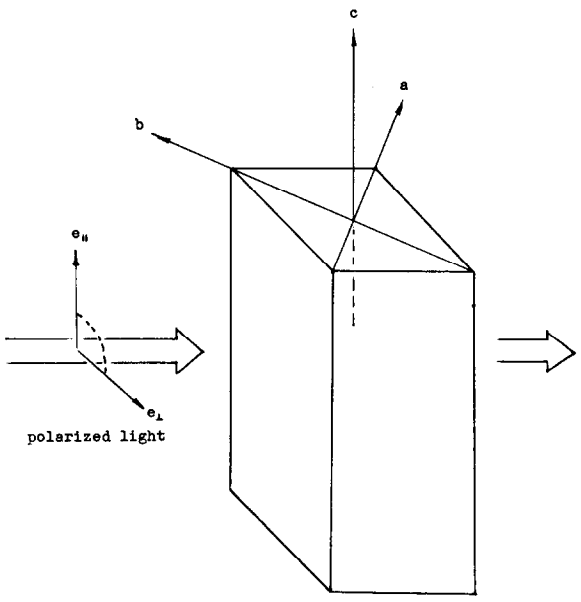


Fig.1. Mutual orientation of electric vectors ($e_{\parallel}$ and $e_{\perp}$) of the polarized light beam and crystallographic axes in the unit cell.

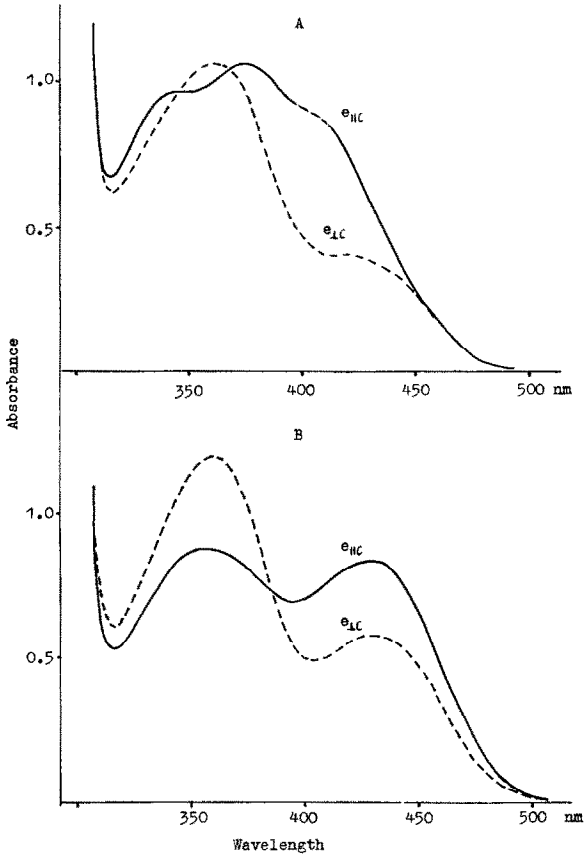


Fig.2. Polarized light spectra of a single orthorhombic crystal of free Asp-transaminase. $e_{\parallel C}$, electric vector of light is parallel to the *c* axis; $e_{\perp C}$, electric vector of light is perpendicular to the *c* axis. (A) A crystal in 0.6 saturated $(NH_4)_2SO_4$ containing 0.3 M potassium phosphate, (pH 7.5). (B) A crystal in 0.6 saturated $(NH_4)_2SO_4$ containing 0.25 M Tris–acetate (pH 7.5).

maxima are different from those of the enzyme in solution. The latter displays absorption bands at 360 and 430 nm; the two bands correspond to the 'internal' PLP-lysine aldimine; i.e., to its non-protonated and protonated forms [2]. In the spectrum of a transaminase crystal, the 430 nm band proved shifted to a shorter wavelength (~405 nm); the position of the 360 nm band is markedly changed in one of the two polarizations.

Spectra of transaminase crystals grown from polyethylene glycol solutions correspond closely to those of the enzyme in solution [4,7,8]. This led us to surmize that the peculiarities of the spectra represented in fig.2A are caused by formation of a Schiff base between PLP and ammonia present in the $(NH_4)_2SO_4$ solutions from which our crystals were grown. Actually we found that transfer of the crystals into 35% (w/v) polyethylene glycol or 2.1 M phosphate solutions led to 'normalization' of the spectrum; i.e., to appearance of the usual bands at 360 and 430 nm. These bands proved to be polarized in opposite directions. Normal spectra were also recorded after transfer of crystals from the stabilizing solution (section 2) to the $(NH_4)_2SO_4$ solution containing 0.15–0.25 M Tris-acetate or ammonium acetate buffer (fig.2B). We suppose that acetate shifts the equilibrium between the 'external' PLP–ammonia aldimine and the 'internal' PLP–lysine aldimine in favor of the latter.

Soaking of crystals in the stabilizing solution containing 0.05 M MeAsp also results in appearance of clearcut absorption maxima at 360 and 430 nm (fig.3A). The same bands are seen in the spectrum of the MeAsp–enzyme complex in solution. Judging

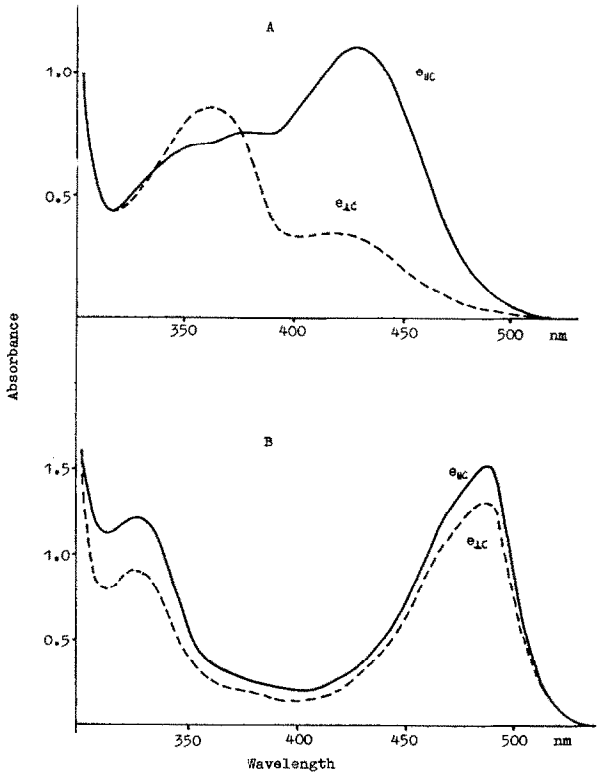


Fig.3. Polarized light spectra of crystalline complexes of Asp-transaminase with substrate analogues. $e_{\parallel C}$ and $e_{\perp C}$ as in the legend to fig.2. (A) A crystal in the stabilizing solution containing 0.05 M 2-methyl-D,L-aspartate. (B) A crystal in the stabilizing solution containing 0.01 M *erythro*-3-hydroxy-L-aspartate.

from absorption and circular dichroism data [9,10], the 360 nm band of this complex corresponds to the non-protonated PLP–lysine aldimine, and the 430 nm band corresponds to the protonated PLP–substrate aldimine. The two bands are polarized in opposite directions (fig.3A).

A crystalline complex of the enzyme with another substrate analogue, *erythro*-3-hydroxyaspartate, has also been prepared. This complex has two absorption bands: one at 493 nm which is due to the quinonoid intermediate and one at 335 nm which is due to the pyridoxamine form of the enzyme (fig.3B). The crystalline complexes of the enzyme with substrate analogues are isomorphous with crystals of the free enzyme [6].

## 4. Discussion

Comparison of our spectra with those in [4] reveals differences in the degree of polarization of identical bands. This is especially noticeable for the 3-hydroxyaspartate–enzeme complex; polarization of the 493 nm band is 6-times lower in our spectra than in Metzler's. This difference may be explained by different orientation of crystallographic axes relative to the light beam and by the different packing of enzyme molecules in the unit cell of crystals of the pig and chicken transaminases.

Direction of polarization in the 360 nm band of a MeAsp-containing crystal was found opposite to that in the free enzyme [4]. In our experiments however, the polarization proved similar in the 360 nm band of the free enzyme and of the MeAsp–complex. This is consistent with the assumption that in both cases the 360 nm band belongs to the internal PLP–lysine aldimine.

The differences in direction of polarization of the absorption bands of crystals at 360 and 430 nm are of great interest. In the case of the MeAsp-complex this difference can be explained either by different orientation of the pyridine ring of PLP in the internal and the substrate aldimines, or by different direction of the transition dipole moment within the pyridine ring. The latter assumption seems unlikely, because both aldimines have similar electronic structure. Quantum mechanical calculations also indicate that direction of the transition dipole moment is very similar for the protonated and the non-protonated aldimines [11]. Therefore, our results support the idea (see [1–3]) that formation of the substrate aldimine, i.e., the transaldimination step, is associated with reorientation of the coenzyme ring.

Opposite polarization of the 360 and 430 nm bands of crystals of the free enzyme seems surprising, since in this case both bands are due to the PLP–lysine aldimine (to its non-protonated and protonated forms, respectively). Note that the 430 nm band is polarized in the same direction, though in somewhat different degree, in the free enzyme and in the MeAsp–enzyme complex. This may be explained by the assumption that protonation of the internal aldimine induces reorientation of the coenzyme ring similar to that postulated for the transaldimination step*. Thus, the following sequence might be proposed for the first steps of the catalytic transamination cycle.

1. Non-covalent binding of an amino acid substrate to the active site of Asp-transaminase.
2. Transfer of a proton from the $NH_3^+$ group of the substrate to the imino nitrogen atom. This produces a positive charge on the imino-N which in turn lowers p$K$ of the pyridine nitrogen. The hydrogen bond between the latter and the protein is broken, and this makes possible rotation of the coenzyme ring, which can be caused by electrostatic interaction between the positively charged imino nitrogen and a nearby negatively charged group of the protein. As a result, the C-4′ atom of PLP moves towards the neutral amino group of the substrate.
3. Transimination reaction in which the PLP-lysine imine bond is replaced by the PLP–substrate imine bond and the $\epsilon$-amino group of the lysine residues is released. In the course of this reaction further rotation of the coenzyme ring may occur. The final angle of rotation relative to the initial state may amount to 25°C (estimate based on the degree of polarization).

The suggested scheme is consistent with our observation that soaking of crystals of the free enzyme in solutions containing 2-oxoglutarate or a high concentration of acetate (>0.25 M) markedly lowers the degree of polarization in the 430 nm band. Strong evidence indicates that the imino-N

* A less likely possibility is that a *trans*-oid to *cis*-oid reorientation of the imine double bond (relative to the 3-OH group) occurs in the protonation step and is accompanied by a change in the direction of the transition dipole moment within the pyridine ring (see [3]). However, is not clear in what way such reorientation could contribute to the catalytic mechanism

serves as a ligand for mono- and dicarboxylate anions [12–14]. Neutralization of the positive charge on this atom may result in complete or partial return of PLP into its position prior to protonation.

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