Comparison of aspartate aminotransferase with other aminotransferases by absorption-spectrum analysis

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The absence of X-ray-crystallographic data for aminotransferases other than the isoenzymes of aspartate aminotransferase makes it difficult to reach any firm conclusions as to the structural basis for the different properties of the many enzymes in this group. Nevertheless the basic mechanism for aspartate aminotransferase action was revealed long before the full structure was known, and much of the evidence in support of the mechanism came from studies of the enzyme’s absorption spectrum in the 300-500nm region, where the coenzyme absorbance is clearly separated from absorbances contributed by the apoprotein. The broad similarities between the spectral properties of aspartate aminotransferase and the other aminotransferases must reflect those features of structure that the whole family of enzymes hold in common. Perhaps more interestingly, it may be possible to make credible proposals for differences in structure on the basis of observed differences in spectral properties.

The 410-430nm absorbance band

The observation of a band absorbing at 430nm in aspartate aminotransferase led very early to the proposal that the aldehyde group of the coenzyme was not free but bound as an internal imine (Jenkins & Sizer, 1957). This deduction has since been amply confirmed (Hughes et al., 1962; Morino & Watanabe, 1969). Examination of the spectra of model imines of pyridoxal derivatives with amino acids led to the generally accepted conclusion that this chromophore is the mesomeric structure which may be considered as the resonance hybrid of the canonical forms IA and IB (Heinert & Martell, 1963; Davis & Metzler, 1972).

\[ \text{I}_A \rightarrow \text{I}_B \]

The value of \( \lambda_{\text{max}} \) for such structures varies from one imine to another. For example, the imine formed with valine has \( \lambda_{\text{max}} \) 414nm, whereas in the tightly restricted internal imine (II) which is constrained to be coplanar, \( \lambda_{\text{max}} = 430 \text{nm} \) (Fisher & Metzler, 1969).

\[ \text{II} \]

Fig. 1 shows the spectra of ornithine aminotransferase and 4-aminobutyrate aminotransferase. The presence of the 418nm band confirms that the coenzyme is bound as an imine, but it seems likely that the resonance system is less rigidly coplanar than in aspartate aminotransferase.

The 410-430nm chromophore in model imines of pyridoxal derivatives is lost when the pH is raised and the \( pK \) for the change is usually high, e.g. 12.2 for the imine of valine with pyridoxal phosphate (Metzler et al., 1980). The high \( pK \) is attributed to chelation of the proton in a hydrogen bond between the imine nitrogen and the phenolic oxygen. Strongly in support of this view is the observation of a band absorbing at 430nm in ornithine aminotransferase (Evangelopoulos, A. E., ed.), Alan R. Liss, New York, in the press.

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that in the cyclic compound (II) the pK is decreased to 6.2 (Fisher & Metzler, 1969). This value exactly matches that observed for the analogous transition observed in aspartate aminotransferase (Jenkins et al., 1958), although it is clear that in the enzyme the imine nitrogen and phenolic oxygen are cisoid as in compound (I) (Ford et al., 1980). In ornithine aminotransferase and 4-aminobutyrate aminotransferase no such transition is seen in the pH range 5–9, indicating that the pK is considerably higher than 9 (John & Fowler, 1976). These observations suggest that in these two enzymes, and in several others which behave similarly, tight bonding of the imine proton remains undisturbed by interactions with the enzyme protein. In aspartate aminotransferase the phenolic OH group of Tyr-225 is hydrogen-bonded to the coenzyme 3′0 atom (Ford et al., 1980; Arnone et al., 1982). If this bond is responsible for making the imine proton more readily dissociable, then such an interaction is presumably absent from ornithine aminotransferase and 4-aminobutyrate aminotransferase.
two or more spectra obtained with solutions containing different proportions of the components. Fig. 3 shows spectra of ornithine aminotransferase taken at 20°C and at 45°C. At the higher temperature \( \lambda_{\text{max}} \) is shifted to slightly higher wavelength, and its absorbance is lower. Analysis of these spectra gave the result shown in Fig. 4. Although not the only explanation of the temperature-dependent spectral changes, this analysis shows that the presence of different proportions of a minor component absorbing maximally at 450 nm would explain the data. In terms of contribution to total absorbance, the rise in temperature has produced an increase in the 450 nm-absorbing component from 18% to 22%. When the temperature of aspartate aminotransferase in 0.1 M succinate buffer (pH 6) was similarly raised, there was no comparable alteration in spectrum.


