Physical Studies Comparing a Genetically Fused Enzyme of the Histidine Operon with Its Component Enzymes*

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SUMMARY

Physical studies have been performed on a genetically fused enzyme and its two component wild type enzymes, L-histidinol dehydrogenase and imidazolylacetol phosphate:1-glutamate aminotransferase. The circular dichroic activity of the peptide chromophore of the fused enzyme is approximately accounted for by a weighted average of the activities of the two wild type enzymes. All three proteins lose circular dichroic activity in dilute alkali.

The fluorescence behavior of the wild type dehydrogenase is significantly modified in the fused enzyme. Tyrosyl fluorescence is quenched and tryptophanyl fluorescence is strongly red-shifted in the fused enzyme compared to their behavior in the wild type dehydrogenase.

The absorption and emission properties of the cofactor (pyridoxal 5'-phosphate) are quite different in the two enzymes. At neutral pH, the wild type aminotransferase exhibits absorption peaks at 330 and 430 nm whereas only the 330-nm band is evident with the fused enzyme. Titration of either enzyme between pH 10 and 12 results in the disappearance of the 330-nm peak with the parallel formation of a band centered at 385 nm. The 430-nm band of the aminotransferase is replaced by a band located at 355 nm when the pH is increased between 7 and 11.

The wild type aminotransferase and the fused enzyme fluoresce with a peak near 400 nm when excited at 330 nm. Three pH-dependent fluorescence transitions are observed in the cofactor of the aminotransferase whereas only two are apparent in the fused enzyme. These occur in the former enzyme, between pH 4 and 6, pH 7.5 and 9.5, and pH 10 and 12.5. The most acidic transition in both enzymes probably represents the ionization of the pyridinium group of the Schiff base form of the pyridoxal 5'-phosphate cofactor. The most alkaline transition appears to result from the release of pyridoxal 5'-phosphate from the two enzymes and the ionization of its phenolic group.

Enzymes—Wild type L-histidinol dehydrogenase (EC 1.1.1.2) and imidazolylacetol phosphate:1-glutamate aminotransferase (EC 2.6.1.9) were purified from a strain of S. typhimurium LT2 containing the histidine operator constitutive mutation, hisO2142, as described by Young and Ino (8) and Martin and Goldberger (6), respectively.

The fused enzyme from S. typhimurium strain TM218 (3) was used in the present studies. It was purified by procedures similar to those used for the fused enzyme from strain TM220 (2). TM218 and TM220 are derived from the same parent strain and yield fused enzymes with indistinguishable properties (2, 3).\footnote{Unpublished results.}

* This work has been supported by the Italy-USA Scientific Cooperation Program.

Mutant strains of Salmonella typhimurium have been obtained in which the enzyme products of the second and third structural genes of the histidine operon are covalently fused (1-3). The molecular weight of the fused enzyme, i.e. 140,000 (1-3), is equal to the sum of the molecular weights of the two component enzymes, i.e. histidinol dehydrogenase, 84,000 (4, 5) and imidazolylacetol phosphate:1-glutamate aminotransferase, 59,000 (6). All three enzymes are dimers (1, 2, 7, 8). The aminotransferase activity of the fused enzyme from one of these strains, TM220, was indistinguishable from that of the wild type enzyme in its kinetic parameters and thermal stability (2). The dehydrogenase activity of the fused enzyme from this strain was less stable to heat and its $K_m$ for L-histidine was 7 times greater than that of the wild type enzyme. The substrates of the aminotransferase or the dehydrogenase did not affect the other enzyme activity in the wild type or fused enzymes. It appears, therefore, that the covalent fusion of two polypeptide chains, encoded by neighboring structural genes in the histidine operon, has no effect on the number of subunits and only a rather small effect on the catalytic properties of each enzyme.

The present study was undertaken to compare the structural properties of one of the fused enzymes with the two wild type component enzymes. The secondary structure was evaluated by the circular dichroism of the peptide chromophore. The fluorescence of the aromatic chromophores, tyrosine and tryptophan, affords information of the tertiary structure organization of the three enzymes. The absorption and fluorescence behavior of the pyridoxal 5'-phosphate reflects its interaction with the wild type aminotransferase and the fused enzyme.

MATERIALS AND METHODS

Enzymes—Wild type L-histidinol dehydrogenase (EC 1.1.1.2) and imidazolylacetol phosphate:1-glutamate aminotransferase (EC 2.6.1.9) were purified from a strain of S. typhimurium LT2 containing the histidine operator constitutive mutation, hisO2142, as described by Young and Ino (8) and Martin and Goldberger (6), respectively.

The fused enzyme from S. typhimurium strain TM218 (3) was used in the present studies. It was purified by procedures similar to those used for the fused enzyme from strain TM220 (2). TM218 and TM220 are derived from the same parent strain and yield fused enzymes with indistinguishable properties (2, 3). $\beta$-Mercaptoethanol (7 mm) was added to all buffers during purification of the TM218 fused enzyme. In place of the acidification
step in the TM220 fused enzyme purification, protamine sulfate (10% suspension adjusted to pH 6.8) was added to the TM218 cell-free extract to a final concentration of 1.7%. The supernatant fraction was collected after centrifugation. The purified TM218 fused enzyme had a histidinol dehydrogenase specific activity of 830 units per mg. The enzyme band represented more than 95% of the protein after sodium dodecyl sulfate-acrylamide gel electrophoresis (9) or disc gel electrophoresis in 6 M urea (10). Ninety milligrams of purified enzyme were obtained from approximately 300 g (wet weight) of bacteria.

Enzyme Assay and Protein Determinations Assays and units for imidazolylacetol phosphate:1L-glutamate aminotransferase and histidinol dehydrogenase were described previously (6, 11). The histidinol dehydrogenase activity of the fused enzyme from TM218 was assayed after preincubation with 28 mM β-mercapto-ethanol (2).

Protein concentration was determined by absorbance at 280 nm based on the following extinction coefficients. E

1
t

m

m

m

= 9.86, 4.70, and 6.83 for the aminotransferase (6), the dehydrogenase (4, 8), and the fused enzyme, respectively. The value for the fused enzyme is based on the weighted average of the extinction coefficients of wild type dehydrogenase and aminotransferase. The extinction coefficients were calculated from the composition of tyrosine and tryptophan using molar extinction coefficients of 1200 and 5500 at 280 nm, respectively (12).

The contents of tryptophan and tyrosine were determined spectrophotometrically in 6 M guanidine hydrochloride (13). Quantum Yields—The quantum yields were measured by comparing the emission spectra of the enzymes with their absorption at 280 nm. The tryptophan and tyrosine contributions to the absorbance at 280 nm were calculated from their known contents in the three enzymes (Table I) and molar extinction coefficient values at 280 nm (12). The emission spectrum of acetyl tryptophanamide (ATA) was used as a standard to determine the quantum yield of the enzymes. The quantum yields were calculated by the equation:

\[
\text{Quantum yield} = \frac{\text{area}_{\text{ATA}}}{\text{area}_{\text{E}}} \times \frac{\text{Abs}_{\text{ATA}}}{\text{Abs}_{E}} \times 0.14
\]

where 0.14 represents the quantum yield of acetyl tryptophanamide (14). The total areas of the emission spectra of the aminotransferase and fused enzyme were attributed to tryptophan emission since very little intensity was present at the tyrosine peak (i.e. 305 nm). The spectrum of the dehydrogenase was resolved into tyrosyl and tryptophanyl emissions as discussed in the text.

Circular Dichroism—CD measurements were made with a Cary model 60 spectropolarimeter equipped with a Pockels cell. Spectra from 260 to 200 nm were taken at room temperature (25°C) in 0.1-cm path length cells. A curve was obtained for the solvent after that for each sample. The CD data are reported as mean residue ellipticities [θ] in units of degree cm² per dmole according to the formula:

\[
[\theta] = \frac{\theta \times 100}{l \times m}
\]

in which θ represents the ellipticity recorded on the instrument, l, the cell path length in centimeters, and m the molarity. A mean residue weight of 115 was used.

Fluorescence—Fluorescence measurements were carried out in a Turner model 210 spectrophotometer in 1 cm² quartz cuvettes. The absorbance of the solution never exceeded 0.1 at the exciting wavelength. The temperature was controlled at 25°C. Acid or alkaline pH titrations were accomplished by the addition to neutral pH enzyme solutions in the fluorescence cuvette of small aliquots of either concentrated HCl or KOH solutions from an Agla syringe while the solution was stirred. The pH was measured with a Radiometer model 25 pH-Meter. Absorption measurements were made in a Beckman DU spectrophotometer.

Results

Secondary Structure

The far-ultraviolet CD spectra of the wild type aminotransferase, wild type dehydrogenase, and fused enzymes are shown in Fig. 1. The spectrum of the fused enzyme agrees approximately with that of a weighted average of the spectra of the two wild type enzymes except for the minimum at 208 nm. The double minima observed with all three proteins indicate that they contain a significant amount of peptide groups in α helical configurations. A rough estimate of the helical content can be obtained by assuming that the enzymes contain only α helical and unordered peptide groups by using the ellipticity values of poly(l-lysine) in these two forms (15). From the mean residue ellipticities at 220 nm, it can be estimated that the helical contents are about 30% for the aminotransferase, 50% for the dehydrogenase, and 40% for the fused enzyme. The latter value is in agreement with that of 42% calculated from a weighted average of the two wild type proteins.

The stability of the helical peptide groups in the three enzymes to alkali was evaluated from the loss of CD activity at 220 nm (Fig. 2). There is a significant reduction in mean residue ellipticity in all three enzymes between pH 8 and 11. The midpoints of the transitions are all near pH 9.5.

Tryptophanyl and Tyrosyl Fluorescence

The fluorescence properties of the intrinsic chromophores of the three enzymes may serve as an indicator of tertiary structure since these residues contribute importantly to the hydrophobic interactions stabilizing the native structure of enzymes (16).

The emission spectra of the aminotransferase and fused enzyme are very similar when excited at 280 nm, exhibiting maxima near 340 nm with very little intensity remaining at 305 nm (Fig. 3). The dehydrogenase, however, has a peak at 315 nm. The relatively intense emission at 305 nm compared to the 315 nm peak indicates that tyrosine emission also contributes importantly to the dehydrogenase spectrum. The dehydrogenase emission peak was displaced to 327 nm when excitation was at 295 nm. The contribution of tyrosyl emission was evaluated by normalizing the tryptophanyl emission spectrum (excitation at 295 nm) to agree with that of the long wavelength wing of the emission spectrum obtained by exciting at 280 nm. The difference be-

<table>
<thead>
<tr>
<th>Table I</th>
<th>Tyrosine and tryptophan composition</th>
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<tr>
<td>Protein</td>
<td>Tyrosine</td>
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<td>Aminotransferase</td>
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<tr>
<td>Dehydrogenase</td>
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<tr>
<td>Fused enzyme (calculated)</td>
<td>14.1</td>
</tr>
<tr>
<td>Fused enzyme (observed)</td>
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* The values cited for the aminotransferase are taken from Martin et al. (7). Those cited for the dehydrogenase are from Loper (4) and Yourno (5).
FIG. 1 (left). The far-ultraviolet CD spectra of wild type aminotransferase (---), wild type dehydrogenase (----), and the fused enzyme (---) in 0.01 M NaHCO₃ and 0.1 M KCl, pH 8.0. Protein concentrations are 0.010, 0.020, and 0.015%, respectively. ---, a calculated curve based on the weighted average of the two wild type enzymes.

FIG. 2 (center). The alkaline pH dependence of the mean residue ellipticities at 220 nm of the wild type aminotransferase ( ), wild type dehydrogenase ( ● ), and the fused enzyme ( ▲ ). Protein concentrations are 0.010, 0.020, and 0.015%, respectively.

FIG. 3 (right). The emission spectra of the wild type dehydrogenase, wild type aminotransferase, and fused enzyme in 0.01 M Tris and 0.01 M KCl, pH 8.4. Excitation is at 280 nm. The absorbances of the three protein solutions at 280 nm were very close.

FIG. 4. The acid pH dependence of tryptophanyl emission (340 nm) of the wild type dehydrogenase (○) and fused enzyme (●) in 0.01 M sodium acetate and 0.01 M KCl. The excitation wavelength is 280 nm.

Table II

<table>
<thead>
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<th>Dehydrogenase</th>
<th>Aminotransferase</th>
<th>Fused enzyme</th>
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<tbody>
<tr>
<td>No. of tryptophan residues per mole</td>
<td>4</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>No. of tyrosine residues per mole</td>
<td>12</td>
<td>16</td>
<td>28</td>
</tr>
<tr>
<td>% tryptophan absorption</td>
<td>0.605</td>
<td>0.63</td>
<td>0.62</td>
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<td>Quantum yields</td>
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<tr>
<td>Tryptophan</td>
<td>0.030</td>
<td>0.032</td>
<td>0.033</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.012</td>
<td></td>
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</table>

*Trp/Trp + Tyr*, based on a molar extinction coefficient at 280 nm of 5500 for tryptophanyl and 1200 for tyrosyl residues.

between the two spectra should represent the contribution of the tyrosyl residues. The quantum yields of both residues in the dehydrogenase were obtained by comparing the areas attributable to the tyrosyl and tryptophanyl residues of the resolved emission spectrum with that of acetyl tryptophanamide (see "Materials and Methods"). The quantum yields of the tryptophanyl residues are very similar in the three enzymes whereas that of the tyrosyl is much greater in the dehydrogenase than in the other two enzymes (Table II). The tyrosine emission present in the dehydrogenase is absent in the fused enzyme. The lack of tyrosine emission in the fused enzyme can result from (a) changes in the environments of the tyrosyl residues or (b) from energy transfer to the pyridoxal 5'-phosphate or tryptophanyl chromophores of the aminotransferase when the dehydrogenase chains are incorporated into the fused enzyme.

The tryptophanyl emission of the dehydrogenase and the fused enzyme is partially quenched in dilute acid (Fig. 4). The quenching in the fused enzyme occurs between pH 6.5 and 4.5 while in the dehydrogenase it is between pH 5.5 and 2.5. Since the emission of the aminotransferase falls by only about 10% between pH 4.5 and 3.0, the tryptophanyl quenching behavior of the fused enzyme does not represent that of either component or their average.

The stability of the three enzymes at pH 8.25 toward urea denaturation was evaluated from their emission behavior. With increasing concentrations of urea between 0 and 10 M, the aminotransferase and the fused enzyme showed only a small, uniform increase in intensity which was accompanied by a slight red shift of the emission peak. In the absence of a definitive transition it is not clear whether the data reflect the enhanced fluorescence of...
Dehydrogenase

Exposure of tryptophanyl residues or configurational changes which produce minor changes in emission intensity.

The configurational transition of the dehydrogenase, however, was very clear (Fig. 5). Very little change in the emission spectra occurred below 6 M urea. In 8 M urea, tyrosyl emission was quenched whereas tryptophanyl emission was enhanced strongly and its peak shifted to longer wavelengths. The quenching at shorter wave lengths and the enhancement at longer wave lengths were even greater in 10 M urea. It is not evident whether the configurational transition is complete in 10 M urea.

Pyridoxal 5'-Phosphate Properties

Absorption—The wild type aminotransferase and the fused enzyme exhibit different absorption spectra at neutral pH. As seen in Fig. 6, at pH 7.2 the aminotransferase has two overlapping absorption bands above 300 nm of approximately equal intensity, both attributable to the pyridoxal 5'-phosphate cofactor. The peaks of these two bands are centered at 330 and 430 nm. In contrast, the fused enzyme exhibits only a single absorption band of pyridoxal 5'-phosphate, with a peak at 330 nm (Fig. 7). The 430-nm band seen in the wild type aminotransferase is very weak or missing in the fused enzyme.

The alkaline pH dependence of the 330-nm band in both enzymes is shown in Fig. 8. The reduction of the 330-nm band between pH 10 and 12 is paralleled by an increase in absorbance at 385 nm for both enzymes. This 330- to 385-nm transition is better resolved for the fused enzyme; in the wild type aminotransferase, the full extent of reduction of the 330-nm band is not seen because of its proximity to the 355-nm band (see below) which should still be increasing between pH 10 and 11. Nevertheless, it is clearly seen in Fig. 9 that the basic features of the pH difference spectrum of the 330- to 385-nm transition of the fused enzyme and the wild type aminotransferase are quite similar. The spectra of the fused enzyme between pH 8.0 and 12.0 show an isosbestic point at 363 nm. This feature strongly suggests that only one single transition occurs in this pH range in the fused enzyme.

The long wave length band, i.e. 430 nm, seen in the wild type aminotransferase, exhibits a different pH dependence from the 330-nm band (Fig. 8). The 430-nm band is eliminated between pH 7.2 and 11.1, and is replaced by a weaker band with a peak near 355 nm (Figs. 6 and 8). This transition is largely complete by pH 10, so that it can be resolved from the 330- to 385-nm transition occurring between pH 10 and 12.

Fluorescence—The fluorescence of pyridoxal 5'-phosphate can be used as a probe to study its interaction with the aminotransferase and the fused enzyme. Excitation was at 330 nm, the absorption maximum that both enzymes have in common at neutral pH. The emission peak for the wild type aminotransferase was at 390 to 400 nm and no emission was observed above 500 nm. It appears that the emission intensity at 400 nm of the fused enzyme at neutral pH is only about 25% of that of the wild type aminotransferase since the fluorescence intensities of pyridoxal 5'-phosphate were nearly identical after treatment with strong alkali and neutralization (see below). The fluorescence of the cofactor is evidently more strongly quenched in the fused enzyme.

A significant difference in the interaction of pyridoxal 5'-phosphate with the two enzymes is also seen in their fluorometric titration curves in mild alkali. Between pH 7 and 10, pyridoxal 5'-phosphate fluorescence of the wild type aminotransferase is
FIG. 8. The effect of pH on the absorbance peaks of pyridoxal 5'-phosphate. A, wild type aminotransferase. B, fused enzyme. The data were obtained from Figs. 6 and 7 and other curves recorded at the same time but not presented in Figs. 6 and 7.

FIG. 9. The change in the absorption spectra of pyridoxal 5'-phosphate in alkali. The continuous line represents the difference in absorbance of the fused enzyme between pH 8.0 and 12.0. The broken line represents 10% of the difference in absorbance of the aminotransferase between pH 10 and 12.

FIG. 10. The alkaline pH dependence of the cofactor emission of the wild type aminotransferase (A) and fused enzyme (B). Both solutions contained 0.01 M lysine and 0.10 M KCl. Excitation was at 340 nm. The data in A and B were obtained with the same fluorometer settings and therefore the fluorescence values of the two enzymes can be compared.

FIG. 11 (left). The pH dependence of free pyridoxal 5'-phosphate emission. Excitation was at 340 nm. The data were obtained in 0.01 M NaHCO₃ and 0.10 M KCl; nearly identical results were obtained in 0.01 M lysine and 0.1 M KCl.

FIG. 12 (right). The acid pH dependence of the cofactor emission of the wild type aminotransferase (○) and fused enzyme (●) in 0.01 M sodium acetate and 0.01 M KCl. Excitation was at 340 nm.

reduced by 60% (Fig. 10). This change is fully reversible if back-titration is initiated from pH 10. In contrast, the pyridoxal 5'-phosphate fluorescence of the fused enzyme, which is already highly quenched at pH 7, shows negligible additional change between pH 7 and 10 (Fig. 10). It should be noted that there is almost no change in absorption at the exciting wavelength, 330 nm, for either enzyme between pH 7 and 10 (Fig. 8).

In strong alkali, i.e. from pH 10 to 12.5, both enzymes show an increase in emission at 400 nm. The increase in quantum yield is even greater than reflected by the fluorescence increase in Fig. 10, since the data have not been corrected for the decreased absorption at the exciting wavelength, 330 nm, above pH 10.

Back-titration of both enzymes from pH 12.5 to neutral pH gives nearly identical pH-fluorescence curves which in both cases differ completely from the forward titration. The fluorescence increase between pH 10 and 7 seen in the reverse titration corresponds closely to the pH dependence of the fluorescence of free pyridoxal 5'-phosphate. Titration of free pyridoxal 5'-phosphate is shown in Fig. 11. Presumably the strong alkaline conditions, i.e. pH 10 to 12.5, liberate free pyridoxal 5'-phosphate from both enzymes.

Pyridoxal 5'-phosphate emission increases strongly with acid-
fication of both the wild type aminotransferase and the fused enzyme. The fluorescence curve for the fused enzyme was shifted about 1 pH unit to more acid values compared to the aminotransferase (Fig. 12).

DISCUSSION

The covalent fusion of histidinol dehydrogenase and imidazolylacetol phosphate:1-glutamate aminotransferase resulting from genetic manipulation causes little alteration of the enzyme kinetic properties or heat stability of the wild type aminotransferase (2). The dehydrogenase activity of the fused enzyme is less stable to heat and its $K_m$ for L-histidinol is 7-fold greater than that of the wild type dehydrogenase (2).

The present results indicate that the conformations of the polypeptide chains of the two wild type enzymes are retained in the fused enzyme since the CD activity of the fused enzyme is accounted for by the properties of the two wild type enzymes. The helical contents of all three enzymes are strongly reduced in alkali.

In contrast to the additivity observed in secondary structure, the fluorescent properties of the aromatic chromophores in the dehydrogenase are significantly modified in the fused enzyme. The emission of the tyrosyl residues is quenched and the wave length maximum of the tryptophanyl residues in the dehydrogenase is strongly red-shifted in the fused enzyme. On the other hand, the aminotransferase and the fused enzyme have very similar emission spectra in water which are only slightly modified in 10 mM urea solutions.

Although the emission properties of the tryptophanyl and tyrosyl residues closely resemble each other in the aminotransferase and fused enzyme, the absorption and emission behavior of the cofactor, pyridoxal 5'-phosphate, are quite different in the two enzymes. Pyridoxal 5'-phosphate is in the form of a Schiff base when bound to the enzymes (17). The absorption bands in the 430- and 330-nm wavelength regions represent two tautomeric forms of the Schiff base (18, 19), the former a quinoid form (eneamine) and the latter a phenolic form (enol-imine). Due to the greater polarity of the quinoid form it is favored by a polar environment whereas a nonpolar environment displaces the equilibrium toward the phenolic forms (17, 20, 21). The environment of the Schiff base in the fused enzyme appears to be less polar than in the wild type aminotransferase since it lacks the 430-nm absorption band. The greater quenching of the cofactor fluorescence at neutral pH in the fused enzyme likewise may reflect a less polar environment than in the wild type aminotransferase (20).

Three pH-dependent transitions were observed in the pyridoxal 5'-phosphate fluorescence of the aminotransferase: between pH 4 and 6, pH 7.5 and 9.5, and pH 10 and 12.5. The most acidic transition is shifted to 1 unit more acid pH in the fused enzyme. The middle transition is not seen in the fused enzyme, and the alkaline transition is approximately the same for both proteins. These transitions can represent proton ionizations, conformational changes in the protein, or dissociation of the Schiff base to its components with release of pyridoxal 5'-phosphate.

By analogy with the 5-deoxypyridoxal L-leucine model system (17), the acid transition probably represents ionization of the pyridinium group. This ionization is not associated with significant absorption change (17). The midpoint of the fluorescence enhancement in acid is at pH 6.5 for the above model compound, pH 5.0 for the aminotransferase, and pH 4.2 for the fused enzyme. The acid shift of the ionization indicates that the uncharged form of the pyridinium group is stabilized by its interaction with the fused enzyme. It should be noted that the quenching of tryptophanyl fluorescence in the fused enzyme which occurs between pH 6.5 and 4.5 is not associated with an increase in pyridoxal 5'-phosphate fluorescence. The latter enhancement first begins at a much lower pH value.

The fluorescence titration between pH 7.5 and 9.5 observed with the wild type aminotransferase but not the fused enzyme probably represents a configurational change in the protein. The Schiff base form of the above model compound does not ionize in this pH range (17). The loss in circular dichroic activity of both enzymes in this pH range suggests that they undergo a structural transition. The transition is not manifested as a quenching of pyridoxal 5'-phosphate fluorescence in the fused enzyme because its emission is already extensively quenched at neutral pH.

The pH 10 to 12.5 fluorescence transition seen with both enzymes appears to reflect release of free pyridoxal 5'-phosphate in its fully ionized form (17). Back-titration of both enzymes from pH 12.5 gives a fluorescence curve essentially indistinguishable from that of free pyridoxal 5'-phosphate, whereas the cofactor remains in its enzyme-bound Schiff base form if pH 10 is not exceeded. The decrease in 330-nm absorption observed with both enzymes above pH 10 is exactly opposite to the absorption change found with the model Schiff bases, namely, 5-deoxypyridoxal L-leucine (17) and pyridoxal 5'-phosphate-$N$-butylamine (21). The decrease in 330-nm absorption and parallel increase at 385 nm in alkali agree, however, with that observed when the phenolic group of free pyridoxal 5'-phosphate is ionized (17).

There are no important differences in the aminotransferase enzyme activities between the fused and wild type enzymes (2). The differences in absorption and fluorescence properties of the pyridoxal 5'-phosphate may therefore depend on interactions with the protein which do not influence its function as a cofactor. The important changes in tryptophanyl and tyrosyl emission properties in the dehydrogenase, however, may be a reflection of the structural alterations responsible for the higher $K_m$ for L-histidinol and the difference in thermal stability of the dehydrogenase moiety of the fused enzyme compared to the wild type dehydrogenase.

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