COMPLEXES OF PYRIDOXAL PHOSPHATE WITH AMINO ACIDS, PEPTIDES, POLYLYSINE, AND APOTRANSAMINASE

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Received February 7, 1967

Glutamic-aspartic transaminase (E. C. 2.6.1.1, L-aspartate-2-oxo-glutarate amino transferase) of pig heart has been shown to contain two pyridoxal phosphate groups per molecule (1). Under certain conditions the enzyme can be resolved to form apoenzyme and pyridoxal-P (2), while partial hydrolysis of transaminase after borohydride reduction yields a peptide which prior to reduction contained pyridoxal-P attached as a Schiff base to the ϵ-amino group of lysine (3). Since pyridoxal-P is located in the active site and functions in the transamination reaction (1, 4, 5) we have studied amino acid and polypeptide complexes of pyridoxal-P which might serve as models of the transaminase system. We have found that pyridoxal-P combines with amino acids and small peptides (6) but does not react readily with large peptides with the exception of polylysine. This polymer reacts in the random coil form but not in the helical configuration (7) to produce a Schiff base involving essentially all the ϵ-amino groups of lysine. Polylysine-pyridoxal-P resembles transaminase in several respects but does not combine with substrates.

Materials and Methods

Chemicals from California Corp. for Biochemical Research were used in all experiments with the exception of the polymers which were supplied by Mann Research Labs. Pyridoxal-P, pyridoxamine-P, and pyridoxal solutions were kept in the dark at 4° and were used at a final concentration of 2×10^{-4} M. The various amino acids, peptides and polymers

were used in a wide variety of concentrations in order to measure their affinity constants (K, the reciprocal of the dissociation constant) with pyridoxal-P. The solutions were buffered at pH 8 with 0.125 M carbonate buffer except in those experiments in which the pH was varied from 8.8 to 11.4 with this same buffer. Experiments were equilibrated at room temperature for 6 minutes before spectra were determined with a Cary recording spectrophotometer. Since an isosbestic point was obtained in all cases it was possible to measure K from the linear plot of the reciprocal of the spectral change at an absorption maximum against the reciprocal of the amino acid, peptide, or polymer concentration.

Results

The absorption spectrum of pyridoxal-P and of its complexes with L-lysine and L-polylysine are shown in Figure 1. Both complexes are characterized by a maximum at about 410 mμ which is characteristic of a Schiff base (6, 8, 9). While the polylysine-pyridoxal-P complex absorbs below 350 mμ in a non-specific (diffuse) manner the lysine-pyridoxal-P compound shows a sharp absorption maximum at 273 mμ as well as at 410 mμ. The polylysine-pyridoxal-P complex is unique, since it displays a yellow-green fluorescence and precipitates from solution in the form of minute granules.

A summary of the spectra and affinity constants (K) of pyridoxal-P complexes with a variety of amino acids, peptides and polymers is shown in Table I. It appears that all the spectra of amino acids-pyridoxal-P resemble closely that of lysine-pyridoxal-P (Figure 1), although the lysine complex is a much more stable one because of the presence of the ε-amino group. The peptides (except for glycyl-lysine) do not form Schiff bases with pyridoxal-P under these conditions and hence do not modify its 388 mμ band. In some cases (tri- and tetraglycine), however, the 310 mμ band is

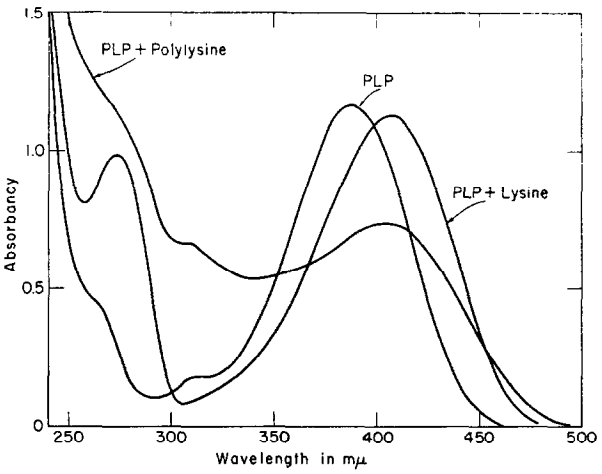

Figure 1: Absorption spectrum of 2×10^{-4} M pyridoxal-P (PLP) alone and in the presence of 1.4×10^{-3} M L-lysine or 7.57×10^{-7} M poly-L-lysine in 0.1 M carbonate buffer, pH 8.85.

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replaced by one at 322 mμ; for all peptide complexes there is a non-specific (diffuse) increase in absorption below 300 mμ. The affinity of pyridoxal-P for peptides decreases with their size and for polyglutamate there is no evidence for complex formation under these conditions. All amino compounds which combine with pyridoxal-P, whether or not they form a typical Schiff base with it, decrease its absorption at 386 mμ and profoundly modify its visible and ultraviolet spectrum.

Special attention has been devoted to the characterization of the polylysine-pyridoxal-P complex, which in some respects resembles transaminase. Like transaminase, this complex is stable to dialysis at pH 5.0 but dissociates slowly to release pyridoxal-P at pH 2.2. Pyridoxal-P combines rapidly (reaction complete in 5 minutes) with polylysine below pH 9.6 but above pH 10.1 there is no interaction; the pK calculated from the pH titration curve is 9.8. This is exactly the pK reported by Apple-

TABLE 1

Addition to Pyridoxal-P	Major Band (mμ)	Minor Band (mμ)	Ultraviolet Band (mμ)	Affinity Constant
None	386	312	diffuse	- - - - -
Polylysine	410	310	diffuse	4.63×10^{-7} M*
Lysine	410	310	273	8.62×10^{-4} M
Glycyl-lysine	410	310	273	4.24×10^{-3} M
Glycine+	410	310	273	9.44×10^{-3} M
Aspartate	410	310	273	1.22×10^{-2} M
Diglycine	388	310	diffuse	- - - - -
Triglycine	388	323	diffuse	- - - - -
Tetraglycine	388	321	diffuse	1.02×10^{-2} M
Trileucine	388	310	diffuse	- - - - -
Polyglutamate	NO INTERACTION			
Apotransaminase (pH 5.5)‡	430	340	diffuse	- - - - -

* 6.66×10^{-4} M when expressed as molarity of lysyl residues assuming a molecular weight of 185,000.

\+ With 1.0 M diglycine the 388 mμ band changes to 410 mμ.

‡ Data of Evangelopoulos and Sizer (10).

quist and Doty (7) for the sharp transition of polylysine from a random coil (below pH 9.8) to an α helix (above pH 9.8). For comparison the pK for the interaction of pyridoxal-P with L-lysine was measured and found to be 10.2. The transition in this case is not abrupt and the pH profile extends over two pH units. Hence it appears that polymer conformation (and not a protonated amino group) is critical, since pyridoxal-P reacts only with the random coil form of polylysine in which all the amino groups are accessible for Schiff base formation.

For purposes of comparison with pyridoxal-P we also studied the interaction of pyridoxal and pyridoxamine-P with polylysine, lysine,

and certain other compounds listed in Table I. Only small and non-specific changes (if any) in spectrum were observed, which were readily reversed by dialysis. It thus appears that both the phosphate and aldehyde groups of pyridoxal-P are important for the reactions listed in the table.

The typical transaminase substrates, L-aspartate and α-ketoglutarate, fail to produce any spectral change when added to polylysine (in coil or helix form) plus pyridoxal-P, pyridoxal or pyridoxamine-P. Hence, although the polylysine-pyridoxal-P complex resembles transaminase in many respects, the complex fails to combine with substrates and catalyze transamination.

ACKNOWLEDGEMENT

We are pleased to report that this research was supported by the National Institutes of Health grant #AM-01680.

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