FEBS Letters 389 (1996) 12–14 FEBS 16909

Minireview

Stereochemical constraint in the evolution of pyridoxal-5′-phosphate-dependent enzymes. A hypothesis

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Received 11 January 1996

Abstract In the transamination reactions undergone by pyridoxal-5′-phosphate-dependent enzymes that act on L-amino acids, the C4′ atom of the cofactor is without exception protonated from the *si* side. This invariant absolute stereochemistry of enzymes not all of which are evolutionarily related to each other and the inverse stereochemistry in the case of D-alanine aminotransferase might reflect a stereochemical constraint in the evolution of these enzymes rather than an accidental historical trait passed on from a common ancestor enzyme. Conceivably, the coenzyme and substrate binding sites of primordial pyridoxal-5′-phosphate-dependent enzymes had to fulfil the following prerequisites in order to allow their development toward effective catalysts: (i) the negatively charged α-carboxylate group of the amino acid substrate had to be positioned as far as possible away from the negatively charged phosphate group of the cofactor, and (ii) the Cα-H bond had to be oriented toward the protein. Compliance with these two criteria implies, under the assumption that C4′ is protonated by an acid-base group of the protein, the observed stereochemical feature.

Key words: Enzyme evolution; Ancestor enzyme; Pyridoxal-5′-phosphate; Stereochemistry

Pyridoxal-5′-phosphate-dependent enzymes (B_6 enzymes) catalyze manifold transformations of amino acids. Comparisons of amino acid sequences [1–3] and protein folds [4–6] have shown that the B_6 enzymes are of multiple evolutionary origin. Apparently, their common mechanistic properties [7], such as the covalent binding of pyridoxal-5′-phosphate to the ε-amino group of an active-site lysine residue and the transimination from this 'internal' to the 'external' aldimine with the amino acid substrate (Fig. 1), are dictated by the chemical properties of the cofactor. Another feature found to be common to all B_6 enzymes studied in this respect is the stereochemistry of protonation at C4′ of the cofactor. Numerous B_6 enzymes are converted, either in their main reaction or in a side reaction with the amino acid substrate, from the pyridoxal-5′-phosphate form to the pyridoxamine-5′-phosphate form. The transamination reaction requires the tautomerization of the aldimine to the corresponding ketimine intermediate by deprotonation at Cα and protonation at C4′. The protonation at C4′ was found to occur on its *si* face in all seven enzymes that were examined [10]. This invariance in absolute stereochemistry was interpreted as evidence for a common ancestor of the B_6 enzymes. For five of the seven enzymes, this supposition holds true in the light of new knowledge on the molecular evolution of B_6 enzymes. Alanine aminotransferase, aspartate aminotransferase, 2,2-dialkylglycine decarboxylase, glutamate decarboxylase, and serine hydroxymethyltransferase indeed belong to the large α family of homologous B_6 enzymes [1–3]. However, the pyridoxal-5′-phosphate-dependent β subunit of tryptophan synthase which shows the same stereochemistry is a member of the β family of B_6 enzymes which is not homologous with the α family [1,4]. (About the seventh enzyme, pyridoxamine pyruvate aminotransferase, no information on primary or tertiary structure is available.) The 3-D structures of five α enzymes that have been reported (aspartate aminotransferase [11], tyrosine phenol lyase [12], tryptophanase [13], ω-amino acid: pyruvate aminotransferase [14] and 2,2-dialkylglycine decarboxylase [15]) as well as the 3-D structure of tryptophan synthase [16] show, consonantly with the stereochemical findings, that the lysine residue engaging in the imine linkage and acting as proton donor/acceptor in the aldimine/ketimine tautomerization approaches the coenzyme from the *si* face.

In this communication, we propose that the invariant stereochemistry might result from a physico-chemical constraint which was effective in the evolution of B_6 enzymes. This notion is based on the following hypothetical scenario of the evolution of B_6 enzymes: the first step in the emergence of every B_6 enzyme family was the reaction of pyridoxal-5′-phosphate with a lysine residue of the progenitor protein. This reaction was facilitated by noncovalent binding of the cofactor. A further prerequisite for the development toward a catalytically effective enzyme was the preexistence of a rudimentary substrate-binding site adjacent to the pyridoxal-5′-phosphate-binding site. The bound coenzyme could thus undergo transimination with an amino acid substrate and produce the 'external aldimine', the starting point for all pyridoxal-5′-phosphate-dependent reactions.

Conceivably, such a primordial binding site for the coenzyme-substrate adduct had to meet two prerequisites: (i) it had to accommodate the adduct in its conformation of minimum energy in order to ensure stronger binding. Calculation of the minimum-energy conformation of the coenzyme-L-alanine aldimine adduct yields a conformer in which the α-carboxylate group with its negative charge is at maximum distance from the phosphate group of the cofactor (Fig. 1), if a dielectric constant of 20 or less is used in the calculations (Fig. 2). Such a value of the dielectric constant within the molecule, i.e. the coenzyme-substrate adduct, seems an appropriate assumption [17]. (ii) The Cα-H bond together with the imine N atom had to lie in a plane close to orthogonal to the plane of the coenzyme-imine π system [11,18] and had to be oriented toward the surface of the protein in order to allow protein-

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SSDI S0014-5793(96)00298-0

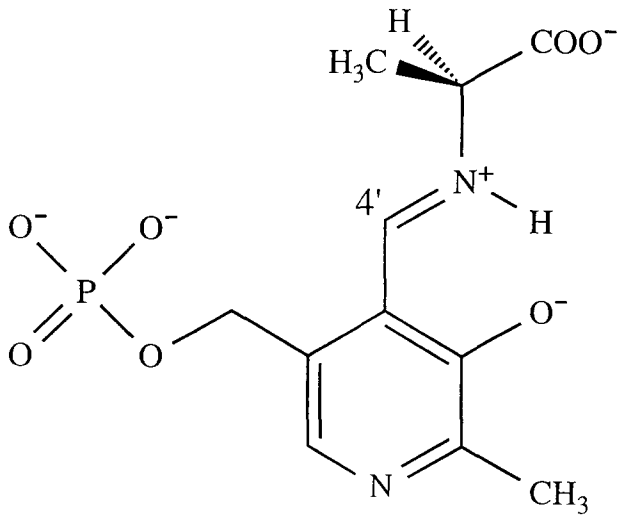

Fig. 1. Aldimine adduct of pyridoxal-5'-phosphate and L-alanine. The dihedral angle (C4', N, Cα, C_{COO}) in this conformation of the adduct is 180°. Model studies and calculations have shown that the 'cisoid' conformation of the aldimine (imine N on the same side of the C4–C4' bond as O3') is energetically favored [8,9].)

assisted deprotonation at Cα, an integral step of transamination and many other reactions catalyzed by B_6 enzymes [7].

If a coenzyme-substrate-binding site meets these two criteria, the *si* side of C4' of the coenzyme-L-amino acid adduct (and in the case of serine hydroxymethyltransferase of the adduct with D-alanine which is the substrate undergoing transamination [19]) will be facing the protein and its *re* side will be exposed to the solvent. Thus, the protonation at C4' will occur from the *si* side if we assume that the protonation is assisted by a side chain group of the protein as it has been found to be the case in aspartate aminotransferase [11]. Indeed, in aspartate aminotransferase [11], tyrosine phenol lyase [20], dialkylglycine decarboxylase [15] and tryptophan synthase [16], the structures of the covalent coenzyme-substrate adducts either determined directly by X-ray crystallography or deduced from the position of the putative substrate binding sites invariably show the coenzyme and substrate moieties to be arranged as displayed in Fig. 1.

As a corollary of the above hypothesis, the inverse stereochemistry of C4' protonation is expected in enzymes acting on D-amino acids. Indeed, in D-alanine aminotransferase (and in homologous branched-chain L-amino acid aminotransferase) C4' has been found to be protonated from the *re* side [21]. The two enzymes are evolutionarily unrelated to the other aminotransferases and appear to have originated from a primordial B_6 enzyme specific for D-amino acids [1,6]. In agreement with C4' protonation from the *re* side, the recently reported crystal structure of D-alanine aminotransferase [6] shows the active-site lysine residue to extend toward the *re* face of the coenzyme.

Acknowledgements: This study was supported in part by the Swiss National Science Foundation, Grant 31-36542.92, and the Bundesamt für Bildung und Wissenschaft as part of the EU program 'Human Capital and Mobility' (Grant BBW 93.0304, EU No. CHRX-CT93-0179). We thank Prof. J.N. Jansonius for valuable discussions.

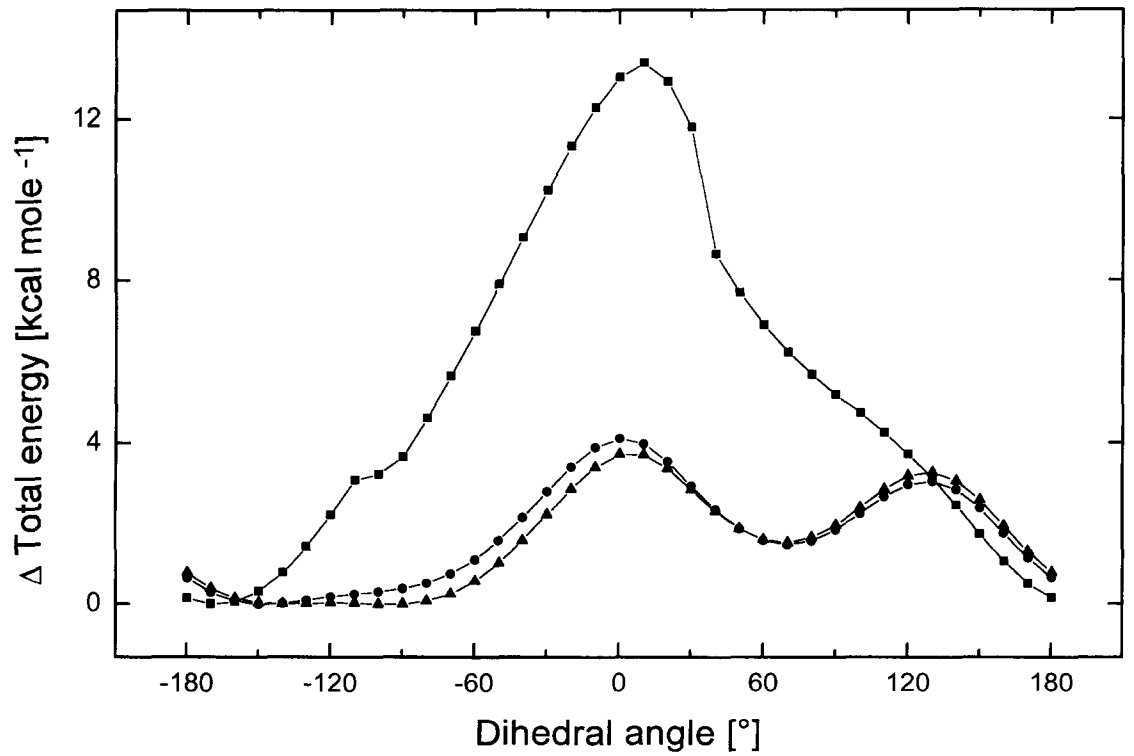

Fig. 2. Dependence of the total energy of the pyridoxal-5'-phosphate-L-alanine adduct on the dihedral angle (C4', N, Cα, C_{COO}). The structure of the compound in Fig. 1 was energy-minimized at the indicated angles, which were kept fixed during computation, with the conjugate gradient method of the program DISCOVER/INSIGHT (Biosym Technologies). The individual atomic charges of the molecule in Fig. 1 (total charge 3−) used in the minimization procedures had been calculated with MOPAC. The energy profile was calculated at dielectric constants of 1 (■), 20 (●) and 80 (▲). The minimum at a dielectric constant of 1 was found to be at an angle of −168°.

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