Structural Basis for the Catalytic Activity of Aspartate Aminotransferase K258H Lacking the Pyridoxal 5′-Phosphate-Binding Lysine Residue[†,‡]

V. N. Malashkevich,[§] J. Jäger,[§,||] M. Ziak,[⊥,#] U. Sauder,[§] H. Gehring,[⊥] P. Christen,[⊥] and J. N. Jansonius*[,§]

Abteilung Strukturbiologie, Biozentrum der Universität Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland, and Biochemisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Received May 19, 1994; Revised Manuscript Received October 24, 1994[⊗]

ABSTRACT: Chicken mitochondrial and *Escherichia coli* aspartate aminotransferases K258H, in which the active site lysine residue has been exchanged for a histidine residue, retain partial catalytic competence [Ziak *et al.* (1993) *Eur. J. Biochem. 211*, 475−484]. Mutant PLP and PMP holoenzymes and the complexes of the latter (*E. coli* enzyme) with sulfate and 2-oxoglutarate, as well as complexes of the mitochondrial apoenzyme with *N*-(5′-phosphopyridoxyl)-L-aspartate or *N*-(5′-phosphopyridoxyl)-L-glutamate, were crystallized and analyzed by means of X-ray crystallography in order to examine how the side chain of histidine 258 can substitute as a general acid/base catalyst of the aldimine−ketimine tautomerization in enzymic transamination. The structures have been solved and refined at resolutions between 2.1 and 2.8 Å. Both the closed and the open conformations, identical to those of the wild-type enzyme, were observed, indicating that the mutant enzymes of both species exhibit the same conformational flexibility as the wild-type enzymes, although in AspAT K258H the equilibrium is somewhat shifted toward the open conformation. The replacement of the active site K258 by a histidine residue resulted only in local structural adaptations necessary to accommodate the imidazole ring. The catalytic competence of the mutant enzyme, which in the forward half-reaction is 0.1% of that of the wild-type enzyme, suggests that the imidazole group is involved in the aldimine−ketimine tautomerization. However, the imidazole ring of H258 is too far away from C_α and $C_{4'}$ of the coenzyme−substrate adduct for direct proton transfer, suggesting that the 1,3-prototropic shift is mediated by a water molecule. Although there is enough space for a water molecule in this area, it has not been detected. Dynamic fluctuations of the protein matrix might transiently open a channel, giving a water molecule fleeting access to the active site.

Vitamin B_6-dependent enzymes catalyze manifold reactions in the metabolism of amino acids. In all these enzymes pyridoxal 5′-phosphate (PLP)[1] is covalently bound to the ϵ-amino group of an active site lysine residue. The amino group of the substrate replaces the ϵ-amino group, while the electron sink of the protonated imine and the pyridine moiety promote such diverse reactions as transamination, α-decarboxylation, and racemization, as well as β- or γ-elimination and replacement reactions.

Aspartate aminotransferase (AspAT; EC 2.6.1.1) is an α_2 dimeric enzyme with, depending on the isozyme, approximately 400 amino acid residues per subunit. It catalyzes the reversible interconversion of dicarboxylic amino and oxo acids:

L-aspartate + 2-oxoglutarate ⇌ oxalacetate + L-glutamate

The ϵ-amino group of K258, the PLP-binding lysine residue in AspATs, is also thought to serve as a proton acceptor and donor in the 1,3-prototropic shift from the aldimine to the ketimine intermediate (Christen & Metzler, 1985) and to assist in the hydrolysis of the ketimine intermediate (Kirsch *et al.*, 1984). In a series of oligonucleotide-directed mutations, this residue was replaced by alanine (Malcolm & Kirsch, 1985; Kirsch *et al.*, 1987), arginine (Kuramitsu *et al.*, 1989; Toney & Kirsch, 1991), and cysteine (Kirsch *et al.*, 1991). All these mutants were virtually inactive. However, if K258 is replaced by histidine, a conversion rate in the forward half-reaction of 0.1% of that of the wild-type (wt) enzyme is retained (Ziak *et al.*, 1990, 1993). The reverse half-reaction from the PMP to the PLP form with oxalacetate as substrate is even faster than that, whereas the half-reaction with 2-oxoglutarate is slowed down by two additional orders of magnitude. Scheme 1 gives the main intermediates of the transamination reaction as catalyzed by

[†] This study was supported in part by the Swiss National Science Foundation (Grants 31-25713.88 and 31-36432.92 to J.N.J. and Grant 31-36542.92 to P.C.).

[‡] The coordinates of the refined structures have been deposited with the Brookhaven Protein Data Bank, Chemistry Department, Brookhaven National Laboratory, Upton, NY, from which copies are available (entry codes 1AKA, 1AKB, 1AKC, 1AIA, 1AIB, and 1AIC).

* Author to whom correspondence should be addressed.

[§] Biozentrum der Universität Basel.

[||] Present address: Department of Molecular Biophysics and Biochemistry, Bass Center, Yale University, New Haven, CT 06511.

[⊥] Biochemisches Institut der Universität Zürich.

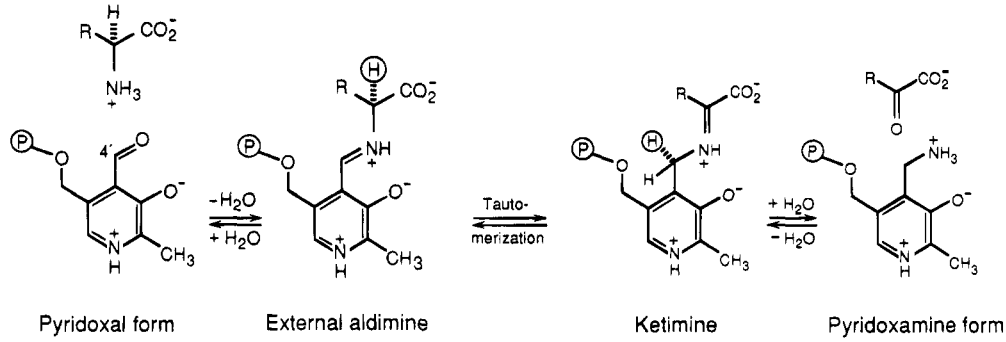
[#] Present address: Abteilung Zell- und Molekularpathologie, Departement Pathologie, Universitätspital, CH-8091 Zürich, Switzerland.

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1994.

[1] Abbreviations: PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; AspAT, aspartate aminotransferase; eAspAT and mAspAT, *Escherichia coli* and mitochondrial AspAT; wt, wild type; PEG, poly(ethylene glycol) of average M_r 4000; AS, ammonium sulfate; PPxy-Asp, *N*-(5′-phosphopyridoxyl)-L-aspartate; PPxy-Glu, *N*-(5′-phosphopyridoxyl)-L-glutamate; F_o and F_c, observed and calculated structure factors; mK258H, mAspAT K258H as free PLP holoenzyme; mK258H PPxy-Asp, PPxy-Asp complex of apo-mAspAT K258H; mK258H PPxy-Glu, PPxy-Glu complex of apo-mAspAT K258H; eK258H PMP, eAspAT K258H as free PMP holoenzyme; eK258H PMP·SO_4, sulfate complex of the PMP form of eAspAT K258H; eK258H PMP·2OG, 2-oxoglutarate complex of the PMP form of eAspAT K258H.

0006-2960/95/0434-405$09.00/0

Scheme 1: Main Intermediates in Catalysis by AspAT K258H[a]



[a] In pyridoxal phosphate the $C_{4'}$-carbon is labeled for clarity.

the AspAT mutant. In the forward reaction, the external aldimine transiently accumulates upon addition of aspartate or glutamate, suggesting that the deprotonation at C_α is rate-limiting. In the reverse half-reaction with 2-oxoglutarate as substrate, the ketimine intermediate is detected, which indicates that the proton abstraction at $C_{4'}$ is at least partially rate-determining (Sterk & Gehring, 1991; Ziak *et al.*, 1993). The relatively high residual activity of AspAT K258H in comparison to the other K258 mutants suggested effective participation of the histidine side chain in the catalytic mechanism.

Mutant enzyme from chicken heart mitochondria (mAspAT K258H) and from *Escherichia coli* (eAspAT K258H) were crystallized as PLP and PMP holoenzymes, as the complexes of the latter with sulfate or 2-oxoglutarate, and as complexes of the apoenzyme with *N*-(5′-phosphopyridoxyl)-L-aspartate (PPxy-Asp) or *N*-(5′-phosphopyridoxyl)-L-glutamate (PPxy-Glu). The three-dimensional structures of all these derivatives were determined by means of X-ray crystallography in order to explore the catalytic function of the histidine side chain in AspAT K258H.

MATERIALS AND METHODS

Crystallization of mAspAT K258H. Cloning, mutagenesis, and purification of the mutant enzyme have been described before (Ziak *et al.*, 1990, 1993). All crystals were grown with the hanging drop method (McPherson, 1982). The 5-μL drop for growing crystals of free PLP holoenzyme contained 8.5−9.5% poly(ethylene glycol) 4000 (PEG), 20 mM sodium phosphate, pH 7.5, and 12 mg/mL enzyme. For cocrystallization with 2-methylaspartate the solution contained in addition 50 mM covalent inhibitor. The reservoir contained 17−19% PEG in the same buffers. Both experiments yielded triclinic crystals (mK258H in Table 1) of maximum size 1.0 mm × 0.5 mm × 0.5 mm and isomorphous with the wt holoenzyme crystals (Gehring *et al.*, 1977). They belonged to the space group $P1$ with unit cell parameters a = 55.9 Å, b = 58.8 Å, c = 75.8 Å, α = 85.3°, β = 109.0°, and γ = 115.6°.

In order to study the closed conformation of the mitochondrial mutant enzyme, the apo form of mAspAT K258H was cocrystallized with PPxy-Asp and PPxy-Glu under conditions similar to those used for crystallization of the wt enzyme (Kirsch *et al.*, 1984; Picot *et al.*, 1991). The drop contained 9−14% PEG, 20 mM sodium phosphate, pH 7.5, 0.9 M NaCl, 20 mM PPxy-Asp (or PPxy-Glu), and 10 mg/mL apoenzyme. The reservoir contained 18−28% PEG in the same buffer. Samples were incubated at 6 °C in the cold room for 2−5 weeks until crystals had reached their maximum size of 1.0 mm × 0.4 mm × 0.4 mm. They belong to space group $C222_1$ with unit cell axes a = 70.1 Å, b = 91.8 Å, c = 129.0 Å and a = 69.8 Å, b = 91.3 Å, and c = 127.8 Å, respectively (mK258H PPxy-Asp and mK258H PPxy-Glu in Table 1), and are isomorphous with the wt enzyme crystals in this space group.

Crystallization of eAspAT K258H. The PMP form of eAspAT K258H was crystallized in the monoclinic space group $P2_1$ by vapor diffusion techniques following the protocols of Smith *et al.* (1986) and Jäger *et al.* (1989) using both ammonium sulfate (AS) and PEG as precipitants. However, the crystallization conditions were slightly modified as follows. In the case of PEG, drops of 20 μL contained 15.1 mg/mL protein, 100 mM maleate, 0.45−0.55 M sodium chloride, 16% (w/v) PEG, and 20 mM sodium phosphate, pH 7.5. The wells were filled with 1 mL of precipitant solution containing 32% (w/v) PEG, 0.9−1.1 M sodium chloride, and 20 mM sodium phosphate. Single crystals (eK258H PMP; see Table 1) appeared within 2−3 days and grew in 2−3 weeks to maximum dimensions of 1.5 mm × 0.5 mm × 0.5 mm. When AS was used (eK258H PMP·SO_4 in Table 1) drops of 20 μL contained 12.0 mg/mL protein, 23−25% saturated AS, and 20 mM sodium phosphate, pH 7.5. The reservoir consisted of 45−50% saturated AS dissolved in 20 mM sodium phosphate.

Soaking Experiments with eAspAT K258H Crystals. Crystals of eAspAT (PMP form), grown from either AS or PEG, were tested in soaking experiments with solutions containing 20, 100, and 200 mM 2-oxoglutarate in an attempt to produce a PMP−enzyme complex or a catalytic intermediate adopting the closed conformation.

The compositions of the buffers used for these experiments were as follows: 20 mM sodium phosphate, pH 7.5, 48% saturated AS or 32% (w/v) PEG, and 2-oxoglutarate in concentrations of 20, 100, and 200 mM. PEG-grown crystals did not change color even when exposed to 200 mM 2-oxoglutarate. However, with AS-grown crystals (eK258H PMP·2OG in Table 1) a color change toward faintly tan could be observed within a few seconds. The soaking experiment was stopped after 120 s, when small cracks in the crystal appeared. Data collection was started immediately.

Data Collection and Processing. Six intensity data sets were collected using a FAST (Enraf-Nonius, Delft) area detector system (see Table 1 for details) and processed with MADNES software (Messerschmidt & Pflugrath, 1987) and the profile-fitting routines PROCOR (Kabsch, 1988). The observed intensities were merged to give a unique set of

Table 1: Data Collection and Refinement Statistics for AspAT K258H Structures

parameter	structure[a] mK258H	mK258H PPxy-Asp	mK258H PPxy-Glu	eK258H PMP	eK258H PMP·SO_4	eK258H PMP·2OG
space group	$P1$	$C222_1$	$C222_1$	$P2_1$	$P2_1$	$P2_1$
resolution range (Å)	10.0−2.1	10.0−2.3	10.0−2.3	10.0−2.2	10.0−2.4	10.0−2.8
reflections						
obsd	56002	72346	38502	89322	72346	56821
unique	41517	18825	16434	49125	39276	27007
completeness (%)	81.1	96.2	88.6	90.1	93.1	98.4
R_{sym}[b]	0.053	0.082	0.075	0.061	0.047	0.091
R-factor[c]						
initial	0.231	0.284	0.305	0.264	0.281	0.284
final	0.167	0.178	0.172	0.210	0.191	0.219
no. of atoms						
protein	6346	3186	3187	6154	6164	6190
solvent	666	312	312	301	286	24
rms deviations from ideal geometry						
bond distances (Å)	0.010	0.010	0.011	0.014	0.013	0.015
bond angles (deg)	1.41	1.38	1.60	3.02	3.12	3.19
torsion angles (deg)	18.8	21.8	21.8	23.9	24.2	24.2
planar groups (Å)	0.009	0.009	0.010	0.028	0.029	0.029
rms coordinate error (Å)[d]	0.24	0.27	0.27	0.43	0.38	0.33
mean B-factor (Å^2)						
main chain	19.5	27.9	27.9	25.2	24.5	19.1
side chains	26.8	35.2	35.2	33.7	27.2	30.1
entire molecule	23.1	31.5	31.5	30.1	26.1	25.8
water molecules	42.1	52.4	52.4	40.8	40.7	30.0

[a] For structure definitions see footnote 1. [b] $R_{sym} = \sum_{hkl}\sum_i|\langle I(hkl)\rangle - I(hkl)_i|/\sum_{hkl}\sum_i I(hkl)_i$, and $\langle I(hkl)\rangle$ is the average of I_i over all symmetry equivalents. [c] $R = \sum_{hkl}|F(hkl)_o - F(hkl)_c|/\sum_{hkl}F(hkl)_o$. [d] Estimated using the program SIGMAA (Read, 1986).

reflections with the ROTAVATA/AGROVATA programs from the CCP4 suite (SERC Daresbury Laboratory). The intensities were converted to structure−factor amplitudes with the program TRUNCATE (French & Wilson, 1978). In order to eliminate scaling problems which arose due to the lack of symmetry elements in triclinic crystals, batches of 4° in ω were scaled to F_c^2 data from the highly refined structure of the wild-type enzyme (McPhalen *et al.*, 1992a). This procedure resulted in more realistic values for scale and temperature factors of individual batches than those obtained with the initial procedure, in which all subsequent batches were scaled to the first one.

Structure Determination and Refinement. The structure of the mAspAT PLP holoenzyme refined earlier at 1.9 Å resolution (McPhalen *et al.*, 1992a) was used as the initial model for mK258H. All 41 500 reflections in the resolution range of 10−2.1 Å were included into positional refinement with the XPLOR program (Brünger, 1988). After seven cycles of overall B-factor refinement, 106 cycles of energy minimization, and 20 cycles of individual B-factor refinement, the process converged at an R-factor value of 17.5% in the resolution range from 10.0 to 2.1 Å. Refinement was continued with PROLSQ (Hendrickson, 1985) until an R-factor of 16.7% and a model with good geometry had been achieved (see Table 1). The structure of the 2-methylaspartate complex of mAspAT (Jansonius & Vincent, 1987; McPhalen *et al.*, 1992b) was used to build the initial model for mK258H PPxy-Asp. Refinement with PROLSQ converged very quickly to an R-factor of 17.8% and a model with good geometry. The initial model for mK258H PPxy-Glu was derived from this final model and refined further with PROLSQ to an R-factor of 17.2%.

All eAspAT K258H structures were solved by difference Fourier techniques, using σ_a weighted $(2F_o - F_c)$, α_c electron density maps, in order to minimize bias toward the models (Read, 1986). Calculated structure factors were derived from a refined model of wt eAspAT in the half-open conformation (Jäger *et al.*, 1994). The cofactor and the side chains of the important active site residues, i.e., Y70* (asterisks indicate residues of the adjacent subunit), W140, N194, Y225, K258, R292*, and R386, were omitted from the phasing model by truncating these residues to glycines prior to structure factor calculation. The difference Fourier maps were inspected, and the models were rebuilt on a graphics display system using FRODO (Jones, 1978), as modified by Pflugrath *et al.* (1984). The strategy of the molecular dynamics refinement with XPLOR was similar to that applied to the half-open wt eAspAT structure described earlier (Jäger *et al.*, 1994).

RESULTS

Unliganded mAspAT K258H in the Open Conformation. The PLP form of the unliganded mutant enzyme yields triclinic crystals characteristic for the open form of wt mAspAT. Its refined structure is indeed in the open conformation and shows only minor deviations from the structure of the wt enzyme (the rms deviation between 814 C_α atoms of the two models is 0.16 Å). Conformational changes are limited to the immediate environment of the exchanged residue.

Clear electron density corresponding to H258 is present in the map calculated with coefficients $(F_o - F_c)$, α_c (Figure 1). However, density corresponding to the pyridine ring of the cofactor is very weak in subunit 1 and is not observed in subunit 2 [for definition of the subunits, see McPhalen *et al.* (1992a)]. The electron density in the latter (not shown) is consistent with four water molecules and a phosphate ion, regardless of whether the coenzyme molecule is included in the structure factor calculation or not. Thus, under the crystallization conditions used, subunit 1 of mK258H exists

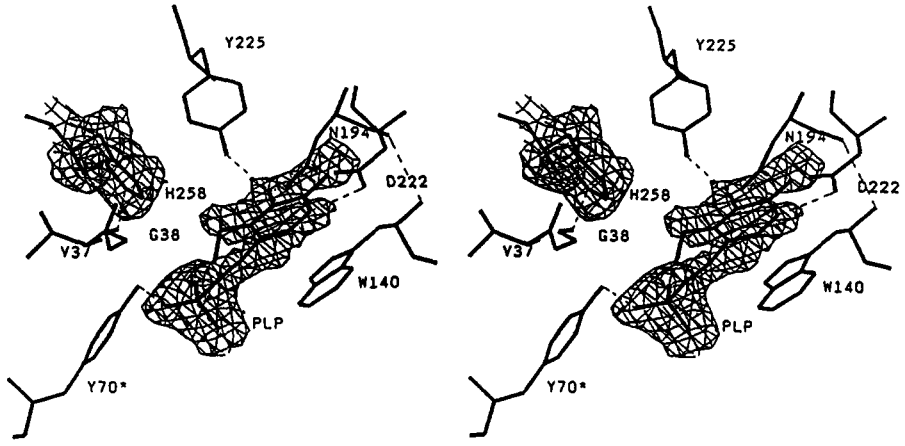


FIGURE 1: Stereoview of the refined mK258H holosubunit active site structure superimposed onto an omit electron density map computed with ($F_o - F_c$) coefficients and calculated phases (omitting H258 and PLP from the calculation). The map is contoured at 3σ. Hydrogen bonds are shown as dashed lines. The density for the imidazole group is well shaped while that for the PLP molecule is rather poor due to low occupancy. The conformation of the 4′ side chain of the cofactor is somewhat ambiguous due to weak electron density.

in the form of PLP holoenzyme, while subunit 2 is in the apo form. The structure of the apo subunit is similar to the structure of the wt apoenzyme, refined at 2.8 Å resolution (T. Schirmer and J. N. Jansonius, unpublished results). The phosphate ion in the apo subunit assumes the position of the phosphate group of the coenzyme and forms identical interactions with the protein side chains. The water molecules that replace the cofactor pyridine ring in the apo subunit are arranged differently in the wt and in the mutant structure, to accommodate the lysine and the histidine side chains, respectively.[2]

The $N_{\epsilon 2}$ atom of H258 forms a short hydrogen bond to the carbonyl oxygen of G38 in both subunits of mK258H. In the apo subunit, a water molecule forms a hydrogen bond to both the $N_{\epsilon 2}$ atom of H258 and O_γ of S255. A water molecule is also found at this position in the wild-type apoenzyme but not in the PLP holoenzyme. The side chains of F360, W140, M359, N194, Y225, and Y70*, which are near H258, change their positions slightly to accommodate the histidine side chain. The loss of the internal aldimine bond in the mutant enzyme results in a rotation of the cofactor pyridine ring of about 16° around the N_1-C_2 bond, improving coplanarity with the indole group of W140 (Figure 1).

Complexes of mAspAT K258H in the Closed Conformation. The PPxy-Asp and PPxy-Glu complexes yield orthorhombic crystals isomorphous with those of the closed form of wt mAspAT. The rms deviations between the C_α atoms of the refined PPxy-Asp and PPxy-Glu structures and the 2-methylaspartate complex of the wt enzyme (Jansonius & Vincent, 1987; McPhalen *et al.*, 1992b) are only 0.18 and 0.17 Å, respectively. The closed conformations are identical in both complexes despite the different lengths of glutamate and aspartate. The coordinates of the 2-methylaspartate complex with the coenzyme−inhibitor adduct removed were used to calculate initial ($F_o - F_c$), α_c electron density maps (Figure 2). In the mK258H PPxy-Asp structure (Figure 2A), the reduced aldimine bond is not coplanar with the cofactor pyridine ring but is rotated out of the plane by approximately −35° around an axis through C_4 and $C_{4'}$. The side chain of H258 can adopt two alternative orientations that differ by a 180° rotation around the $C_\beta-C_\gamma$ bond, but in contrast to the mK258H structure, it cannot form strong hydrogen bonds in either orientation due to a shift of G38 by about 1 Å. Upon formation of the closed conformation of the enzyme the carbonyl oxygen of G38 pushes the imidazole ring of H258 to a position where their relative orientation (the O−N distance is 2.9 Å, but the H258 $N_{\delta 1}-H$···O G38 angle is about 110°) is geometrically unfavorable for hydrogen bond formation (Burley & Petsko, 1988). The closest possible hydrogen-bonding partners are O_η of Y70* and O_η of Y225 at distances of 3.5 and 3.9 Å, respectively. The shortest distances of the $N_{\epsilon 2}$ atom of H258 (in the two possible side-chain conformations) to C_α and $C_{4'}$, respectively, are 4.2 and 3.7 Å. The average *B*-factor value for the H258 side chain is 30 Å^2, similar to the average value of its close neighbors. There is room in this area for a water molecule, which, however, was not detected. Selected interatomic distances in the active site of the PPxy-Asp complex are given in Table 2. Similar to the 2-methylaspartate complex of the wt enzyme, the β-carboxylate is nearly coplanar with the guanidinium group of R292* and forms "end-on-symmetric"-type ion pair−hydrogen bond interactions with it (Mitchell *et al.*, 1992). The α-carboxylate makes similar interactions with the side chain of R386. The χ_1 torsion angle of the substrate moiety is −56°, corresponding to a *gauche* conformation.

If the mK258H PPxy-Asp structure is superimposed onto the external aldimine structure of the 2-methylaspartate complex of mAspAT (Jansonius & Vincent, 1987), the positions of all active site residues are similar except, of course, those of the side chain of residue 258 (Figure 3A). The N_ζ atom of K258 in the external aldimine structure is pointing away from the methyl group and is in a position favorable for protonation/deprotonation of $C_{4'}$ rather than C_α. In the 2-methylaspartate adduct of mAspAT the N_ζ atom of K258 can be brought into close hydrogen-bonding distances to C_α and $C_{4'}$, while in the mK258H PPxy-Asp structure the $N_{\epsilon 2}$ atom of H258 is too far away (see above).

The initial model for solving the structure of mK258H PPxy-Glu was the protein part of the mK258H PPxy-Asp structure. Further positional and individual temperature

[2] The low occupancy of the PLP-binding site in mK258H is most likely an artifact of crystallization. The mutant enzyme in solution still tightly binds PLP and PMP; neither form of the coenzyme is removed by gel filtration at pH 7.5 (Ziak *et al.*, 1990).

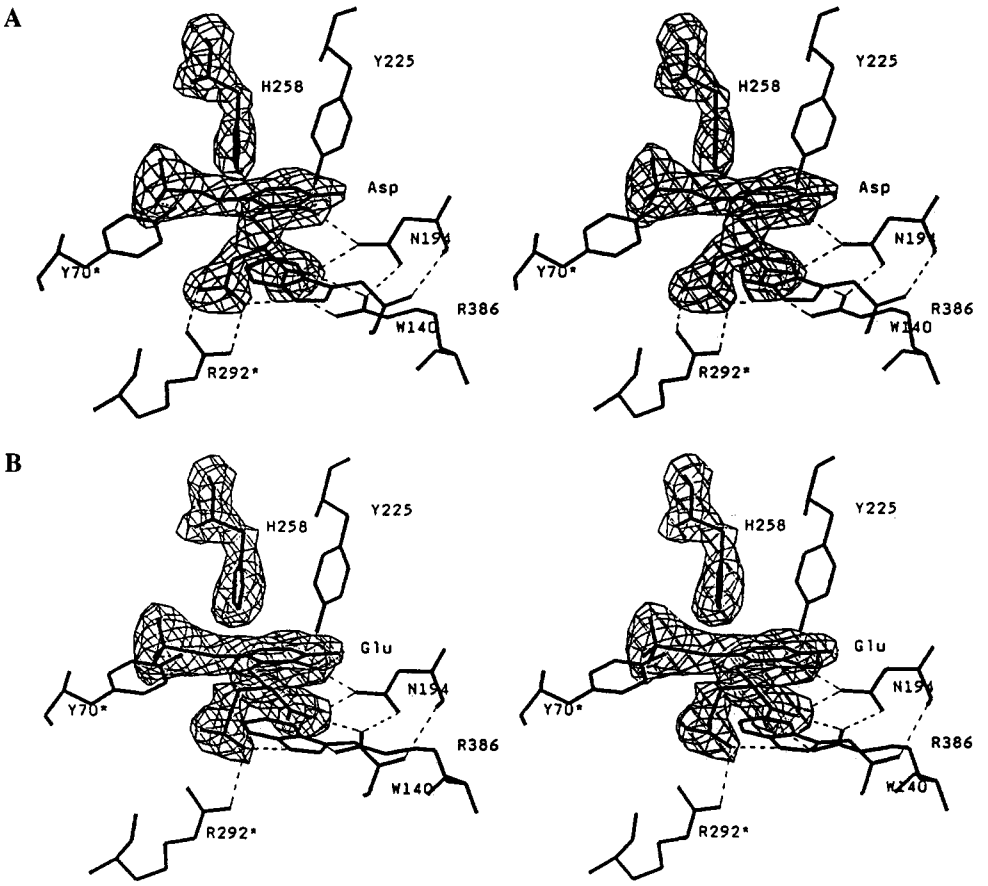


FIGURE 2: Stereoviews of the active sites of the refined mK258H PPxy-Asp and mK258H PPxy-Glu structures superimposed onto omit electron density maps calculated with ($F_o - F_c$) coefficients and calculated phases. The maps are contoured at 5σ. (A) mK258H PPxy-Asp; (B) mK258H PPxy-Glu. Clear, continuous densities at the substrate−coenzyme binding site indicate high occupancy with a covalent coenzyme−substrate adduct. The conformations of the adducts and the side chain of H258 are well-defined by the density. Gly38 is not shown.

Table 2: Selected Active Site Interatomic Distances for the PPxy-Asp and PPxy-Glu Complexes of mAspAT K258H

		dist (Å)				dist (Å)	
source atom	target atom	−Asp	−Glu	source atom	target atom	−Asp	−Glu
C_α PPxy-	$N_{\epsilon 2}$ H258	4.20	4.10	$C_{4'}$ PPxy-	$N_{\epsilon 2}$ H258	3.70	3.80
	$N_{\epsilon 1}$ W140	3.71	3.94	N PPxy-	$N_{\epsilon 1}$ W140	3.36	3.28
$O_{\delta 1}(O_{\epsilon 1})^a$ PPxy-	$N_{\eta 1}$ R292*	3.56	3.81		O_η Y225	3.51	3.55
	$N_{\eta 2}$ R292*	2.85	3.22	$O_{3'}$ PPxy-	O_η Y225	2.55	2.43
	$N_{\epsilon 1}$ W140	2.98	2.90		$N_{\delta 1}$ N194	2.62	2.52
$O_{\delta 2}(O_{\epsilon 2})$ PPxy-	$N_{\eta 1}$ R292*	3.04	3.33	N_1 PPxy-	$O_{\delta 1}$ D222	3.31	3.33
	$N_{\eta 2}$ R292*	3.78	4.11		$O_{\delta 2}$ D222	2.89	2.67
	O Wat57	2.74	2.38	C PPxy-	$C_\gamma(C_\delta)$ PPxy-	3.16	3.49
O_1 PPxy-	$N_{\eta 2}$ R386	2.95	2.71	$N_{\epsilon 2}$ H258	O_η Y70*	3.54	3.42
	N G38	3.09	2.78		O_η Y225	3.87	4.10
O_2 PPxy-	$N_{\eta 2}$ R386	3.31	3.31	$N_{\delta 1}$ H258	O G38	2.88	2.89
	$N_{\eta 1}$ R386	2.85	2.56				
	$N_{\delta 1}$ N194	2.91	2.73				
	$N_{\epsilon 1}$ W140	3.29	3.31				

[a] $O_{\delta 1}$ and $O_{\delta 2}$ are the distal carboxylate oxygens in PPxy-Asp, and $O_{\epsilon 1}$ and $O_{\epsilon 2}$ are the distal carboxylate oxygens in PPxy-Glu.

factor refinement led to the final model (Figure 2B) with good geometry and a low *R*-factor value (Table 1). In mK258H PPxy-Glu the dihedral angle defined by C_3, C_4, $C_{4'}$, and N is −33°, similar to that in mK258H PPxy-Asp (Figure 3B). The side chain of glutamate in PPxy-Glu is not extended ($\chi_1 = -43°$ and $\chi_2 = -67°$). The following differences distinguish the structure of the PPxy-Glu complex from that of the PPxy-Asp complex. The planes of the γ-carboxylate and the guanidinium group of R292* deviate from coplanarity by 45−50°. The side chain of V37 is rotated by 48° around the $C_\alpha - C_\beta$ bond in order to avoid too close van der Waals contact with the C_γ atom of the substrate side chain. This brings it from a sterically favorable staggered conformation in the mK258H PPxy-Asp structure ($\chi_1 = 44°$) to an eclipsed one ($\chi_1 = -4°$). The electron density for V37 is weak, presumably due to multiple conformations. H258 does not form any strong hydrogen bonds. As in mK258H PPxy-Asp, the closest possible hydrogen-bonding partners of H258 are O_η of Y70*, O_η of Y225, and O of G38 at distances of 3.4, 4.1, and 2.9 Å (with unfavorable geometry), respectively. The overall *B*-factor of the H258 side chain is 34 Å^2, similar to the average of its

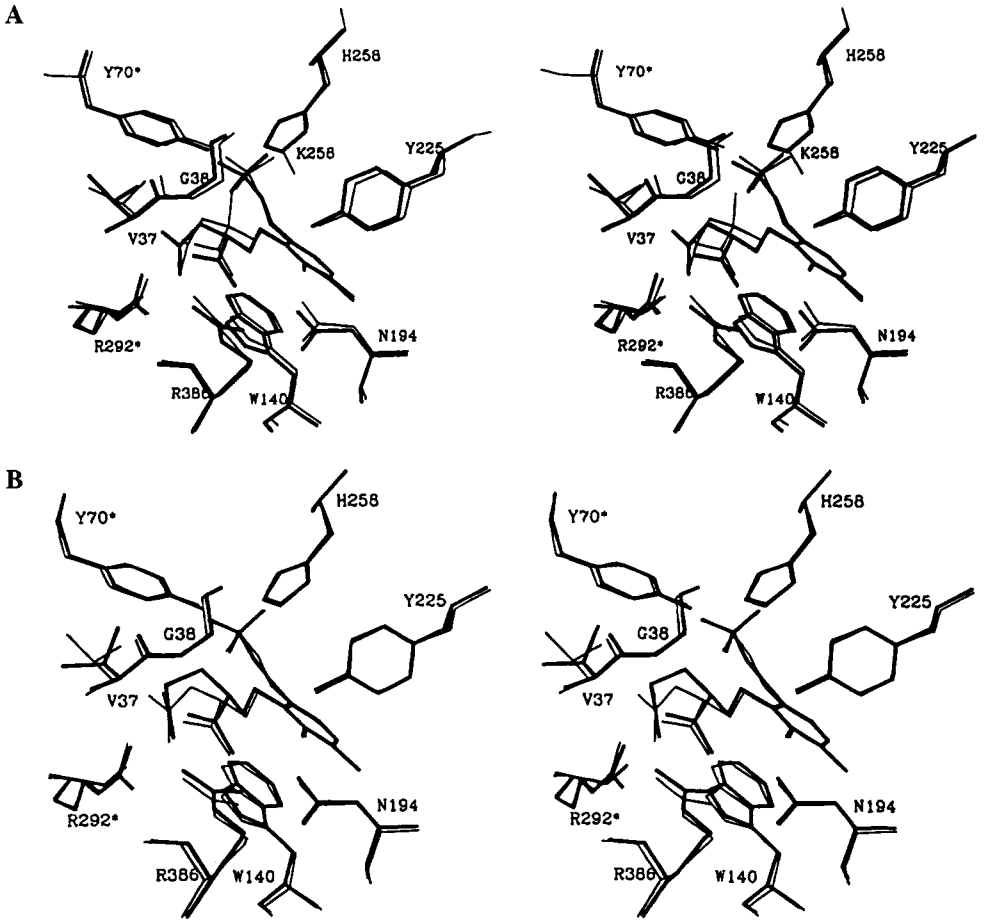


FIGURE 3: (A) mK258H PPxy-Asp structure (thick bonds) superimposed onto the 2-methylaspartate external aldimine structure of wt enzyme (thin bonds; Jansonius & Vincent, 1987). Conformational changes are limited to the immediate environment of H258. (B) Superposition of the PPxy-Asp (thin bonds) and PPxy-Glu (thick bonds) complexes of mAspAT K258H. The side chain of glutamate in PPxy-Glu is longer than that of aspartate and adopts a puckered conformation, relative to that of aspartate, to make the substrate moiety fit between R292* and R386. The V37 side chain assumes an unfavorable conformation in mK258H PPxy-Glu in order to accommodate the pucker.

closest neighbors. The shortest distances between the $N_{\epsilon 2}$ atom of H258 (in the two alternative conformations) and C_α and $C_{4'}$ are 4.1 and 3.8 Å. Further selected active site distances are listed in Table 2.

Half-Open Structures of eAspAT K258H. Two of the mutant structures, eK258H PMP and eK258H PMP·SO_4, adopt the half-open conformation of the wt enzyme crystallized from AS (Jäger *et al.*, 1994). The rms deviation between all backbone atoms (dimer) of the mutant (eK258H PMP·SO_4) and the wt eAspAT structure crystallized from AS is 0.32 Å.

In the eK258H PMP structure the H258 side chain is very well defined in the σ_a-weighted ($2F_o - F_c$) electron density, with average *B*-factors of only ~11 Å^2 in both subunits. This can be explained by interactions with the neighboring residues G38, Y225, and Y263. The carbonyl oxygen atom of G38 forms a hydrogen bond of 3.0 Å with $N_{\epsilon 2}$ of the imidazole ring, which is enclosed between the aromatic rings of Y225 and Y263 (see Figure 4A). The electron density of the cofactor PMP is also well-defined. The shape of the density at higher σ levels and the refined *B*-values for the PMP atoms suggest full occupancy of the cofactor binding site. The coenzyme is tightly bound to the protein through nine hydrogen bonds, the same as observed in the PLP form of the wt crystal structure (Jäger *et al.*, 1994). The torsion angles of the phosphate group deviate slightly from those of the wt PLP enzyme crystal structures. The angle ϕ around $C_5-C_{5'}$ is about 80°; i.e., the phosphate ester oxygen is behind the pyridine ring. In the structures of both eK258H PMP and eK258H PMP·SO_4, the pyridine ring plane of the cofactor is tilted by 10° toward the substrate binding site with respect to the wt PLP enzyme. In the wt PLP enzyme the pyridine ring tilting is restricted by the covalent aldimine bond to K258. This is not the case in the wt PMP enzyme, which assumes the most relaxed conformation and orientation as manifested by an ~10° increase in the tilt angle of the pyridine ring. The amino group of PMP in the active site of the eK258H PMP structure is close to H258 and Y70* (Figure 4A). In this structure, the amino group does not interact with negatively charged substrates or competitive inhibitors such as sulfate anions bound to the substrate binding pocket. The torsion angle χ around $C_4-C_{4'}$ is about −85°, indicating that the amino group is not charged, since the protonated amino group would be expected to form a strong ion pair with the ionized $O_{3'}$ hydroxyl group, with a χ-angle near 0°. Only the imidazole ring of H258 is within hydrogen-bonding distance, i.e., 2.95 Å, from the PMP amino group. The $N_{\epsilon 2}$ atom of the imidazole ring could only form a hydrogen bond to the PMP amino group upon rotation of 180° around $C_\beta-C_\gamma$ and would have to break the (weak) hydrogen bond to G38 O first. The electron density in the substrate binding pocket of eK258H PMP can be interpreted

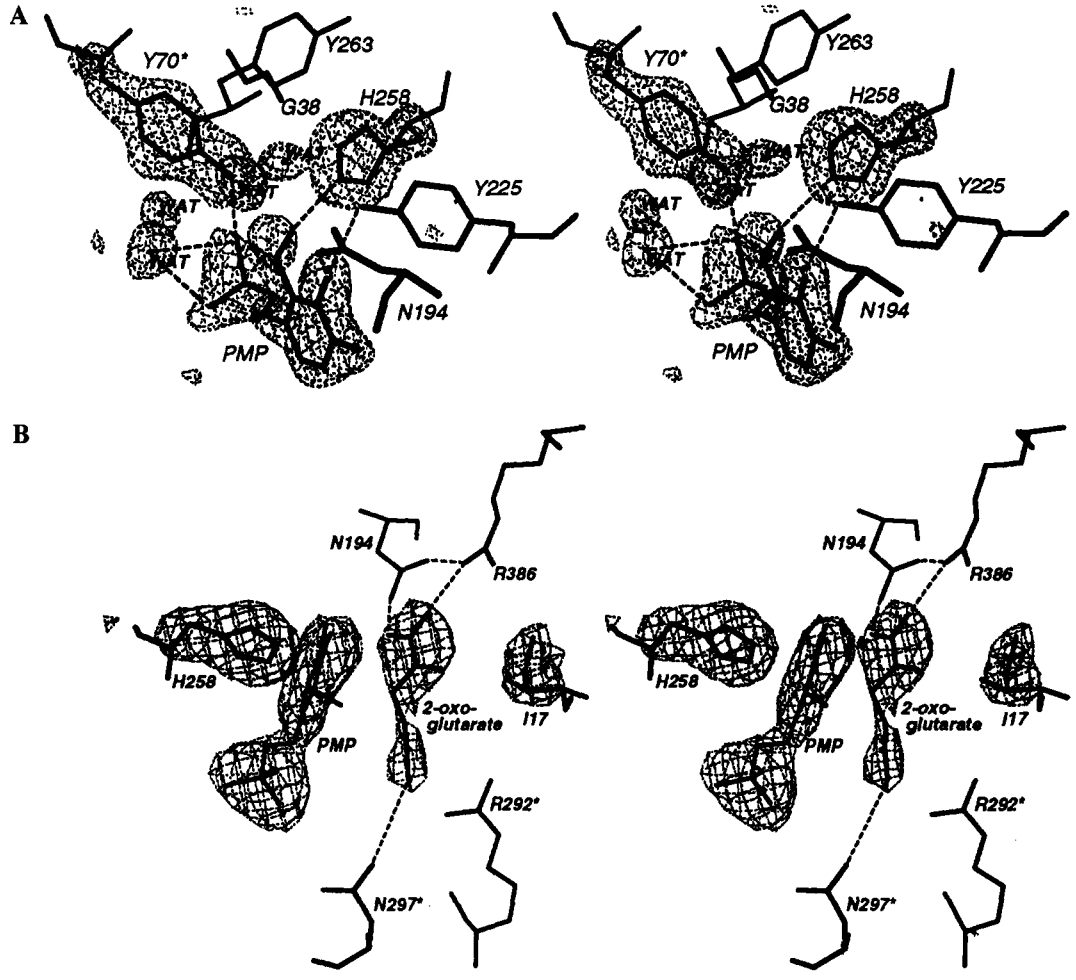


FIGURE 4: Stereoviews of the refined eAspAT K258H structures superimposed onto omit electron density maps calculated with ($F_o - F_c$) coefficients and calculated phases. The map is contoured at 2.6σ. (A) eK258H PMP structure. H258, PMP, and water molecules have been omitted from the electron density calculation. Four water molecules are found in the vicinity of the PMP amino group, which does not form strong hydrogen bonds. (B) Nonproductive binding of the substrate 2-oxoglutarate to the active site of eK258H PMP·2OG (subunit S1). The cofactor, 2-oxoglutarate, and the I17 side chain have been omitted from the electron density calculations. Unexpectedly, the substrate molecule has not formed a ketimine intermediate with the PMP enzyme. The orientation and conformation of the substrate molecule in subunit 2 is somewhat different (Jäger, 1991; not shown).

as four bound water molecules. Although maleate was supplied with the crystallization buffer, it obviously did not bind. The solvent molecules form a hydrogen-bonding network with manifold interactions between themselves and the polar or charged active site residues. The active site geometry of this structure corresponds to that of the unliganded PMP holoenzyme.

A sulfate ion has been identified in the active site of eK258H PMP·SO_4 through the shape and the high electron density level of a feature in a ($F_o - F_c$) difference Fourier map. A sulfate ion was always found in eAspAT structures of AS-grown crystals which adopt the half-open conformation and was located near the guanidinium group of R386 (Smith *et al.*, 1989; Jäger *et al.*, 1994). In eK258H PMP·SO_4, this ion occupies a different site, in the vicinity of R292*, where the distal carboxyl group of a putative substrate would bind. The shift in the sulfate position relative to R386 is likely due to the different chemical nature of the coenzyme in eAspAT K258H. As the PMP amino group is rotated away from H258 and Y70*, a positive charge on the amino nitrogen can be assumed to attract the sulfate anion toward the cofactor, to which it is hydrogen bonded (Jäger, 1991; not shown).

Nonproductive Binding of 2-Oxoglutarate in eAspAT K258H. The PMP form of eAspAT K258H is a poor catalyst with 2-oxoglutarate (Ziak *et al.*, 1993). Hence, complex formation between oxo acids and the PMP form of this mutant enzyme results in a relatively stable reaction intermediate. Conceivably, in the crystal the reaction is slowed down further, so that it might not proceed beyond the stable intermediate in the time span of X-ray intensity data collection. The electron density in the eK258H PMP·2OG structure is, unexpectedly, most consistent with a nonproductively bound 2-oxoglutarate molecule (Figure 4B). The shapes of the densities in the substrate binding pockets of the two subunits differ somewhat. In both subunits the proximal carboxylate groups form single hydrogen bonds to N194 as well as R386, and the distal carboxylate group of the substrate forms a hydrogen bond to N297 (not shown). R292* does not directly interact with 2-oxoglutarate. The oxo group of the substrate points away from the PMP amino group and in one subunit forms a hydrogen bond to the indole ring nitrogen of W140. In the other subunit, the carbonyl group is in contact with I17 $C_{\gamma 2}$. The electron densities of the cofactor and the substrate are clearly separated. The density corresponding to the PMP amino group does not

clearly indicate whether this group is hydrogen-bonded to one of the carboxylate groups of 2-oxoglutarate or whether it is oriented more toward Y70* and H258. It might shuttle between different positions. The binding of 2-oxoglutarate within the substrate-binding pocket of eK258H PMP·2OG induces a partial active site closure, presumably mainly caused by van der Waals interactions with residues I17 and L18. These interactions result in a shift of helix 1 (residues 16−26). Helix 15 (residues 367−377) and β-strands A (residues 33−35) and C (residues 378−380) follow this movement which brings P16 and P141 into van der Waals contact with one another. The side chain of I37 remains in the same position as observed in the half-open wt eAspAT structure, however. In this position, its side-chain atoms are accommodated in a hydrophobic pocket formed by N34, L35, N388, and G391.

All but one solvent molecule (mediating a hydrogen bond between S296* and the phosphate group of the cofactor) are expelled. The position of the remaining water molecule next to the phosphate group is identical to that found in the liganded, closed structures of both wt and eAspAT V39L and, in fact, also in mAspAT (McPhalen *et al.*, 1992a).

DISCUSSION

Domain Closure in mAspAT K258H. The occurrence of two conformations of AspAT was predicted on the basis of solution studies (Birchmeier *et al.*, 1973; Gehring & Christen, 1978) and was subsequently demonstrated by crystallographic determinations of three-dimensional structures of the enzyme in its open and closed conformations [see *e.g.*, Christen and Metzler (1985), Picot *et al.* (1991), McPhalen *et al.* (1992a,b), and Hohenester and Jansonius (1994)]. In solution, the unliganded enzyme exists in the open conformation. Upon binding of dicarboxylate ligands, the equilibrium is shifted toward the closed conformation. Both the open (mK258H) and the closed conformations (mK258H PPxy-Asp and mK258H PPxy-Glu) were observed in the mutant mAspAT. They are identical to those of the wt mitochondrial enzyme. The equilibrium between the two conformations of mAspAT in solution can be monitored by the reaction of C166 with thiol reagents, which is faster by 1 order of magnitude in the closed form of the enzyme (Gehring & Christen, 1978). The reactivity of C166 is 1.5−2 times lower in the liganded forms of mAspAT K258H than in the corresponding complexes of the wild-type enzyme (Ziak *et al.*, 1993). This difference indicates a slight destabilization of the closed structure in the K258H mutant, perhaps resulting from tighter packing in the region of the H258 side chain, an effect that may be assumed to be more pronounced in the closed than in the open structure. This conclusion corroborates the previously observed relative destabilization of the closed conformation in complexes of mAspAT with 5-carbon with respect to 4-carbon substrates (Malashkevich *et al.*, 1993).

Half-Open Conformation in eAspAT K258H. It is interesting to note that the eK258H PMP structure, in which the active site is filled with water molecules only, does not adopt the fully open conformation of the chicken mAspAT holoenzyme. In all eAspAT structures known so far, the half-open conformation had been observed only in crystals grown from AS (Smith *et al.*, 1989; Jäger, 1991; Jäger *et al.*, 1994), the substrate binding site consequently being occupied by a sulfate ion. It was not *a priori* clear whether the partial closure of the active site cleft, which was intermediate between the open and closed forms of mAspAT, was caused by this sulfate ion. Since eK258H PMP contains neither sulfate ions nor inhibitors and nevertheless is found in the half-open conformation just like the sulfate complexes of wt eAspAT and the K258H mutant, it now seems clear that the *half-open* eAspAT structure corresponds in fact to the open form of this enyzme. Apparently, a domain rotation of 6° in eAspAT affords sufficient access for a substrate molecule to the active site.

The overall conformation of the eK258H PMP·2OG structure deviates somewhat from the half-open conformation found in eK258H PMP, eK258H PMP·SO_4, and wt eAspAT. It turns out that the C-terminal half of helix 13 and also helices 14 and 16 [see Jäger *et al.* (1994) for definitions] remain in positions almost identical to those in the half-open conformation, whereas the N-terminal part of the small domain (residues 15−46), the small β-sheet (strands A and C), and helix 15 in the C-terminal part of the small domain are shifted by 0.5−0.9 Å toward the conformationally invariant PLP-binding domain. This only partial closure can probably be attributed to the fact that the 2-oxoglutarate complex was produced by soaking rather than cocrystallization. Lattice constraints presumably prevented the full domain movement. The character of this partial domain movement illustrates the small domain malleability revealed before by temperature factor analyses and structural comparisons between unliganded and liganded forms of the enzyme. These analyses had indicated that the small domain in eAspAT can be subdivided into three regions which change their relative orientation to some extent (Jäger *et al.*, 1994). Closure of the active site *via* a rigid body rotation of the small domain is thus only an approximate description of the transition from the unliganded, open to the liganded, closed conformation. This has been found for wt mAspAT as well (McPhalen *et al.*, 1992b).

Substrate Specificity of mAspAT. The mitochondrial isozyme has a clear preference for 4- over 5-carbon substrates with a $\Delta\Delta G^{\circ\prime} = 1.8$ kcal/mol [$(k_{cat}/K_M)_{Asp} = 4.5 \times 10^5$ M^{-1} s^{-1}; $(k_{cat}/K_M)_{Glu} = 2.3 \times 10^4$ M^{-1} s^{-1}; Michuda & Martinez-Carrion, 1969; Gehring, 1985]. For mAspAT K258H a very similar value of $\Delta\Delta G^{\circ\prime} = 2.0$ kcal/mol applies [$(k_{cat}/K_M)_{Asp}$ = 350 M^{-1} s^{-1}; $(k_{cat}/K_M)_{Glu} = 12$ M^{-1} s^{-1}; Ziak *et al.*, 1993]. In both cases the K_M values are responsible for most of the difference. A plausible explanation for this preference for C_4 substrates based on the refined structures of the oxalacetate and 2-oxoglutarate ketimines of mAspAT has been given by Malashkevich *et al.* (1993). The structural results presented here for the PPxy-Asp and PPxy-Glu complexes of mAspAT K258H support the previous conclusions. Exactly as in the ketimine intermediate structures, which were obtained by diffusion of aspartate or glutamate into the mAspAT crystals, mK258H PPxy-Asp and mK258H PPxy-Glu have virtually identical structures.[3] This also holds for the positions and conformations of R386 and R292*. The

[3] Diffusion of 4- or 5-carbon dicarboxylic substrates and inhibitors into the mAspAT crystals as well as cocrystallization with these ligands results in identical (closed) conformations of the enzyme, independently of the crystal form obtained (Picot *et al.*, 1991; Malashkevich *et al.*, 1993; Hohenester & Jansonius, 1994). This is further evidence that the observed conformation is not imposed by crystal lattice forces.

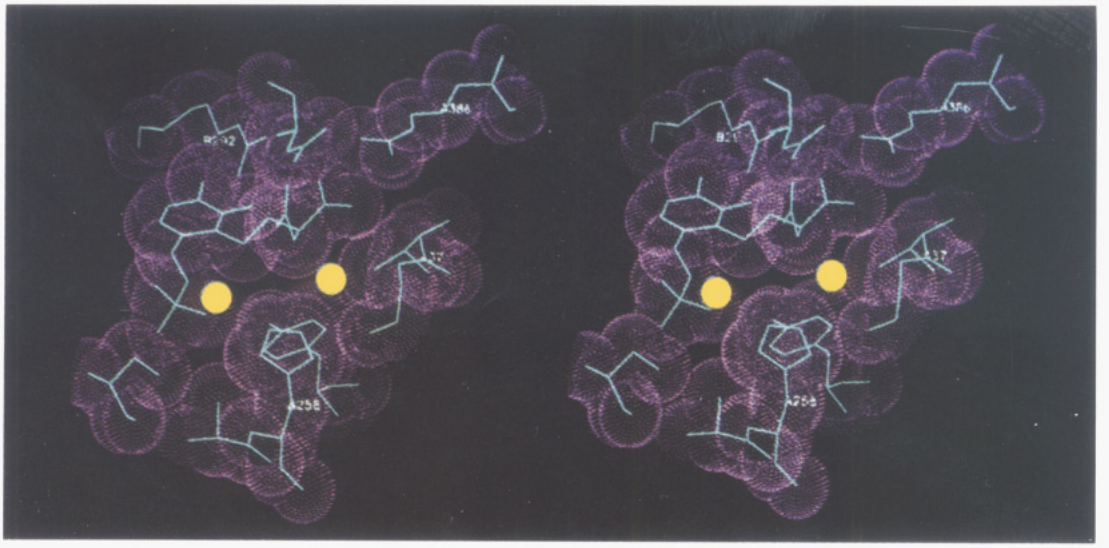


FIGURE 5: Stereo diagram of van der Waals surfaces in the active site of mK258H PPxy-Asp. The substrate-binding arginine residues are labeled B292 and A386. The channel that can be used by a transient water molecule protrudes from the surface (the opening is tuned by the side chain of V37, on the right) to the side chain of H258 and the coenzyme−substrate adduct. Yellow spheres denote the productive positions of the transient water molecule suitable for proton exchange with C_α (on the right) and $C_{4'}$ (on the left), respectively. The channel is somewhat narrower in the PPxy-Glu complex due to the puckering of the glutamate side chain (see Figure 3B). In both static structures, it is too narrow to accommodate bound water molecules.

L-Asp moiety fits exactly between them, but the extra methylene group in the side chain of L-Glu can be only accommodated by puckering C_β and C_γ out of the path followed by the aspartate side chain (Figure 3B) as was observed before in the ketimine structures (Malashkevich *et al.*, 1993). Likewise, in both structures of the glutamate adduct a χ_1 rotation of 30−45° of the V37 side chain, necessary to prevent too close contact with C_γ of the substrate, causes its C_β−C_γ bonds to be eclipsed with N−C_α and C_α−C (Malashkevich *et al.*, 1993; Figure 3B). This effect, together with the less ideal geometry of the hydrogen bonds between the γ-carboxylate and R292*, may cause the increase in K_M.

Reaction Mechanism of AspAT K258H. The half-reactions of AspAT K258H with aspartate, glutamate, and oxaloacetate are only 3 orders of magnitude slower than the corresponding reactions of the wild-type enzyme. However, the half-reaction with 2-oxoglutarate is slower by 5 orders of magnitude. One of the possible reasons for this discrepancy is the nonproductive binding of 2-oxoglutarate to the PMP form of the mutant enzyme as described above. The following reaction mechanism can be proposed on the basis of functional studies (Ziak *et al.*, 1993) and the structural data presented here. In AspAT K258H, the substrate amino group attacks the aldehyde group of PLP, expelling a water molecule (Scheme 1). This reaction replaces the transaldimination step from internal aldimine to external aldimine in the wt enzyme. In the reverse half-reaction of the mutant enzyme, this step is a hydrolysis, similar to the hydrolysis of the ketimine intermediate. Involvement of the H258 side chain in the proton-transfer reaction within the coenzyme−substrate adduct is indicated by the undiminished rate of stereospecific ^3H release from [4′-^{3}H]PMP (Ziak *et al.*, 1993). In contrast, the K258A enzyme failed to release ^3H (Kochhar *et al.*, 1987). The structural studies of ketimine intermediates of wt mAspAT formed with L-Asp and L-Glu (Malashkevich *et al.*, 1993) demonstrated that the ϵ-amino group of K258 has large conformational freedom, as indicated by an increased *B*-factor of this group. The K258 side chain can rotate to within hydrogen-bonding distances of its N_ζ to C_α and $C_{4'}$. The side chain of H258 is shorter and appears to have much less conformational flexibility, the *B*-factor of this group being not significantly higher than those in its immediate environment. In models of the PPxy-Asp and PPxy-Glu complexes of mAspAT K258H, the $N_{\epsilon 2}$ atom of H258 can be brought to ~4.2 Å from C_α and to ~3.8 Å from $C_{4'}$ (as compared to 2.7 and 2.9 Å, respectively, for K258). These distances are too long for the imidazole group to be able to act directly as a general acid/base catalyst. Most probably, this function is performed by a water molecule situated between H258 and the coenzyme−substrate adduct. The question is how the water molecule gets there. One can think of three possibilities.

A bound water molecule is present in the active site at the right position to perform this function. In fact, it could be the water molecule that is released upon formation of the external aldimine. However, no electron density that could account for such a bound water molecule is seen near the cofactor−substrate adduct in ($F_o - F_c$), α_c electron density difference maps of the mK258H PPxy-Asp and mK258H PPxy-Glu structures. Likewise, Malashkevich *et al.* (1993) did not observe such a bound water molecule near the substrate moiety in their structures of oxalacetate and 2-oxoglutarate ketimines of wt mAspAT, although space for it was available. Thus, this first possibility seems unlikely. Alternatively, the water molecule could gain access upon an occasional domain opening after the external PLP−substrate aldimine has been formed. Although the open−closed conformational equilibrium of the K258H mutant lies more toward the open form than that of the wt enzyme (Ziak *et al.*, 1993), it is not likely that domain movement would provide access for the required water molecule. The third and most plausible possibility is that water molecules gain fleeting access to the area between H258 and the substrate moiety. Local small conformational changes which constantly take place in protein molecules in thermal equilibrium with their environment produce short-lived channels through which a water molecule may gain access to the active site

of the (statically closed) enzyme. One such possible channel in mAspAT K258H which connects the area between the *si* side (with respect to $C_{4'}$) of the cofactor substrate adduct and the surface of the enzyme is seen in the mK258H PPxy-Asp structure and is illustrated in Figure 5. It is delineated by the residues R386, Y70*, V37, A39, Y263, and H258. The side chain of V37 partially blocks its entrance, more so in mK258H PPxy-Glu than in mK258H PPxy-Asp (compare Figure 3B). The substrate C_α position is more easily accessible than $C_{4'}$ of the cofactor. Such not statically, but only dynamically available ligand-binding sites in proteins are well-known. A good example is hemoglobin, in which the oxygen-binding site is obstructed in the static molecule [for a review, see Petsko and Ringe (1984)]. NMR studies also indicate that even internally bound water molecules have high exchange rates with bulk solvent (Otting *et al.*, 1991).

The proton-transfer reactions in the K258H mutant enzyme could take place as follows. In deprotonation, either at C_α in the external aldimine or at $C_{4'}$ in the ketimine intermediate, a water molecule between this atom and the imidazole group of H258 would in a concerted process deliver a proton to the imidazole group and abstract a proton from the stereoelectronically activated (Dunathan, 1966; Kirsch *et al.*, 1984; Malashkevich *et al.*, 1993) cofactor−substrate adduct, producing the quinonoid intermediate. According to this mechanism, a decrease in the rate of the reaction by 3 orders of magnitude (5 for 2-oxoglutarate as substrate, see above) for the K258H mutant would result from the chemically less favorable and sterically less flexible catalyst pair H_2O/imidazole as compared to K258 in the wt enzyme.

Both the functional studies of the K258H mutant (Ziak *et al.*, 1990, 1993) and the present work show that covalent binding of the coenzyme, i.e., the internal aldimine linkage, appears not to be essential for enzymic transamination. Histidine, at least to some extent, can replace the ubiquitous active site lysine residue as a proton donor/acceptor in the aldimine−ketimine tautomerization, although it is somewhat shorter and lacks the conformational flexibility of the lysine necessary for efficient catalysis.

REFERENCES

Birchmeier, W., Wilson, K. J., & Christen, P. (1973) *J. Biol. Chem. 248*, 1751−1759.

Brünger, A. T. (1988) *J. Mol. Biol. 203*, 803−816.

Burley, S. K., & Petsko, G. A. (1988) *Adv. Protein Chem. 39*, 125−189.

Christen, P., & Metzler, D. E., Eds. (1985) *Transaminases*, John Wiley & Sons, New York.

Dunathan, H. C. (1966) *Proc. Natl. Acad. Sci. U.S.A. 55*, 712−716.

French, G. S., & Wilson, K. S. (1978) *Acta Crystallogr. A34*, 517−525.

Gehring, H. (1985) in *Transaminases* (Christen, P., & Metzler, D. E., Eds.) pp 373−375, John Wiley & Sons, New York.

Gehring, H., & Christen, P. (1978) *J. Biol. Chem. 253*, 3158−3163.

Gehring, H., Christen, P., Eichele, G., Glor, M., Jansonius, J. N., Reimer, A.-S., Smit, J. D. G., & Thaller, C. (1977) *J. Mol. Biol. 115*, 97−101.

Hendrickson, W. A. (1985) *Methods Enzymol. 115*, 252−270.

Hohenester, E., & Jansonius, J. N. (1994) *J. Mol. Biol. 236*, 963−968.

Jäger, J. (1991), Ph.D. Thesis, University of Basel, Basel, Switzerland.

Jäger, J., Köhler, E., Tucker, P., Sauder, U., Housley-Markovic, Z., Fotheringham, I., Edwards, M., Hunter, M., Kirschner, K., & Jansonius, J. N. (1989) *J. Mol. Biol. 209*, 499−501.

Jäger, J., Moser, M., Sauder, U., & Jansonius, J. N. (1994) *J. Mol. Biol. 239*, 285−305.

Jansonius, J. N., & Vincent, M. G. (1987) in *Biological Macromolecules and Assemblies* (Jurnak, F. A., & McPherson, A., Eds.) Vol. 3, pp 187−285, John Wiley & Sons, New York.

Jones, T. A. (1978) *J. Appl. Crystallogr. 11*, 268−272.

Kabsch, W. (1988) *J. Appl. Crystallogr. 21*, 916−924.

Kirsch, J. F., Eichele, G., Ford, G. C., Vincent, M. G., Jansonius, J. N., Gehring, H., & Christen, P. (1984) *J. Mol. Biol. 174*, 497−525.

Kirsch, J. F., Finlayson, W. L., Toney, M. D., & Cronin, C. N. (1987) in *Biochemistry of Vitamin B_6* (Korpela, T., & Christen, P., Eds.) pp 59−67, Birkhäuser, Basel.

Kirsch, J. F., Planas, A., & Toney, M. D. (1991) in *Enzymes Dependent on Pyridoxal Phosphate and Other Carbonyl Compounds as Cofactors* (Fukui, K., Kagamiyama, H., Soda, K., & Wada, H., Eds.) pp 161−167, Pergamon Press, Oxford.

Kochhar, S., Finlayson, W. L., Kirsch, J. F., & Christen, P. (1987) *J. Biol. Chem. 262*, 11446−11448.

Kuramitsu, S., Inoue, K., Tanase, S., Morino, Y., & Kagamiyama, H. (1989) *Ann. N.Y. Acad. Sci. 585*, 331−338.

Malashkevich, V. N., Toney, M. D., & Jansonius, J. N. (1993) *Biochemistry 32*, 13451−13462.

Malcolm, B. A., & Kirsch, J. F. (1985) *Biochem. Biophys. Res. Commun. 132*, 915−921.

McPhalen, C. A., Vincent, M. G., & Jansonius, J. N. (1992a) *J. Mol. Biol. 225*, 495−517.

McPhalen, C. A., Vincent, M. G., Picot, D., Jansonius, J. N., Lesk, A. M., & Chothia, C. (1992b) *J. Mol. Biol. 227*, 197−213.

McPherson, A. (1982) *Preparation and analysis of protein crystals*, pp 119−121, John Wiley & Sons, New York.

Messerschmidt, A., & Pflugrath, J. W. (1987) *J. Appl. Crystallogr. 20*, 306−315.

Michuda, C. M., & Martinez-Carrion, M. (1969) *J. Biol. Chem. 244*, 5920−5927.

Mitchell, B. O., Thornton, J. M., Singh, J., & Price, S. L. (1992) *J. Mol. Biol. 226*, 251−262.

Otting, G., Liepinsh, M., & Wüthrich, K. (1991) *Science 254*, 974−980.

Petsko, G. A., & Ringe, D. (1984) *Annu. Rev. Biophys. Bioeng. 13*, 331−371.

Pflugrath, J. W., Saper, M. A., & Quiocho, F. A. (1984) in *Methods and applications in crystallographic computing* (Hall, S., & Ashida, T., Eds.) pp 404−407, Clarendon Press, Oxford, U.K.

Picot, D., Sandmeier, E., Thaller, C., Vincent, M. G., Christen, P., & Jansonius, J. N. (1991) *Eur. J. Biochem. 196*, 329−341.

Read, R. J. (1986) *Acta Crystallogr. A42*, 140−149.

Smith, D. L., Ringe, D., Finlayson, W. L., & Kirsch, J. F. (1986) *J. Mol. Biol. 191*, 301−302.

Smith, D. L., Almo, S. C., Toney, M. D., & Ringe, D. (1989) *Biochemistry 28*, 8161−8167.

Sterk, M., & Gehring, H. (1991) *Eur. J. Biochem. 201*, 703−707.

Toney, M. D., & Kirsch, J. F. (1991) *J. Biol. Chem. 266*, 23900−23903.

Ziak, M., Jaussi, R., Gehring, H., & Christen, P. (1990) *Eur. J. Biochem. 187*, 329−333.

Ziak, M., Jäger, J., Malashkevich, V. N., Jaussi, R., Gehring, H., Jansonius, J. N., & Christen, P. (1993) *Eur. J. Biochem. 211*, 475−484.

BI941098V