Active-Site Labeling of Aspartate Aminotransferases by the β,γ-Unsaturated Amino Acid Vinylglycine†

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ABSTRACT: The pyridoxal form of both cytosolic and mitochondrial aspartate aminotransferase is irreversibly inactivated consequent to its interaction with the β,γ-unsaturated substrate analogue vinylglycine. Per catalytic cycle, 90% of the enzyme molecules are inactivated while 10% escape inactivation by transamination to the pyridoxamine form. In the presence of vinylglycine plus 2-oxoglutarate, inactivation is complete because of retransamination of the pyridoxamine form to the susceptible pyridoxal form. Peptide analyses after inactivation with [1-^{14}C]vinylglycine showed that vinylglycine alkylates the active-site lysine residue 258 which forms the internal aldimine with the coenzyme pyridoxal 5′-phosphate. The coenzyme itself is left intact; resolution of the inactivated enzyme by base or trichloroacetic acid yields pyridoxal-5′-P. The absorption spectrum of the inactivated enzyme (λ_{max} 335 nm) suggests that the cofactor is bound as a substituted aldimine. The proposed pathway of alkylation of Lys-258 involves abstraction of the α proton from vinylglycine, isomerization to the α,β-unsaturated enamine, and subsequent nucleophilic attack of the ε-amino group of the lysyl residue at the β carbon of the inhibitor. The determination of the amino acid sequence around the coenzyme-binding lysyl residue in the mitochondrial isoenzyme from chicken gave Ala-(ε-Pxy)Lys-Asn-Met-(Gly,Leu,Tyr) which is identical with the other mitochondrial transaminases examined so far.

Amino acids with a double bond in the β,γ position can be irreversible inhibitors of pyridoxal 5′-phosphate dependent enzymes (Rando, 1975; Soper et al., 1977). These compounds belong to a class of enzyme inhibitors that require catalytic conversion by the target enzyme. They are relatively unreactive substrate analogues which, once activated, may react in situ with a neighboring functional group and thus inactivate the enzyme. Since these enzyme-activated or k_{cat} inhibitors (Rando, 1974a; Abeles and Maycock, 1976) rely both on binding and catalytic conversion by the target enzyme, they are highly specific in their action and thus have great potential for in vivo applications.

The simplest β,γ-unsaturated amino acid, vinylglycine (CH_2=$CHCHNH_2COOH$), has been shown previously to be a k_{cat} inhibitor of cytosolic aspartate aminotransferase (Rando, 1974b) as has its 4-methoxy analogue which is a bacterial toxin (Rando et al., 1976). The present study investigates the mode of inactivation of the cytosolic and the mitochondrial isoenzymes of aspartate aminotransferase by vinylglycine and characterizes the enzyme–inhibitor adducts. The reactions of these pyridoxal-5′-P dependent enzymes are of special interest since the determination of their spatial structures is currently under way (Arnone et al., 1977; Gehring et al., 1977).

Experimental Procedure

Materials. The α subform of the cytosolic isoenzyme of aspartate aminotransferase (sp act. 350 U/mg) was isolated from pig heart (K. Pfister and P. Christen, manuscript in preparation). The mitochondrial isoenzyme (sp act. 150 U/mg) was isolated from chicken heart (Gehring et al., 1977). Enzymatic activity was measured in the coupled assay with malate dehydrogenase (EC 1.1.1.37, obtained from Boehringer) as described previously (Birchmeier et al., 1973). The pyridoxamine form of the enzyme was prepared by addition of 2 mM cysteine sulfinate (Jenkins and D'Ari, 1966) and subsequent gel filtration on Sephadex G-25 equilibrated with 50 mM sodium phosphate (pH 7.5). Alkaline phosphatase was prepared from *Escherichia coli* (Simpson et al., 1968). L-Aspartic acid, 2-oxoglutaric acid, and iodoacetic acid were obtained from Fluka, L-cysteine sulfinic acid was from Sigma, cyanogen bromide and dansyl chloride were from Pierce, guanidine hydrochloride was from Mann, and pyridoxal-5′-P and pyridoxamine-5′-P were from Merck. Carboxypeptidase C and aminopeptidase M were from Roehm/Roth. D,L-Vinylglycine and D,L-[1-^{14}C]vinylglycine were prepared by the previously published procedure (Rando, 1974, 1977).

Preparation and Separation of Labeled Peptides. The cytosolic enzyme (0.2 mM subunit concentration) was incubated for 8 h with [1-^{14}C]vinylglycine (10 mM, sp radioact. 0.18 μCi/μmol) under the conditions of Figure 1. After inactivation the reagent was removed on a Sephadex G-25 column (50 mM sodium phosphate (pH 7.5)) followed by extensive dialysis against water. The carboxymethylated protein (Hirs, 1967) was cleaved with cyanogen bromide (Steers et al., 1965). The fragments were chromatographed on a Sephadex G-75 column (1.9 × 130 cm, 10% formic acid). The radioactive fractions were pooled, lyophilized, suspended in 0.1 M ammonium bicarbonate, and digested with chymotrypsin (total 4%, from Worthington) at pH 8.5 and 37 °C for 7 h. The reaction was terminated by acidification with formic acid (10%) and the peptide mixture was fractionated on a Sephadex G-25 column (1.1 × 110 cm, 10% formic acid). The mitochondrial enzyme was processed the same way with the exception that the concentration of [1-^{14}C]vinylglycine was 6 mM and its specific radioactivity 0.09 μCi/μmol.

High-voltage electrophoresis was performed on Whatman No. 3MM paper at pH 1.9 (acetic acid–formic acid–water, 4:1:45), pH 3.5 (pyridine–acetic acid–water, 1:10:89), and pH 6.5 (pyridine–acetic acid–water, 25:1:225). Peptides were

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eluted from the paper with 50% acetic acid. Amino acid analyses were performed on a Durrum D-500 analyzer using the standard sodium citrate buffers for protein hydrolysates. Dansylation was carried out by the standard procedure (Hartley, 1970). Aminopeptidase M digestions were performed in 0.2 M *N*-ethylmorpholine acetate (pH 8.5, 37 °C) and carboxypeptidase C digestions in 50 mM sodium citrate (pH 5.3 and 37 °C).

Identification of the Cofactor of the Inactivated Enzyme. Cytosolic aspartate aminotransferase (20 mg) was inactivated as indicated in Figure 1. Excess vinylglycine was removed on a Sephadex G-25 column. The enzyme was precipitated with 10% trichloroacetic acid (Christen and Riordan, 1970) and the resolved cofactor was identified by absorption spectra at pH 2 and 12 (Peterson and Sober, 1954) as well as by paper electrophoresis at pH 1.9. Pyridoxamine-5′-P was detected by its fluorescence or by spraying with ninhydrin, resulting in an orange spot; pyridoxal-5′-P was detected by exposure of the paper to ammonia vapors, which produced a yellow spot, and by soaking the paper in 25 mM sodium borohydride in ethanol, which produced an intensive bluish fluorescent spot.

Isolation of the Phosphopyridoxyl Peptide of Mitochondrial Aspartate Aminotransferase from Chicken Heart. A previously described electrophoresis procedure (Torchinsky et al., 1974), based on the altered electrophoretic mobility of the phosphopyridoxyl peptide after cleaving the phosphoester bond with alkaline phosphatase (Strausbauch and Fischer, 1970), was used. Before electrophoretic separation, the phosphopyridoxyl peptide was prepurified by chromatography on the Sephadex G-25 column described above. Fluorescence was detected with a Perkin-Elmer spectrofluorimeter (excitation at 335 nm, emission at 400 nm). The peptide with bluish fluorescence was eluted from the paper with 50% acetic acid.

Results

Inactivation and Transamination of Aspartate Aminotransferase by Vinylglycine. As reported previously, addition of vinylglycine to the pyridoxal form of cytosolic aspartate aminotransferase results in a time-dependent loss of the enzymatic activity (Figure 1). The mitochondrial isoenzyme behaves almost identically (Figure 1). The pyridoxamine forms of the enzymes are not susceptible to inactivation by vinylglycine. The inactivation is reversed neither by gel filtration nor dialysis, nor by addition of pyridoxal-5′-P. Over two-thirds of its course, the inactivation follows pseudo-first-order kinetics, and then reaches a plateau of 10 ± 3% residual activity. The residual activity is due to enzyme which has transaminated to its pyridoxamine form. Subsequent addition of 2-oxoglutarate to the enzyme solution converts the pyridoxamine form back to the inactivation-susceptible pyridoxal form and initiates complete inactivation. In the presence of 2-oxoglutarate the reaction follows pseudo-first-order kinetics over its entire course (Figure 1). The slower rate of inactivation indicates that the substrate competitively inhibits the interaction of the enzyme with vinylglycine (see Velick and Vavra, 1962).

The absorption spectrum of the coenzyme chromophore in the completely inactivated enzymes had a maximum at 335 nm, suggestive of the pyridoxamine or ketimine form of the enzyme (Fasella et al., 1966). However, under conditions normally used for the resolution of the pyridoxamine form (no amino acid substrate added, 0.5 M sodium phosphate, pH 5.1 to 5.5 (Schlegel and Christen, 1974)) only ~5% of the coenzyme was released from the inactivated cytosolic isoenzyme. Since the enzyme used in these experiments had a residual activity of ~7%, the released coenzyme probably originated from unmodified enzyme. When the inactivated enzyme was

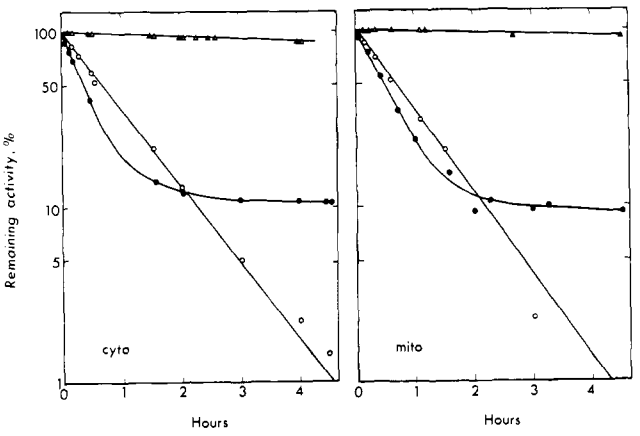


FIGURE 1: Comparison of the inactivation of the cytosolic (cyto) and the mitochondrial (mito) aspartate aminotransferase by vinylglycine. Vinylglycine (18 mM) was added at zero time to a solution of enzyme (0.3 mg/mL in 100 mM sodium phosphate (pH 7.5), 25 °C) in the absence or presence of substrates: pyridoxal form of the enzyme (●); pyridoxal form plus 3.6 mM 2-oxoglutarate (○); pyridoxal form plus 33 mM aspartate (△); pyridoxamine form (▲). The pyridoxal form was prepared by addition of 6 mM 2-oxoglutarate and subsequent gel filtration.

precipitated with 10% trichloroacetic acid, the cofactor was released with >90% yield and was identified as pyridoxal-5′-P by its absorption spectrum and by high-voltage electrophoresis. Addition of 0.1 M NaOH (Peterson and Sober, 1954) to the inactivated enzyme also released pyridoxal-5′-P in stoichiometric quantities. If a solution of inactivated enzyme is acidified to pH <5 by addition of HCl, a yellow color transiently develops which is probably due to the acidic form of enzyme-bound pyridoxal-5′-P absorbing at 430 nm (Jenkins and Sizer, 1960). When [1-^{14}C]vinylglycine was used, 1.0 ± 0.1 mol of vinylglycine per mol of subunit was incorporated in both isoenzymes, concomitant to the inactivation.

Isolation and Amino Acid Sequence of the Labeled Peptide from the Cytosolic Isoenzyme. The radioactively labeled protein was carboxymethylated and then cleaved with cyanogen bromide. The fragments were solubilized in 99% formic acid and applied to a Sephadex G-75 column (see Experimental Procedure). The elution pattern obtained had less material in the first peak than that obtained previously with the cyanogen bromide fragments of aspartate aminotransferase (Birchmeier et al., 1973). Although the cleavage of the modified enzyme with cyanogen bromide was complete, the radioactivity was not located in a single peak (Figure 2). The radioactive fractions were combined in three pools, A, B, and C. Pool A contains peptide 213–287 (Ovchinnikov et al., 1973). Its amino acid composition gave the first indication as to the type of amino acid residue which was modified by vinylglycine. Instead of four lysine residues, only three were detected. In the elution diagram of the amino acid analyzer, a new peak, VGM-Lys,[1] was detected which cochromatographed with homoserine lactone. In order to decide whether the modification with vinylglycine was nonspecific or whether the radioactivity in the three pools was due to the same radioactive peptide distributed over the elution diagram by some chromatographic artefact, such as partial aggregation of the peptide, the material of each of the three pools was digested with chymotrypsin. The radioactive chymotryptic peptides from pools A, B, and C behaved identically on gel filtration. The radioactive fractions were pooled and subjected to two-di-

[1] Abbreviations used are: VGM-Lys, vinylglycine modified lysine, the amino acid derivative produced in the inactivation of the enzyme by vinylglycine; Pxy, pyridoxyl.

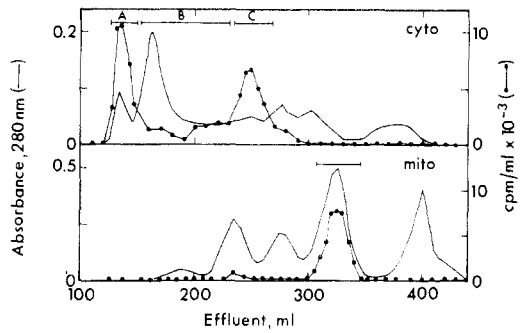


FIGURE 2: Chromatography of the cyanogen bromide fragments of vinylglycine-inactivated cytosolic (cyto) and mitochondrial (mito) isoenzyme of aspartate aminotransferase. The fractions that were pooled are indicated by solid bars. The procedures for preparation of the derivatives and for chromatography are detailed under Experimental Procedure.

mensional high-voltage paper electrophoresis at pH 3.5 and 1.9. On autoradiography there was one main radioactive spot at the same position in all three peptide maps. It contained 95% of the total radioactivity that could be eluted from the paper (yield of elution, 60–70%). Acid hydrolysis and amino acid analysis showed it to be a tetrapeptide containing VGM-Lys (Table I). The spot originating from pool B was inhomogeneous. A peptide with the same amino acid composition was isolated by paper electrophoresis at three different pH values from a chymotryptic digest of a sample of inactivated enzyme that had not been subjected to cyanogen bromide cleavage.

Dansylation (—), digestion with aminopeptidase M (—), and digestion with carboxypeptidase C (—) of the isolated peptide gave the following sequence:

$$\underset{\rightarrow\!\!\rightarrow}{\text{Ser}}\text{-}\underset{\rightarrow}{\text{VGM}}\text{-}\underset{\rightarrow}{\text{Lys}}\text{-Asx-}\underset{\leftarrow}{\text{Phe}}$$

This sequence uniquely corresponds to that of the section Ser_{257}-Lys-Asn-Phe_{260} of cytosolic aspartate aminotransferase from pig heart (Ovchinnikov et al., 1973). The vinylglycine derivative is thus a derivative of lysine residue 258, the residue which forms the internal Schiff base with pyridoxal-5′-P (Morino and Watanabe, 1969).

Isolation and Amino Acid Sequence of the Labeled Peptide from the Mitochondrial Isoenzyme. The peptides obtained by cyanogen bromide cleavage of the radioactively labeled and carboxymethylated enzyme were fractionated on a Sephadex G-75 column. The radioactivity appeared in a single peak (Figure 2). The pooled radioactive fractions were digested with chymotrypsin, prepurified by Sephadex G-25 chromatography, and separated by two-dimensional high voltage paper electrophoresis at pH 3.5 and 1.9. Two radioactive spots, I and II, were detected. Their amino acid compositions (Table I) indicated that the different electrophoretic mobility was due to the presence of homoserine or homoserine lactone at the C terminus. Digestion with aminopeptidase M gave the following amino-terminal sequence Ala-VGM-Lys-(Asn,Met). The amino acid composition and the sequence correspond to the sequence of the active-site peptide Ala-(ϵ-Pxy)Lys-Asn-Met containing the lysine residue which forms the internal aldimine with pyridoxal-5′-P (see below).

The sequence of the active-site phosphopyridoxyl peptide was determined in independent experiments. After reduction with sodium borohydride, carboxymethylation, and digestion with chymotrypsin the resulting peptides were fractionated on a Sephadex G-25 column. The pooled fluorescent fractions were subjected to paper electrophoresis twice at pH 6.5 with intermittent phosphatase treatment (see Experimental Procedure). Two fluorescent bands (1, 2) were found which were

TABLE I: Amino Acid Composition of the Chymotryptic Peptides Labeled with [1-^{14}C]Vinylglycine.

	Molar ratios			
	Cytosolic enzyme		Mitochondrial enzyme	
	Peptide A	Peptide C	Peptide I	Peptide II
Asp	1.00	1.00	1.00	1.00
Ser	0.9	0.8		
Hse			0.4[b]	0.4[b]
Ala			0.9	1.1
Phe	0.9	0.8		
Hse lactone			0.6[c]	0.6[c]
VGM-Lys	1.0[a]	1.0[a]	1.0[a,d]	0.8[a,d]

[a] Calculated with the ninhydrin constant at 590 nm for Asp. [b] Calculated with a ninhydrin constant at 590 nm of 90% of that of Leu. [c] Calculated as 1 minus the value of homoserine. [d] Calculated from the total area minus the area corresponding to homoserine lactone (determined from footnote *c*) by using a ninhydrin constant at 590 nm of 57% of His).

purified further by paper electrophoresis at pH 1.9. Dansylation (—), digestion with aminopeptidase M (—), and digestion with carboxypeptidase C (—) gave the following sequence.

$$\underset{\rightarrow\!\!\rightarrow}{\text{Ala}}\text{-}\underset{\rightarrow}{(\epsilon\text{-Pxy})\text{Lys}}\text{-}\underset{\leftarrow}{\text{Asn-Met}}$$

1

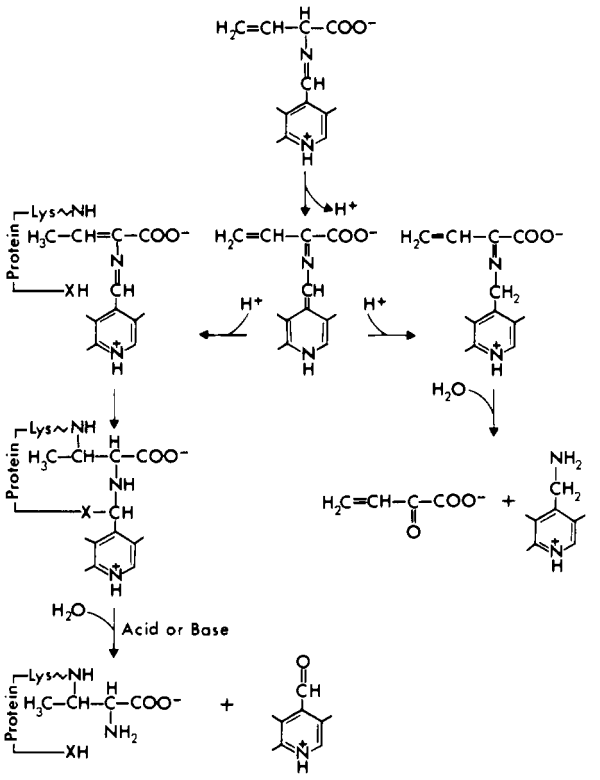
$$\underset{\rightarrow\!\!\rightarrow}{\text{Ala}}\text{-}\underset{\rightarrow}{(\epsilon\text{-Pxy})\text{Lys}}\text{-(Asn,Met,Gly,Leu,Tyr)}$$

2

Discussion

The present investigation shows that the inactivation of both the cytosolic and the mitochondrial aspartate aminotransferase by vinylglycine is due to the selective alkylation of the active-site lysyl residue 258 which covalently binds the coenzyme pyridoxal-5′-P. Since the behavior of the two isoenzymes toward vinylglycine is almost identical, the following exposition is valid for both of them. A description of the reactions of the inhibitor with the enzyme which is compatible with the present data is given in Scheme I. The pathway leading to alkylation of Lys-258 is shown on the left-hand side of the scheme. An enamine is formed by a 1,3-prototropic shift after formation of the aldimine intermediate and α-C–H bond cleavage. The enzyme-bound enamine is an electrophilic Michael acceptor which alkylates the ϵ-amino group of Lys-258. Subsequent hydrolysis yields pyridoxal-5′-P and the alkylated enzyme. The identification of the coenzyme of the inactivated enzyme as pyridoxal-5′-P is in apparent contradiction to its absorption spectrum. The absorption maximum at 335 nm suggests the existence of the pyridoxamine form or of a ketimine bond between the substituted vinylglycine and the coenzyme which on hydrolysis would yield pyridoxamine-5′-P and the keto acid derivative of VGM-Lys. However, a similar absorption spectrum of protein-bound pyridoxal-5′-P has been found in phosphorylase *b* (Kent et al., 1958) and other proteins (Boeker and Snell, 1972). In these cases, the linkage between pyridoxal-5′-P and the protein was assigned the structure of a substituted aldimine. This linkage is, as in the present case, quite tight and resists resolution under the usual conditions. However, on denaturation of the protein pyridoxal-5′-P is released (Kent et al., 1958). In aspartate aminotransferase, the presence of such a substituted aldimine has been postulated to occur in the so-called β subform which is enzymatically less active than the main α subform (Martinez-Carrion et al., 1967). Apparently, a similar bond arrangement develops after the modification with vinylglycine.

SCHEME I



The structure of VGM-Lys proposed in Scheme I is deduced from mechanistic considerations and is consistent with its behavior on electrophoresis and ion exchange chromatography. On high-voltage electrophoresis at pH 6.5, the chymotryptic peptide derived from the cytosolic isoenzyme appears to be positively charged and moves between lysine and glycine. In view of its amino acid composition this indicates the presence of both an amino and a carboxyl group in the vinylglycine moiety of the peptide. Similar conclusions apply to the chymotryptic peptide from the mitochondrial isoenzyme. Correspondingly, the VGM-Lys (released from the chymotryptic peptide either by aminopeptidase M digestion or by acid hydrolysis) co-elutes on the amino acid analyzer with homoserine lactone.

An alternative pathway for the inactivation mechanism would involve reaction of the ϵ-amino group with the ketimine of vinylglycine (Rando, 1974b). This pathway would result in the formation of pyridoxamine-5′-P and the keto acid derivative of vinylglycine substituted at the γ carbon with the lysine side chain. The experiments reported here rule out this possibility in the present case. Interestingly, this pathway applies to the inactivation of aspartate aminotransferase with the methoxy derivative of vinylglycine (L-2-amino-4-methoxy-*trans*-3-butenoic acid) (Rando et al., 1976). The different pathway observed with the methoxy derivative is likely due to the stabilizing effect of the methoxy group on the β,γ-double bond, decreasing the probability of isomerization. This effect has indeed been observed in studies with model compounds (Rando et al., 1976).

The pathway leading to inactivation is taken by 90% of the enzyme molecules undergoing a catalytic cycle with vinylglycine. Every tenth enzyme vinylglycine complex, however, transaminates and yields the pyridoxamine form of the enzyme and the corresponding keto acid (right-hand side of Scheme I). Thus, per catalytic cycle, about 10% of the enzyme molecules interacting with vinylglycine escape the inactivation. The inactivation/transamination ratio is virtually the same in the cytosolic and the mitochondrial isoenzyme of L-aspartate aminotransferase. This correspondence must reflect a close geometric similarity between the active sites of the two homologous isoenzymes. In contrast, the inactivation/transamination ratios of the reaction of D-vinylglycine with bacterial D-amino acid transaminases are 2 to 3 orders of magnitude lower (Soper et al., 1977) and L-alanine aminotransferase has been reported to transaminate vinylglycine without any accompanying loss of enzymic activity (Soper et al., 1977).

The reaction of the ϵ-amino group of Lys-258 with the β carbon of catalytically activated vinylglycine at the active site shows that this group remains within reaction distance of the β carbon during the bond rearrangement steps of transamination. This conclusion is also consistent with previous experiments with β-chloroalanine which have shown that this three-carbon k_{cat} inhibitor alkylates the same residue (Morino and Okamoto, 1973). Lys-258 might thus be close enough to serve as the proton abstracting group in the deprotonation at the α carbon (Morino and Okamoto, 1973). However, if this hypothesis were generally true, a similar spatial relationship of transaminating substrate and the ϵ-amino group of the coenzyme bonding lysyl residue, and hence an inactivation/transamination ratio of the same order of magnitude, would be expected in different transaminases. The large range of the values of the partition ratio (from 0 to 90%) would seem to exclude a general role of this residue in the deprotonation at the α carbon.

The sequence of the phosphopyridoxyl peptide of the mitochondrial asparate aminotransferase from chicken heart which had not been determined previously corresponds to the sequence of the coenzyme-binding peptide of all other mitochondrial isoenzymes reported so far, viz. from pig (Morino and Watanabe, 1969; Doonan et al., 1974; Kagamiyama et al., 1975), sheep (Campos-Cavieres and Milstein, 1975), and beef (Bossa et al., 1976).

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Effect of H^+ on the K^+ Activation of Adenosine-5′-monophosphate Aminohydrolase†

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ABSTRACT: The activation of adenosine-5′-monophosphate aminohydrolase from rabbit skeletal muscle by H^+ has been demonstrated. Evidence is presented which indicates that the binding of H^+ and K^+ is linked, in that the dissociation constant (K_A) for K^+ activation is reduced as the pH is lowered. Concomitantly, the pK of several enzyme functional groups is changed when K^+ is added to a solution of enzyme. This change in pK results in an uptake or release of H^+, depending on the pH, and shows that K^+ interacts with the enzyme to achieve its effect. The uptake or release of H^+ provides a simple method of following conformational changes in the enzyme following interaction of K^+. The K_D for K^+ interaction monitored by following pH changes is the same within experimental error as that measured from kinetic data.

Adenosine-5′-monophosphate aminohydrolase is found in most mammalian tissues in varying levels of activity. It functions to regulate some aspects of purine nucleotide metabolism, ammonia metabolism, energy charge, and gluconeogenesis or glycolysis. The latter stems from the role that AMP[1] has on many enzyme reactions which is not shared by IMP. Most preparations of this enzyme are activated by adenine nucleotides and monovalent cations and inhibited by guanine nucleotides and inorganic phosphates (Zielke and Suelter, 1971a). Our recent studies indicated that H^+ also exerts an important effect on the enzyme activity (Suelter et al., 1968). This paper describes our attempts to determine whether H^+ and monovalent cations such as K^+ interact at the same site, or, if not, to determine the way in which H^+ affects the binding of K^+. A method for detecting conformation changes of 5′-AMP aminohydrolase not discernible by other methodologies is noted.

Materials and Methods

A. *Enzyme Purification.* 5′-AMP aminohydrolase (EC 3.5.4.6) was purified from mature rabbit back muscle in the manner of Smiley et al. (1967). All buffers and reagents were of the same composition as those used by Smiley et al. (1967). The purification was achieved by direct elution of the enzyme from cellulose phosphate with 1.0 M KCl, 1 mM 2-mercaptoethanol, pH 7.0, rather than a 0.45 to 1.0 M KCl gradient as in the original procedure. Specific activities of between 80 and 130 units per mg of protein were obtained at 50 μM 5′-AMP, pH 6.3. The enzyme was routinely stored at 4 °C, in the presence of 1.0 M KCl, 1 mM 2-mercaptoethanol, pH 7.0, under a nitrogen atmosphere. There was negligible loss of activity after 3 weeks. In all experiments, enzyme was used within 2 weeks of preparation.

B. *Enzyme Assays.* 5′-AMP aminohydrolase was assayed using the spectrophotometric assay of Kalckar (1947). For

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[1] Abbreviations used: Mes, 2-(*N*-morpholino)ethanesulfonate; 5′-AMP, adenosine 5′-monophosphate; IMP, inosine 5′-monophosphate; EDTA, ethylenediaminetetraacetic acid; ORD, optical rotatory dispersion; CD, circular dichroism.