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Identification of Methionine-110 as the Residue Covalently Modified in the Electrophilic Inactivation of D-Amino-acid Oxidase by *O*-(2,4-Dinitrophenyl)hydroxylamine†

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ABSTRACT: The reaction of *O*-(2,4-dinitrophenyl)hydroxylamine with D-amino-acid oxidase leads to complete inactivation which can be protected against by the competitive inhibitor benzoate [D'Silva, C., Williams, C. H., Jr., & Massey, V. (1986) *Biochemistry 25*, 5602–5608]. The residue modified has been identified as methionine-110. Differential high-performance liquid chromatography mapping of tryptic digests of D-amino-acid oxidase modified in the absence and presence of benzoate allows the isolation of a single methionine-containing tryptic peptide corresponding to residues 100–115 and referred to as T_6-T_7. In unmodified enzyme, the bond involving Arg-108 is readily cleaved and T_6 and T_7 are isolated. Brief treatment of peptide T_6-T_7 with carboxypeptidase Y released residues 112–115, and the residual peptide was isolated in good yield. Further treatment of this peptide (residues 100–111) with carboxypeptidase Y released Val and an unknown amino acid that comigrated with synthetically prepared *S*-aminomethionine sulfonium salt. The unknown compound and *S*-aminomethionine break down to methionine on treatment with dithiothreitol.

D-Amino-acid oxidase (EC 1.4.3.3) is a flavoprotein that has been the target of numerous mechanistic and structural investigations aimed at understanding the roles that specific amino acid residues play in its catalysis. In an effort to identify these amino acid residues, a number of different chemical modifications have been reported (Williams et al., 1984). Recently we reported the use of *O*-(2,4-dinitrophenyl)-hydroxylamine (DNPHA)[1] in the covalent modification of pig kidney D-amino-acid oxidase (D'Silva et al., 1985, 1986).

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[1] Abbreviations: CPY, carboxypeptidase Y; DNPHA, *O*-(2,4-dinitrophenyl)hydroxylamine; FAD, flavin adenine dinucleotide; HPLC, high-performance liquid chromatography; PTC, phenylthiocarbamoyl; TFA, trifluoroacetic acid.

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DNPHA was shown to cause a 95% loss in enzymatic activity, with the incorporation of 1–1.5 mol of amine residue/mol of enzyme-bound FAD. Benzoate, a competitive inhibitor, protected the enzyme against inactivation, suggesting that DNPHA reacted in or near the active center. The 7–9-nm blue shift of the flavin spectrum of enzyme modified with DNPHA relative to that of native enzyme further supported modification within the active site. The methylation of His-217 results in an enzyme of properties similar to that obtained with DNPHA. The histidine residue (217) methylated by methyl 4-nitrobenzenesulfonate (Swenson et al., 1984a) was eliminated as the target of this modification by isolation of the peptide T_{22} containing His-217 and by recovery of unmodified His-217 (D'Silva et al., 1986). The reaction of DNPHA has been tentatively assigned, from the pH characteristics of the modification reaction, model studies on the specificity of the reagent to different functional groups, and the susceptibility of the modification to thiolysis, to be located on a methionine residue (D'Silva et al., 1986).

While the identity of several amino acid residues at the active center of D-amino-acid oxidase has been established (Williams et al., 1984), prior to this work no evidence to support the presence of a methionine residue had been presented. A total of five methionine residues are present in D-amino-acid oxidase, at positions 1, 87, 110, 124, and 342 in the sequence (Ronchi et al., 1982). Precedence exists for the presence of a methionine residue at the active center of flavoproteins. In the flavodoxin from *Clostridium MP*, Met-56 has been shown from X-ray crystallographic studies to be in van der Waals contact with the bound flavin (Smith et al., 1977), while in *p*-hydroxybenzoate hydroxylase, Met-347 forms part of the wall of the substrate binding site (Hofsteenge, 1981). In view of our interest in the role active site residues play in catalysis and because few reagents for modifications of methionine residues have been reported (Shechter, 1986), we have characterized the properties of this modification and have identified its location in the primary structure of the enzyme.

Experimental Procedures

Materials and Methods. D-Amino-acid oxidase was purified from pig kidney as described previously (Curti et al., 1973). L-1-(Tosylamido)-2-phenylethyl chloromethyl ketone treated trypsin was from Worthington. Acetonitrile (UV spectral grade) was from Fisher Chemical Co. Chemicals used in amino acid analysis as well as carboxypeptidase Y (CPY) and *Staphylococcus aureus* protease were obtained from Pierce Chemical Co. DNPHA was synthesized as described previously (D'Silva et al., 1986). All other chemicals were analytical reagent grade.

Enzyme assays were carried out as described previously (D'Silva et al., 1986).

Enzyme Modification. Modification was carried out as described previously (D'Silva et al., 1986). Enzyme (200 μM with respect to bound FAD) was reacted with a 5-fold excess of DNPHA at 25 °C in 50 mM sodium phosphate, pH 7.4, containing 0.1 mM added FAD, in the absence or presence of 10 mM sodium benzoate. Reaction was carried on until the activity in the standard assay dropped to the stable level of 5% of the initial level. This required a 20-min reaction in the absence of benzoate. In the presence of benzoate, less than 10% activity loss occurred.

Tryptic Digestion, Peptide Mapping, Isolation, and Characterization. Modified protein was denatured under N_2 in 6 M guanidine hydrochloride containing 1 mM ethylenediaminetetraacetic acid, and cysteine thiols were carboxymethylated as described previously (D'Silva et al., 1986), except that dithioerythritol in a 2-fold molar excess over protein thiols was included in a 5-h incubation at pH 8.0 (25 °C) prior to addition of recrystallized iodoacetic acid (1.05-fold molar excess over total thiols in the reaction mixture). Digestion of the modified protein and isolation of the modified peptide were carried out as described previously (D'Silva et al., 1986); Swenson et al., 1982, 1983). Amino acid sequences were determined by the Edman degradation procedure with an Applied Biosystems Model 470A gas-phase sequencer or by the manual Edman degradation procedure described by Tarr (1986).

Amino Acid Analysis. The acid hydrolysates (6 N HCl, 110 °C, 24 h) of purified peptide were derivatized with phenyl isothiocyanate/triethylamine reagent (Tarr, 1986) prior to separation on a Waters Pico Tag column eluted with a two-part linear gradient as described previously (D'Silva et al., 1986). Alternatively, gas-phase hydrolysis of purified peptide was used (Tarr, 1986) prior to derivatization with phenyl isothiocyanate/triethylamine reagent. Methionine sulfoxide analysis of purified peptide used 2 N sodium hydroxide as described by Neumann (1972), and the amino acid composition was determined by conventional ion-exchange amino acid analysis.

S. aureus Protease Cleavage of T_6-$T_7(A)$ or T_6-$T_7(B)$. Peptide (6 nmol) was hydrolyzed with *S. aureus* protease (4% w/w) at 37 °C, in 50 mM phosphate buffer, pH 7.6, for 22 h. Peptides were separated by reverse-phase HPLC at low pH.

Carboxypeptidase Y (CPY) Sequence Analysis of T_6-$T_7(A)$. Peptide (8 nmol) was hydrolyzed with CPY (4% w/w) at 37 °C, in 0.1 M sodium acetate, pH 5.7, for 1 h. Aliquots were removed for PTC-amino acid analysis. The remaining peptide, separated by reverse-phase HPLC at low pH (4.2 nmol), was further digested with CPY (4% w/w) at 37 °C for 7 h, with removal of aliquots at hourly intervals for PTC-amino acid analysis. After 7 h the residual material was purified by reverse-phase HPLC at low pH, and the major peptide was hydrolyzed with acid for amino acid analysis.

Synthesis of S-Aminomethionine Sulfonium Salt. *S*-Aminomethionine sulfonium salt was prepared by two different methods: (a) by incubation for 16 h at room temperature of equal volumes of molar solutions of methionine in water and DNPHA in acetonitrile, the solution then being diluted and used in PTC-amino acid analysis; (b) by adaptation of the method of Tamura et al. (1973). The resultant *S*-aminomethionine 2,4-dinitrophenol salt, obtained as a yellow solid, was freed from traces of methionine by passage through a column of Amberlite IRC-50 in water: ^{1}H NMR (D_2O) δ 1.63 (2 H, m, CH_2), 2.13 (3 H, s, CH_3), 2.46 (1 H, m, CH), 3.2, 3.5 (2 H, m, CH_2), 6.85 (1 H, d, H–Ar), 8.47 (1 H, d, H–Ar), 9.37 (1 H, s, H–Ar); ^{13}C NMR (D_2O) (Bruker) δ 24.2 (CH_3), 36.9 (CH_2), 48.7 (CH_2–S), 53.8 (CH), 125.6, 126.0, 129.7, 132.3, 136.4 (Ar), 173.6 (C=O). The trifluoroacetic acid salt of *S*-aminomethionine sulfonium salt was prepared by acidifying a solution of the 2,4-dinitrophenol salt until the yellow color was discharged and extracting 3 times with ethyl acetate. The aqueous layer was evaporated to afford on repeated evaporation from ethanol a colorless solid which was then thoroughly dried over P_2O_5 and used in the preparation of an *S*-aminomethionine calibration curve.

Results

Identification of the Site of Amination Responsible for Enzyme Inactivation. As the nature of the modification did not permit the use of radioactive techniques, the site of am-

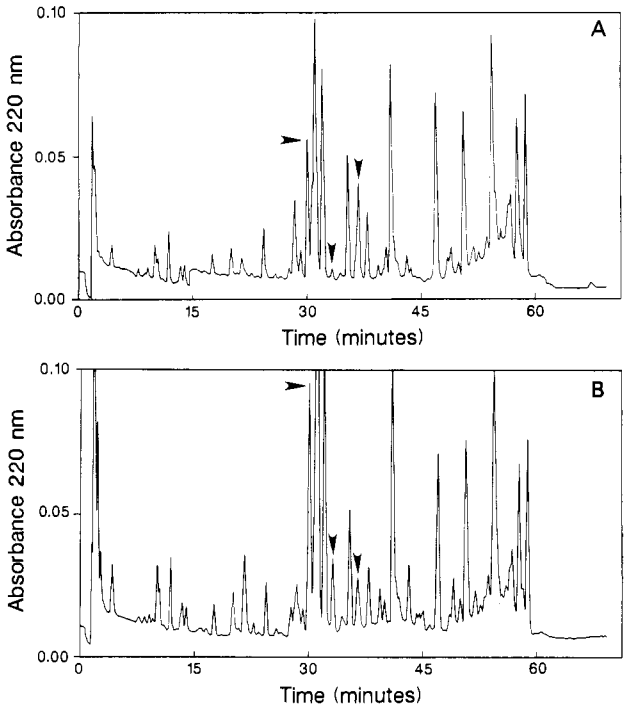


FIGURE 1: High-performance liquid chromatographic peptide maps of tryptic digests of D-amino-acid oxidase that had been reacted with a 5-fold excess of DNPHA in the absence (A) and presence (B) of 10 mM benzoate. Tryptic digests of reduced and carboxymethylated protein were chromatographed in 0.1% phosphoric acid on a Waters Associates μBondapak C_{18} reverse-phase column, using a linear acetonitrile gradient. The peaks discussed in the text eluting at approximately 31, 33, and 37 min have been marked with arrows.

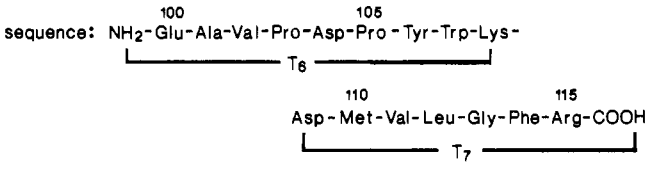
ination responsible for inactivation was determined by comparison of HPLC tryptic peptide maps of protein modified with a 5-fold excess of DNPHA at pH 7.5 in the absence (A) and presence (B) of benzoate. Representative tryptic chromatograms from reverse-phase HPLC at low pH, monitored at 220 nm, are shown in Figure 1. The tryptic map of enzyme reacted in the presence of benzoate (Figure 1B) is very similar to those obtained previously with unmodified enzyme (Swenson et al., 1982, 1984a). The tryptic map of modified enzyme (Figure 1A) differs in several important respects from that of protected enzyme. The peak normally associated with T_7 (33 min) is missing, and the peak normally containing a mixture of T_{22} and T_6 (31 min) is significantly reduced. The peak running near 37 min is markedly larger and is indeed slightly displaced (36.6 min in the protected sample and 36.8 in the modified sample). Two peptides from the modified enzyme were purified from the material in this peak by HPLC at pH 6.9 and were identified as T_{10} and T_6-T_7 by amino acid analysis. The amino acid composition of T_6-T_7 is given in Table I and its sequence is given in Table II, showing that it represents residues 110–115 in the amino acid sequence (Ronchi et al., 1982).

In other experiments where the protection by benzoate had not been so complete and where tryptic hydrolysis was less efficient, intact T_6-T_7 could be isolated by the same sequence of steps as described above and eluted at approximately 45 min in the low-pH chromatograms. Pure T_6-T_7 was separated from contaminating T_{11} at high pH. Its amino acid composition and sequence are shown in Tables I and II, respectively. At pH 6.9 the two purified peptides designated T_6-T_7(A) (obtained from enzyme modified in the absence of benzoate) and T_6-T_7(B) (obtained from enzyme reacted in the presence of benzoate as described above) had similar HPLC retention times of 36.7 and 38.6 min, respectively. The marked difference in retention times between the two peptides at low pH, but not at high pH, suggests the ionization of a modified residue in peptide T_6-T_7(A). This is consistent with the modification of a methionine residue to a *S*-aminosulfonium salt, which would be expected to increase the polarity of the modified peptide [T_6-T_7(A)] relative to the unmodified peptide [T_6-T_7(B)].

Table I: Amino Acid Composition of Peptides T_6-T_7(A) and T_6-T_7(B) from D-Amino-acid Oxidase

amino acid	T_6-T_7(A)[a]	T_6-T_7(B)[a]	amino acid	T_6-T_7(A)[a]	T_6-T_7(B)[a]
Asp (2)	1.7	2.41	Tyr (1)	1.1	0.41
Glu (1)	1.1	1.11	Val (2)	2.0	1.67
Gly (1)	1.1	1.20	Met (1)	0.4	0.95[c]
Arg (1)	1.2	1.02	Leu (1)	1.1	1.23
Ala (1)	1.0	0.95	Phe (1)	1.0	0.93
Pro (2)	2.4	nd[b]	Lys (1)	0.84	1.01

location: 100–115



[a] Hydrolysis in 6 N HCl. [b] nd, not determined. [c] Hydrolysis in 6 N HCl + 1% ethanethiol.

Table II: Automated Gas-Phase Sequence Analysis of Peptides T_6-T_7(A) and T_6-T_7(B) from D-Amino-acid Oxidase

Edman cycle	PTH-amino acid[a] identified	recovery (nmol) T_6-T_7(A)	recovery (nmol) T_6-T_7(B)
1	Glu	3.3	2.8
2	Ala	3.0	2.5
3	Val	2.5	2.1
4	Pro	2.5	1.7
5	Asp	2.2	1.7
6	Pro	2.0	1.7
7	Tyr	1.7	1.5
8	Trp	0.6	0.7
9	Lys	1.4	1.2
10	Asp	1.2	1.3
11	Met	1.0	1.2
12	Val	1.2	1.2
13	Leu	1.2	1.0
14	Gly	1.2	0.8
15	Phe	1.0	0.8
16	Arg	0.3	0.2

[a] PTH, phenylthiohydantoin. The amount of initial peptide coupled was determined by PTC-amino acid analysis as 3 nmol in both cases.

In the amino acid analysis reported in Table I, no unusual amino acid peaks were observed, indicating that the modification is unstable to the strong acid conditions used for hydrolysis. In the automated Edman sequence analysis shown in Table II, all cycles yielded an identifiable phenylthiohydantoin derivative in good yield. No unusual derivative attributable to an aminated methionine was observed, indicating that the modification was also unstable under the acid conditions used repetitively during sequence analysis.

The peptide bond between T_6 and T_7 is normally cleaved by trypsin digestion of native enzyme. The resistance to tryptic cleavage of T_6-T_7(A) may indicate that the modification is close to Lys-108 and has an inhibitory effect on tryptic cleavage at this residue. In well-protected enzyme, such as that shown in Figure 1B, tryptic hydrolysis proceeds normally with T_7 easily visible in the HPLC map (peak at 33 min). It was also observed that while the digestion of T_6-T_7(B) with *S. aureus* protease proceeded normally, with hydrolysis at

Table III: Amino Acid Composition of CPY Peptides Isolated from Cleavage of T_6-T_7(A)

amino acid	peptide fragment 100–111,[a] CPY, 1 h	peptide fragment 100–109,[b] CPY, 7 h	amino acid	peptide fragment 100–111,[a] CPY, 1 h	peptide fragment 100–109,[b] CPY, 7 h
Asp	2.0 (2)	2.3 (2)	Tyr	1.0 (1)	0.9 (1)
Glu	0.8 (1)	1.2 (1)	Val	1.9 (2)	1.0 (1)
Gly			Met	0.6 (1)	
Arg			Leu		
Ala	0.8 (1)	0.9 (1)	Phe		
Pro	2.5 (2)	1.9 (2)	Lys	0.3 (1)	0.8 (1)

[a] Glu-Ala-Val-Pro-Asp-Pro-Tyr-Trp-Lys-Asp-Met-Val. [b] Glu-Ala-Val-Pro-Asp-Pro-Tyr-Trp-Lys-Asp.

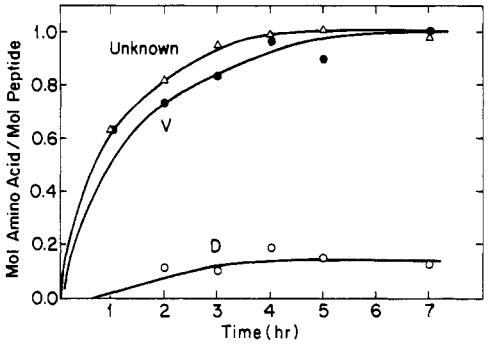


FIGURE 2: Time course of CPY-catalyzed release of amino acids from the purifed peptide fragment 100–111 of T_6-T_7(A).

Asp-109 (and Glu-100), the digestion of T_6-T_7(A) was markedly inhibited (results not shown). Presumably, the introduction of a charged residue close to the hydrolysis points decreases significantly the catalytic activity of both trypsin and *S. aureus* protease.

Identification of Methionine-110 as the Site of Amination by CPY Analysis of Peptide T_6-T_7(A). The identification of the site of modification as Met-110 was achieved by C-terminal analysis of T_6-T_7(A) with carboxypeptidase Y. As PTC-*S*-aminomethionine sulfonium salt elutes in the same location as PTC-arginine under PTC-amino acid analysis conditions, it was advantageous to first remove arginine with subsequent purification of the peptide prior to removal of the modified methionine residue for identification. Treatment of T_6-T_7(A) (8 nmol) with CPY (4% w/w) for 1 h at 37 °C and assay by PTC-amino acid analysis showed the removal of Arg (1.0), Phe (1.0), Gly (0.8), and Leu (0.6). Separation of the hydrolysis mixture at low pH (2.0) by using volatile buffers afforded a single peptide eluting at 25.5 min whose amino acid composition was consistent with the loss of the above residues as shown in Table III. Treatment of the isolated fragment 100–111 with CPY (4% w/w) at 37 °C resulted in a time-dependent release of both valine and an unknown amino acid eluting at 5.7 min and a small amount of aspartic acid as shown in Figure 2. The unknown peak at 5.7 min was assigned as PTC-*S*-aminomethionine on the basis of its retention relative to the standard shown in Figure 3. The standard and the unknown were treated with dithioerythritol (0.1 M) at pH 9.0. Amino acid analysis revealed the appearance of methionine in both cases. It should be noted that both high pH and high concentration of thiol are required for thiolysis of the derivative. These conditions differ greatly from those employed in reduction and carboxymethylation of the protein prior to tryptic hydrolysis (see Materials and Methods). Further substantiation of the loss of both valine and modified methionine from peptide fragment 100–111 was achieved by purification of the peptide remaining after a 7-h CPY hydrolysis by reverse-phase HPLC at low pH (2.0) with volatile buffers and determination of the amino acid composition of the major peptide. The result obtained shown in Table III is consistent with the recovery of peptide fragment 100–109 and the loss of both valine and methionine.

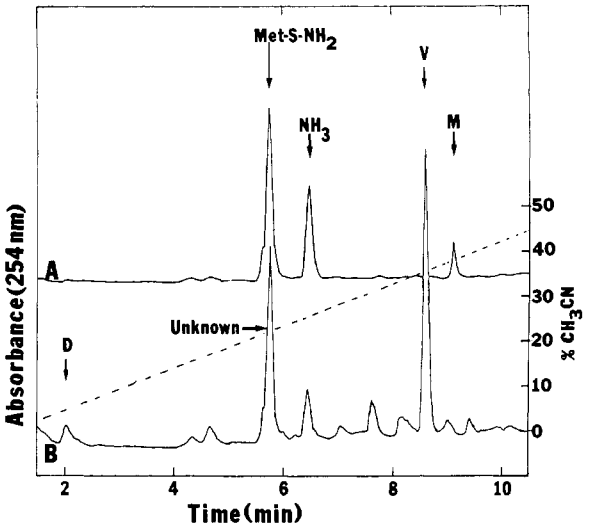
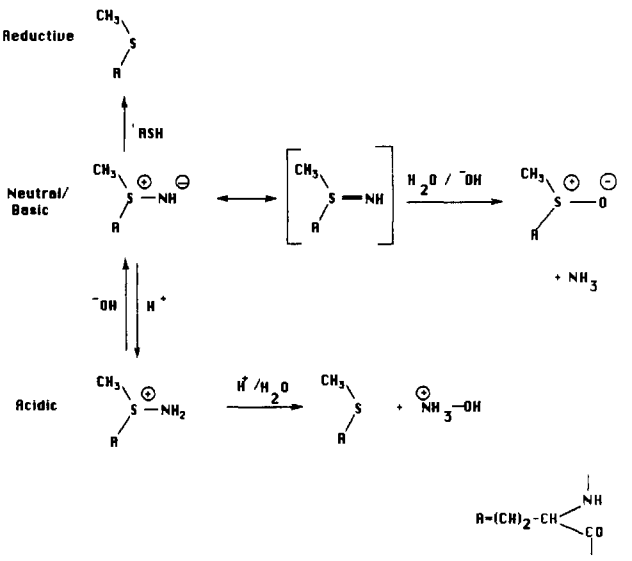


FIGURE 3: (A) Chromatogram of *S*-aminomethionine sulfonium salt prepared by method a (see Synthesis of *S*-Aminomethionine Sulfonium Salt). (B) Chromatogram from the CPY analysis of 4-h digests of purified fragment 100–111 of T_6-T_7(A).

Scheme I



Discussion

DNPHA is a unique modifying agent that achieves a minimal modification by the introduction of an amino group. This reagent, on the basis of model studies, has been shown to exhibit a marked specificity for amino acid side chains containing a soft nucleophilic center, e.g., sulfur (D'Silva et al., 1986). In the inactivation of D-amino-acid oxidase the residue modified was postulated to be a single methionine residue on the basis of kinetic studies (D'Silva et al., 1986). The modification of a methionine residue by DNPHA converts a neutral nonpolar amino acid side chain into a charged polar side chain whose properties are dependent on both the pH and the nature of the environment in which it is located. The properties of such a modification, based on the chemistry of related compounds and observations made in the study of T_6-T_7(A), are summarized in Scheme I. At pH 2.0 the cationic form of the *S*-aminosulfonium salt is predominant, accounting for the 8-min difference in retention time between

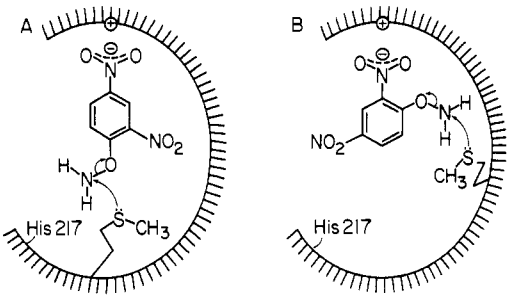


FIGURE 4: Model proposed for interaction of DNPHA with the cationic residue located within the enzyme.

T_6-T_7(A) and T_6-T_7(B), while at pH 7.0 the zwitterionic form may predominate. The presence of a cationic charge, as a result of the introduction of an amino group on the thioether side chain of a methionine residue, significantly alters the acidity of protons in this region, as determined from the downfield shifts relative to methionine of the proton NMR spectra of the CH_3 and CH_2–S groups of 0.43 and 1.46 ppm, respectively. The ^{13}C NMR spectra showed similar downfield shifts relative to methionine of 9.85 and 18.6 ppm for the CH_3 and CH_2–S groups, respectively. The zwitterionic form is neutral, and this property is reflected at pH 6.9 in the very small difference in the retention times on HPLC that is found between T_6-T_7(A) and T_6-T_7(B) in comparison to that which exists at low pH. The pK_a for formation of the zwitterionic species may be estimated to be >4.2, as the pK_a reported for the amino group of (aminooxy)amino acids (NH_2–O–R) (Kisfaludy et al., 1976) is in this range. On the basis of these observations we may assume that the modified residue on the enzyme (Met-110) exists primarily as the neutral species, assuming that deprotonation of the *S*-aminosulfonium salt is not prevented in any way due to the dielectric properties associated with its environment. Both strong acids and thiol reductants regenerate methionine. The latter effect is also observed with *S*-methylsulfonium salts (Christie et al., 1979; Naider & Bohak, 1972). Gas-phase Edman sequencing of T_6-T_7(A) results in the recovery of methionine and not *S*-aminomethionine sulfonium salt, which is presumably due to the TFA cleavage reactions. Breakdown of the modification may therefore be a result of the accumulative effects of repeated treatment of the modified residue for 15 min at 44 °C with TFA for 11 cycles, including a 4-fold increase in time for cleavage of the Asp–Pro bond at cycle 6. This results in an accumulated time of 3.5 h in which the modification is in constant contact with hot TFA prior to its removal at cycle 11 for analysis. The assignment of Met-110 as the site of amination by DNPHA was achieved by a two-stage hydrolysis of T_6-T_7(A) with CPY. The identification of the unknown peak as *S*-aminomethionine (comigration with standard) confirms the location of the modification on the methionine residue at position 110 in the primary structure of the enzyme.

This study extends the number of amino acid residues identified at or near the active center of D-amino-acid oxidase. The relative location of this residue to other residues such as His-217 may be inferred from the nature of binding utilized by the reagent in the inactivation of the enzyme (D'Silva et al., 1986). Two alternative orientations for the interaction of DNPHA with the substrate binding cationic center of the enzyme are possible, as shown in Figure 4. If inactivation is a result of the orientation shown in Figure 4A, then Met-110 is adjacent to His-217, assuming methyl 4-nitrobenzenesulfonate utilizes a similar mode of binding in its inactivation of the enzyme (Swenson et al., 1984a; Williams et al., 1984). However, if modification is a result of the interaction of the 2-nitro group at the cationic center (Figure 4B), then Met-110 is somewhat removed from His-217 and is nearer the cationic center, presumably an arginine residue.

The role of methionine in proteins is unclear. It is believed that this residue mainly fulfills a structural role, but in a few enzymes participation in the catalytic process has been postulated. Modification of a methionine residue in chymotrypsin, for example, alters the affinity of the enzyme for substrate without affecting its ability to hydrolyze substrate (Lawson & Schramm, 1965; Weiner et al., 1966); in *Pseudomonas*

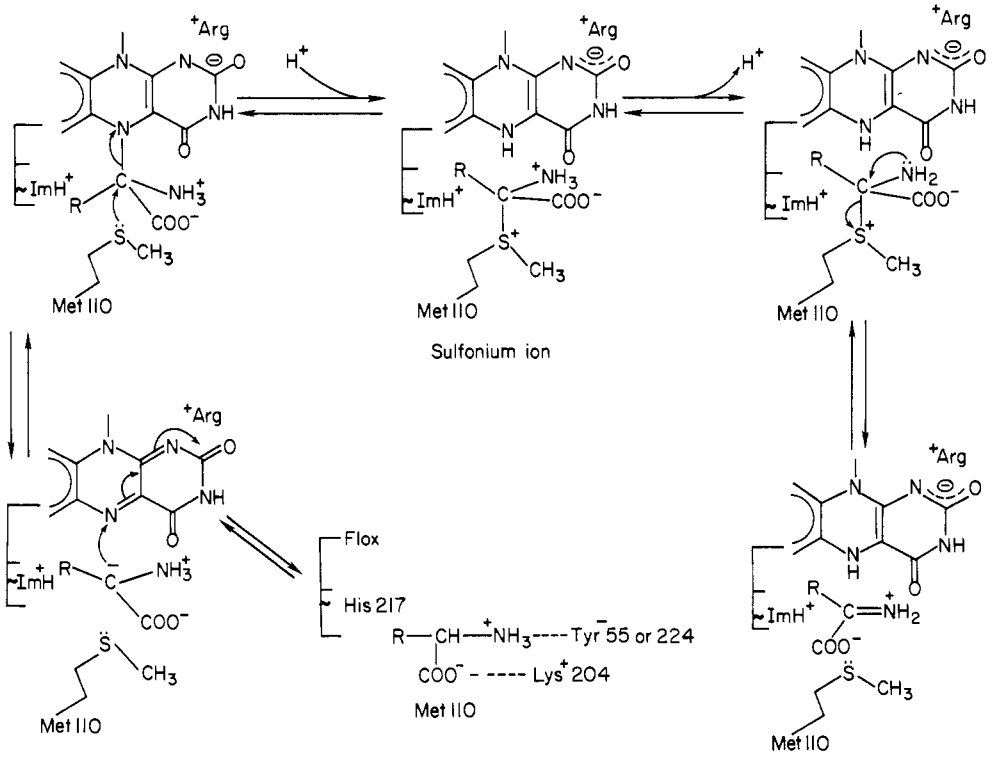


FIGURE 5: Proposed mechanism for catalytic involvement of Met-110 in the reductive half-reaction of D-amino-acid oxidase.

cytochrome *c*, methionine is implicated in coordination of the heme iron (Fanger et al., 1967), while in ribonuclease, alkylation of a methionine residue decreases the ability of *S*-protein and its *S*-peptide to refold into its native conformation (Stark & Stein, 1964; Vithayathil & Richards, 1960). Other enzymes for which the participation of methionine in catalytic function has been proposed are phosphoglucomutase (Ray & Koshland, 1962), myokinase (Kress & Noda, 1967), peroxidase (Brill & Weinryb, 1967), isocitrate dehydrogenase (Colman, 1968), and lipoxygenase (Kuhn et al., 1984). Our data indicate that modification of a single methionine residue located at position 110 in the primary sequence results in a significant loss in activity and the formation of a catalytically crippled form of the enzyme exhibiting 5% of the activity of native enzyme. Despite its small size, this modification, like the methylation of His-217 (Swenson et al., 1984a,b), causes a marked spectral change in the flavin absorption and significant weakening in the binding of benzoate (D'Silva et al., 1986). The 70-fold rate enhancement for the reaction of DNPHA with a methionine residue at the active site of the enzyme relative to a methionine residue in solution may be a consequence of binding and proximity effects or the enhanced reactivity of this particular methionine residue. We do not know which of the two locations Met-110 occupies relative to His-217 (see Figure 4). If the location is adjacent to His-217 as shown in Figure 4A, we may infer a catalytic role for this residue. The thioether group of a methionine residue has no acidic or basic properties in aqueous solution but is a powerful neighboring group as indicated by the reactivity of mustard gases (Hine, 1956). The presence of a sulfur group increases the reactivity of these compounds by several orders of magnitude by displacing chloride ion, forming a sulfonium ion which then rapidly reacts with water to yield the product. On the basis of this mechanism an analogous role may be perceived for Met-110 in which it participates in the breakdown of the proposed N_5 flavin adduct (Massey & Ghisla, 1983) to form a sulfonium salt as shown in Figure 5. The amine group would be expected to deprotonate spontaneously and then donate its lone pair of electrons to the electron-deficient C center, resulting in the collapse of the sulfonium salt and the regeneration of methionine and the product imine. The participation of the methionine residue in the catalytic mechanism would facilitate deprotonation of the amine residue and accelerate the rate of formation of product from the N_5 adduct.

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Registry No. D-Amino-acid oxidase, 9000-88-8; *O*-(2,4-dinitrophenyl)hydroxylamine, 17508-17-7; methionine, 63-68-3.

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