DAVID M. GREENBERG

Metabolic Pathways

VOLUME II

Metabolic Pathways

(Second Edition of *Chemical Pathways of Metabolism*)

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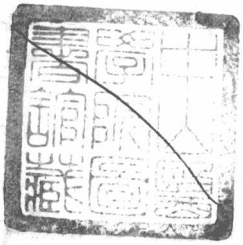

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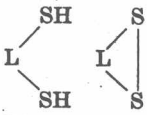
LIST OF COMMON ABBREVIATIONS AND SYMBOLS

AMP, GMP, IMP, XMP, UMP, CMP, OMP	The 5′-phosphates of ribosyladenine, guanine, hypoxanthine, xanthine, uracil, cytosine and orotic acid.
dAMP, dGMP, dUMP, dCMP	The 5′-phosphates of deoxyribosyl adenine, etc.
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
NMN	Nicotinamide mononucleotide
DPN_{ox}, DPN^+, DPN	Diphosphopyridine nucleotide (cozymase, coenzyme I)
DPN_{red}, DPNH	Reduced form of above
TPN_{ox}, TPN^+, TPN_{red}, TPNH	Triphosphopyridine nucleotide (coenzyme II)
FMN	Flavin monophosphate
FAD, $FADH_2$	Flavin adenine dinucleotide and its reduced form
TPP	Thiamine pyrophosphate
CoA, CoASH	Coenzyme A
GSH, GSSG	Glutathione and its reduced form
	Lipoic acid, thioctic acid
FA	Folic acid, pteroyl glutamic acid
FH_2, FH_4	Dihydro and tetrahydrofolic acid, respectively
PRPP	5′-Phosphoribosyl-1′-pyrophosphate
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
P	Inorganic phosphate
PP	Inorganic pyrophosphate
PPP	Inorganic triphosphate
PLP, pyridoxal-P	Pyridoxal phosphate
EDTA	Ethylenediamine tetraacetic acid
Kcal	Kilocalories
Kj	Kilojoules
Q_{O_2}, $Q_{acetate}$, etc.	Metabolic quotients expressed in microliters metabolite/mg dry weight/hour
ΔF	Increment of free energy
ΔF^0	Standard free energy change
$\Delta F'$	Standard free energy change at pH 7

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CONTENTS

CHAPTER 13

Nitrogen Metabolism of Amino Acids

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I. Scope

This chapter is concerned primarily with the enzymic systems involved in the transformation or transfer of the amino, amine, and amide nitrogen moiety of amino acids, amino acid amides, and amines. Reactions involved in purine biosynthesis and degradation will be discussed in Chapter 18. Complete coverage of the literature since the publication of the first edition of this chapter (*1*) has not been attempted. Of necessity there has been a selection of the literature references dictated for the most part by the interests of the authors. In general, preference has been given to studies which emphasize the enzymic aspects of chemical transformations.

Recent reviews which cover this general area are those of Cohen and Brown (*2*), which deals with the comparative biochemical aspect, and Meister (*3*), which is a comprehensive review of the biochemistry of amino acids.

II. Deamination

A. Oxidative

A large number of enzyme systems has been recorded in the literature in support of the concept that amino acids as a group are oxidatively deaminated in accordance with the following general over-all reaction:

$$\begin{array}{l} \mathrm{RCHCOOH} \\ \quad | \\ \quad \mathrm{NH_2} \end{array} + \tfrac{1}{2}\mathrm{O_2} \rightarrow \begin{array}{l} \mathrm{RCCOOH} \\ \quad \| \\ \quad \mathrm{O} \end{array} + \mathrm{NH_3} \qquad (1)$$

In a general way these enzyme systems may be classified as follows:

(*a*) L-Amino acid oxidases (or dehydrogenases)

(*b*) D-Amino acid oxidases (or dehydrogenases)

(*c*) Specific amino acid oxidases (or dehydrogenases)

This classification indicates that the L- and D-amino acid oxidases have broad substrate specificities, whereas the other enzymes have a narrower specificity. For the purpose of simplicity the above classification will be used. The amino acid oxidase systems may be subclassified according to the nature of the hydrogen acceptor under two categories, aerobic and anaerobic. The former have been more commonly referred to as amino acid oxidases, whereas the latter are frequently referred to as dehydrogenases.

The term oxidase is usually considered to refer to a system in which oxygen is an obligatory hydrogen acceptor. The term dehydrogenase is used for that enzymic component of an oxidative reaction system which is concerned with the activation of the substrate and its dehydrogenation. Thus, the distinction between the succinoxidase system and succinic dehydrogenase is clearly recognized under this terminology. In the case of the amino acid oxidative enzymes, the distinction is somewhat more difficult to establish, owing chiefly to the fact that most of the measurements of activity have been based on oxygen consumption and thus it is not certain in most instances that the rate-limiting reaction is the dehydrogenase step. It is clear from the formulation of the mechanism of the oxidation of amino acids and amines that the primary reaction is that of a dehydrogenation and that the ultimate fate of the hydrogen (or electrons) is determined either by the autoxidizability of the primary acceptor, i.e., coenzyme, or by its participation in the chain of hydrogen or electron transport systems of the cell. The term oxidase will be retained in referring

to most of the amino acid oxidizing enzyme systems because of precedent and for the reasons given above. The term dehydrogenase will be included parenthetically in those instances in which in the authors' opinion the term is preferable to the more commonly used term, oxidase. It is beyond the scope of this discussion to reclassify and rename the many enzymes mentioned. It should be clear to the reader, however, that there is a great need for a more systematic basis for nomenclature.

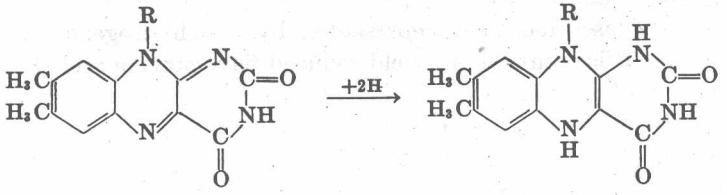
Reaction (1) should be considered as a two-step reaction as follows:

$$\begin{array}{l} \mathrm{RCHCOOH} \\ \quad\vert \\ \mathrm{NHR_1} \end{array} \rightleftharpoons \begin{array}{l} \mathrm{RCCOOH} \\ \quad\Vert \\ \mathrm{NR_1} \end{array} + 2\mathrm{H} \tag{2}$$

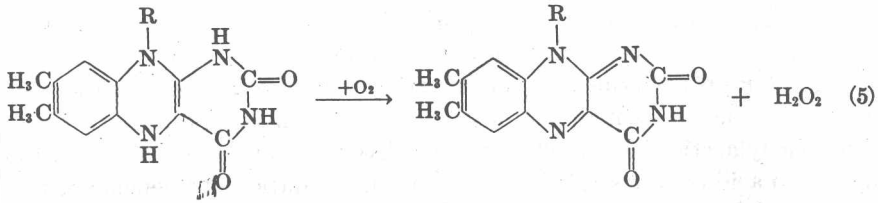
$$\begin{array}{l} \mathrm{RCCOOH} \\ \quad\Vert \\ \mathrm{NR_1} \end{array} \underset{-\mathrm{H_2O}}{\overset{+\mathrm{H_2O}}{\rightleftharpoons}} \mathrm{RCOCOOH} + \mathrm{NH_2R_1} \tag{3}$$

Thus, the primary oxidative step is a dehydrogenation (and thus the term dehydrogenase is preferable) to form an imino acid which in turn is non-enzymically hydrolyzed to form the keto acid and ammonia or a derivative thereof. In the formulation of reactions (2) and (3), R_1 is intended to represent either a hydrogen atom or a substituent, such as an alkyl group. If R_1 is a hydrogen atom, ammonia is formed; if R_1 is an alkyl group, an alkyl amine is formed.

The aerobic oxidases, as far as they have been investigated, are all flavoproteins, and the hydrogen acceptor for this group is molecular oxygen. The nature of the oxidative steps following reaction (2) may be represented as follows:



(4)



(5)

The net effect of reactions (4) and (5) is:

$$2\mathrm{H} + \mathrm{O_2} \rightarrow \mathrm{H_2O_2} \tag{6}$$

Thus, the aerobic oxidases form hydrogen peroxide. In the absence of catalase, the peroxide formed may react with the keto acid as follows:

$$RCOCOOH + H_2O_2 \rightarrow RCOOH + CO_2 + H_2O \quad (7)$$

The over-all formulation of amino acid oxidation by an aerobic oxidase in the absence of catalase is as follows:

$$\begin{matrix} RCHCOOH \\ | \\ NHR_1 \end{matrix} + O_2 \rightarrow RCOOH + NH_2R_1 + CO_2 \quad (8)$$

In the presence of catalase which decomposes the hydrogen peroxide, the over-all reaction is:

$$\begin{matrix} RCHCOOH \\ | \\ NHR_1 \end{matrix} + \tfrac{1}{2}O_2 \rightarrow RCOCOOH + NH_2R_1 \quad (9)$$

Keilin and Hartree (*4*) demonstrated the coupling of D-amino acid oxidase with ethyl alcohol in the presence of catalase and were able to show that in the presence of catalase ethyl alcohol was oxidized in preference to the α-keto acid to form acetaldehyde as follows:

$$CH_3CH_2OH + H_2O_2 \xrightarrow{\text{catalase}} CH_3CHO + 2H_2O \quad (10)$$

The total coupled system is thus formulated as follows:

$$\begin{matrix} RCHCOOH \\ | \\ NHR_1 \end{matrix} + O_2 + CH_3CH_2OH \rightarrow RCOCOOH + NH_2R_1 + CH_3CHO + H_2O \quad (11)$$

and exhibits the theoretical oxygen uptake without destruction of the α-keto acid.

As indicated above, the reaction catalyzed by the general D- and L-amino acid oxidases has been represented by a dehydrogenation of an amino acid by a flavoenzyme to yield reduced flavoenzyme and the corresponding imino acid [reactions (2) + (4)]. Indirect support for the formation of the hypothetical imino acid has been provided by a number of studies which exclude α,β-unsaturation in the course of the reaction. For example, it has been shown that (*a*) the four isomers of isoleucine are enzymically oxidized by the appropriate amino acid oxidase to the corresponding optically active α-keto-β-methylvaleric acids (*5*, *6*), (*b*) the L-isomers of β-phenylserine are converted by L-amino acid oxidase to the respective isomers of mandelic acid (*7*), (*c*) the L- and D-isomers of α-aminophenylacetic acid, which have no β-hydrogen atom, are attacked by the amino acid oxidases (*8*, *9*), and (*d*) on the oxidation of L-leucine in the presence of D_2O by L-amino acid oxidase, no deuterium is found in the isolated α-ketoisocaproic acid (*10*). More direct evidence for the formation of the α-imino acid as an intermediate has been provided by Pitt (*10a*) in studies on the oxidation of aromatic amino acids by *ophio*-L-amino acid oxidase in the presence of a tautomerase.

Until recently, the amino acid oxidase reaction has been studied only in the direction of ammonia and α-keto acid formation. In the presence of air the reaction proceeds to completion and is essentially irreversible because of the reoxidation of the reduced flavoprotein by molecular oxygen [reaction (5)]. Meister and his associates (*11*, *12*) have provided a clear demonstration of the reversibility of the amino acid oxidase reaction with D-amino acid oxidase (from sheep kidney) and L-amino acid oxidase (from snake venom). When an amino acid, ammonia, and the α-keto acid analog of a second amino acid are incubated with either amino acid oxidase under anaerobic conditions, the formation of the second amino acid is observed:

$$\begin{array}{l} \mathrm{RCHCOOH} \\ \quad\mid \\ \quad\mathrm{NH_2} \end{array} + \mathrm{R'COCOOH} \rightleftharpoons \mathrm{RCOCOOH} + \begin{array}{l} \mathrm{R'CHCOOH} \\ \quad\mid \\ \quad\mathrm{NH_2} \end{array} \qquad (12)$$

The reaction is markedly accelerated by the addition of ammonia; $N^{15}H_3$ leads to the formation of the N^{15}-labeled amino acid; and spectrophotometric studies show that the reduced amino acid oxidase can be reoxidized anaerobically by the addition of ammonia and α-keto acid. All of these experiments indicate that the reaction observed does not involve a transamination. It has been suggested that under appropriate physiological conditions a reversal of the amino acid oxidase reaction may be responsible for amino acid synthesis.

The anaerobic oxidases (dehydrogenases) represent enzymes which have as coenzymes nonautoxidizable hydrogen acceptors and thus are linked with the cytochrome system.

1. *L-Amino Acid Oxidases (Dehydrogenases)*

Four L-amino acid oxidases have been partially purified and studied from such widely different sources as snake venom, rat kidney, turkey liver, and molds.

a. SNAKE VENOM OR OPHIO-L-AMINO ACID OXIDASE (DEHYDROGENASE). Zeller and Maritz (see *13*) described an enzyme, originally discovered in snake venoms and later shown to be present in various tissues of both venomous and nonvenomous snakes, which they termed *ophio*-L-amino acid oxidase. The enzyme is widely distributed in a large number of snake venoms (*13*). The L-amino acid oxidase from moccasin venom has been highly purified and shown to be a homogeneous protein with a molecular weight of 62,000 (*14*). It has a turnover number of 3100, a Q_{O_2} of 68,400, and a pH optimum at 7–7.5, with a sharp decline in activity on either side (*14*). The prosthetic group has been shown to be flavin adenine dinucleotide (FAD), which is present in a concentration of 1 mole per mole of protein (*15*).*

* However, recent studies by Wellner and Meister [D. Wellner and A. Meister, *J. Biol. Chem.* **235**, 2013 (1960)] indicate a molecular weight of 130,000 and 2 moles of FAD for crystalline L-amino acid oxidase from venom of *Crotalus adamanteus*.

TABLE I

COMPARISON OF REACTIVITY OF AMINO ACIDS WITH DIFFERENT AMINO ACID OXIDASES (*16*, *46*)

Type of amino acid	D-Amino acid oxidase of sheep kidney	L-Amino acid oxidase of cobra venom	L-Amine acid oxidase of *N. crassa*	D-Amino acid oxidase of *N. crassa*	L-Amino acid oxidase of *Mytilus edulis*
Straight-chain aliphatic monoaminomonocarboxylic acids	3-C > 6-C > 4-C > 5-C > 8-C 11-C, 12-C, 18-C not attacked	6-C > 8-C > 5-C 3-C, 4-C, 11-C, 12-C, 18-C not attacked	4-C > 5-C > 6-C > 3-C > 8-C 11-C, 12-C, 18-C not attacked	4-C > 5-C > 6-C > 3-C > 18-C 8-C, 11-C, 12-C not attacked	6-C > 5-C > 4-C > 8-C > 3-C
Branched-chain monoaminomonocarboxylic acids	Valine > isoleucine > leucine	Only leucine oxidized	Leucine > isoleucine > valine	Leucine > isoleucine, valine	Only leucine oxidized
Aliphatic monoaminodicarboxylic acids	13-C rapidly oxidized 4-C slowly oxidized 5-C, 6-C, and 7-C not attacked	Only 13-C oxidized — —	7-C > 6-C > 13-C > 5-C > 4-C All oxidized —	5-C > 7-C > 4-C > 6-C 13-C not attacked —	C-11 > C-7 > C-6 4-C and 5-C not attacked
Diaminomonocarboxylic acids	Ornithine > lysine	Not attacked	Ornithine > lysine	Not attacked	Lysine, Ornithine > citrulline > arginine, canavanine > histidine > hydroxylysine (α-γ-diaminobutyric not attacked)
Amino acids with cyclic substituent	Tyrosine > aminophenylalanine > dimethylaminophenylalanine > phenylalanine	Tyrosine > aminophenylalanine and phenylalanine > dimethylaminophenylalanine	Dimethylaminophenylalanine > phenylalanine > aminophenylalanine > tyrosine	*p*-Aminophenylalanine > β-pyridyl-(4)alanine > phenylalanine > tyrosine	Tyrosine > phenylalanine > tryptophan > 5-hydroxytrytophan

TABLE I—CONTINUED

Type of amino acid	D-Amino acid oxidase of sheep kidney	L-Amino acid oxidase of cobra venom	L-Amino acid oxidase of *N. crassa*	D-Amino acid oxidase of *N. crassa*	L-Amino acid oxidase of *Mytilus edulis*
	Dimethylamino-phenylalanine consumed > 1 atom O, ε-*N*-Sulfanilyllysine not attacked	Tyrosine, amino-phenylalanine, dimethylamino-phenylalanine, tryptophan, and β-furyl(2)alanine consumed > 1 atom O	Tyrosine, aminophenyl-alanine, dimethylamino-phenylalanine, ε-*N*-sulfanilyllysine consumed > 1 atom O	*p*-(β-Aminoethyl)-phenylalanine, *p*-aminophenylala-nine, *p*-dimethyla-minophenylalanine, pyridyl(4)alanine, tyrosine consumed > 1 atom of O	
	β-Quinolyl(2)al-anine > β-quin-olyl(4)alanine	Histidine and ε-*N*-sulfanilyllysine not attacked	—		
	—	β-Quinolyl(4)alanine > β-quinolyl(2)-alanine	β-Quinolyl(4)alanine and β-quinolyl(2)-alanine attacked at similar rates	—	—
Unclassified amino acids	Proline > methio-nine > serine > threonine > cystine	Only methionine oxidized	Serine > cystine > methionine > threonine	Only methionine at-tacked	Proline not attacked Methionine > ethio-nine > cystathio-nine > *meso*-cys-teine, L-cystine, LL-djenkolic acid > homocysteine
	—	—	Proline and piperi-dine-α-carboxylic acid not attacked	—	
	—	—	Cysteine consumed > 1 atom O	—	Cysteic acid, homo-cysteic acid, cys-teinesulfinic acid not attacked α,α′-Diaminopimelic (*meso* and LL) oxi-dized
Total number amino acids tested	38	38	38	38	42
Total number amino acids oxidized	31	19	33	23	31

i. *Specificity.* Early specificity studies have been reviewed by Zeller (*13*) and have been studied in greater detail by Bender and Krebs (*16*). In Table I are listed the reactivities of different amino acids with the different amino acid oxidases. In general, *ophio*-L-amino acid oxidases, although showing variation in substrate specificity from one species to another, are highly specific for L-amino acids. Zeller (*13*) has summarized the specificity requirements as follows: "The substrate must possess a free carboxyl group, an unsubstituted α-amino group, and an organic radical. A second amino or carboxyl group inhibits a substance otherwise suitable as a substrate for the enzyme." If the second amino group is acylated, or if the second carboxyl is converted to an amide or ester, however, oxidation occurs.

A number of amino acid analogs (β-arylalanines) (*17*) and derivatives of α-L-aminodicarboxylic acids (β-aspartylalanine, β-aspartylglycine, γ-glutamylalanine, etc.) (*18*) are oxidized by snake venom L-amino acid oxidases. The rates of oxidation of the β-arylalanines are comparable to that of the corresponding naturally occurring amino acids, supporting the generalization of Zeller (*13*) that the β-group exerts relatively little influence on the substrate activity of different amino acids. However, in studies with α,β-diasymmetric amino acids as substrates for ten different *ophio*-L-amino acid oxidases, it was found that substrates with the α-L, β-L configuration were more readily oxidized than were the corresponding diastereoisomers (*19*, *20*).

ii. *Mechanism of action.* With the clear demonstration by Singer and Kearney (*15*) that FAD is the coenzyme of *ophio*-L-amino acid oxidase, the oxidative steps are those represented by reactions (2), (4), and (5). In the absence of catalase, the over-all reaction is that shown by reaction (8); in the presence of catalase, it is that shown by reaction (9). The reaction proceeds more rapidly in pure oxygen than in air (*14*).

On the basis of pH activity data and reversible inactivation by phosphate and other ions, Kearney and Singer (*21–23*) have suggested that one of the active groups of the enzyme is an ionizable imidazole.

In his investigations of the mechanism of action of the flavoproteins, Beinert (*24*) studied the spectral changes induced by enzymic or chemical reduction and oxidation of FMN and FAD. Evidence has been obtained for two intermediates, a semi-quinoid free radical, monomeric form, and a dimeric (possibly quinhydrone-like) form of the free radical, which is formed from the monomer in a relatively slow reaction. The semi-quinones are formed rapidly enough to permit them to function kinetically as intermediates. A reaction scheme has been proposed (*24*).

iii. *Inhibitors.* Zeller and co-workers (*13*, *25–29*) have studied a variety of carboxylic and sulfonic acids as possible inhibitors of *ophio*-L-amino