all and the ^{15}N found in glycine after feeding ^{15}N -labelled amino-acids other than glycine and serine, may, as already suggested by Shemin (1946), arise by synthesis of glycine from a substance other than glyoxylic acid.

SUMMARY

1. Glycine containing excess ^{15}N was fed to rabbits and rats, and various amino-acids were isolated from protein of muscle and internal organs respectively. The glycine, serine, glutamic acid, arginine and tyrosine contained varying concentrations of isotopic nitrogen. Lysine and threonine had no excess ^{15}N .

2. It is concluded that threonine, like lysine, does not take part in the reversible transfer of nitrogen which is observed with other amino-acids, both 'essential' and 'non-essential'.

3. The glycine and serine isolated contained similar proportions of ^{15}N . This is taken to indicate that the conversion of glycine to serine is very fast compared with other reversible reactions in which the two amino-acids participate.

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The Oxidation of Various Synthetic α -Amino-acids by Mammalian D-Amino-acid Oxidase, L-Amino-acid Oxidase of Cobra Venom and the L- and D-Amino-acid Oxidases of *Neurospora crassa*

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Through the courtesy of Sir Charles Harington, F.R.S., National Institute for Medical Research, Hampstead (Elliott, Fuller & Harington, 1948; Elliott & Harington, 1949) and of Dr B. A. Hems, Glaxo Laboratories Ltd. (Elks, Hems & Ryman, 1948) we obtained twenty-seven synthetic α -mono-amino-acids not commonly available. The behaviour

of these substances towards D-amino-acid oxidase of sheep kidney, L-amino-acid oxidase of cobra venom and the L- and D-amino-acid oxidase of *Neurospora crassa* has been investigated. The rates of oxidation of the common amino-acids already examined by previous authors were also measured in order to obtain series of directly comparable data.

EXPERIMENTAL

Substrates

All amino-acids used were DL- unless otherwise stated. The following special DL-amino-acids were examined.

Straight chain α -amino-monocarboxylic acids. α -Aminocaprylic, α -aminohendecanoic, α -aminolauric, α -aminostearic acids.

Aliphatic α -aminodicarboxylic acids. α -Aminoadipic, 1-aminohendecane-1:11-dicarboxylic, α -aminopimelic, α -amino- β -n-octylsuccinic, α -amino- β -n-butylsuccinic, α -amino- α -n-propylsuccinic acids.

α -Amino-acids with no hydrogen atoms attached to the α -carbon. α -Amino- α -methyltridecanoic, α -amino- α -methylcaprylic, α -amino- α -methylvaleric, α -amino- α -methylhendecanoic, α -amino- α -n-heptylpelargonic, α -amino- α -n-amyloenanthic, α -amino- α -ethylbutyric acids.

α -Amino-acids with a cyclic substituent attached to the β -carbon. *p*-Amino-phenylalanine, *p*-dimethylaminophenylalanine, β -quinolyl(4)alanine, β -quinolyl(2)alanine, *p*-(β -aminoethyl)phenylalanine,* β -pyridyl(4)alanine, β -6-methoxyquinolyl(4)alanine, *p*-guanidinomethylphenylalanine, β -furyl(2)alanine,* ϵ -*N*-sulphanilyllysine.*

α -Amino-acids having no hydrogen atom attached to the α -carbon were not oxidized by any of the enzymes tested, as was to be expected (Wieland & Bergel, 1924). Of the dicarboxylic amino-acids those having branched chains (β -substituted aspartic acids) were not attacked. In view of the negative results obtained with these substances these amino-acids are not listed in the following tables.

EXPERIMENTS WITH D-AMINO-ACID OXIDASE
OF SHEEP KIDNEY*Procedure*

A crude extract of acetone-dried sheep kidney cortex (Krebs, 1935) was prepared immediately before use by shaking 1 g. of dried kidney for 5 min. with 20 ml. of 0.1 M-pyrophosphate buffer, pH 8.4, and filtering through an agar filter paper. The rate of oxidation was measured manometrically in Warburg flasks of about 23 ml. capacity provided with centre well and side arm. The quantity of enzyme solution used varied from compound to compound and is stated in Table 1. It was so adjusted as to give pressure changes not exceeding 20 mm. in 5 min. Control experiments showed that under the conditions used the rate of oxidation was directly proportional to the quantity of enzyme solution used. The main compartment of the Warburg flasks contained the enzyme solution and sufficient 0.1 M-pyrophosphate buffer to bring the volume to 4 ml. The buffer was prepared by adding 1.6 ml. of *N*-HCl to 20 ml. of 0.2 M-sodium pyrophosphate and diluting to 40 ml. (Warburg & Christian, 1938). Of the DL-amino-acids 4.5×10^{-5} g.mol. were weighed into the side arm; the powder was moistened with 0.5 ml. of water or, in the case of acidic substances, with 0.5 ml. of NaOH solution of sufficient strength to neutralize the acid. This procedure was adopted because the limited solubility made the use of solutions impracticable

* Synthesized in the laboratory of Sir Charles Harington (unpublished).

in many cases. The gas space of the flasks contained O_2 . The bath temperature was 40° . The contents of the side arm and main compartment were mixed after equilibration and readings were taken at 5 min. intervals. Undissolved material was present at the end of the experiments with α -aminocaproic, α -aminohendecanoic, α -aminolauric, α -aminostearic, α -amino- α -methyltridecanoic, α -amino- α -methylhendecanoic, α -amino- α -n-heptylpelargonic, α -amino- α -n-amyloenanthic acids, and with cystine.

Differences in the potency of enzyme preparations from different kidneys

Wide variations were observed in the potency of the enzyme solutions prepared from different sheep kidneys. For the test of activity 1 ml. of extract was used and DL-alanine served as substrate. The conditions were as described in the preceding paragraph. The O_2 absorption for the first 5 min. varied between 6 and 145 μ l. No correlation was found between D-amino-acid oxidase activity and breed, age or sex of the animals, but the limited information so far available suggests a seasonal factor. Eight kidneys tested between April and June all showed low activity (13–22 μ l. of O_2 absorbed in 5 min.). Of sixteen specimens collected between August and October seven gave very high values (107–145 μ l. of O_2 in 5 min.); four fairly active (78–90 μ l. of O_2 in 5 min.); four were moderately active (25–45 μ l. of O_2 in 5 min.) and only one had an activity below 25 μ l. of O_2 in 5 min. For the following experiments an acetone powder of high potency was used. The cause of the variations in potency is being further investigated.

*Results**Rate of oxidation of various amino-acids*

The data obtained are shown in Table 1. All available amino-acids were examined with the same enzyme solution which contained 2.025% dry matter (excluding buffer) and which gave a blank oxygen absorption of 3 μ l./ml. in 5 min. and 4.5 μ l./ml. in 10 min. The figure in the last column (q_{O_2} = μ l. of O_2 absorbed/mg. dried kidney in enzyme solution/hr.) is calculated from the oxygen-uptake values for 10 min. and the dry matter (excluding buffer) of the enzyme solution. It represents a relative measure of the rate of oxidation of various amino-acids.

It will be noted that in most cases the 5 min. value for the oxygen uptake was less than half of the 10 min. value. The slow initial rate was probably due to a true lag period, the cause of which is not clear. In a few cases, for example, that of phenylalanine, the rate of reaction decreased during the second 5 min. period.

Straight-chain α -aminomonocarboxylic acids. The data in Table 1 show that the rates of oxidation of the C_4 and C_6 compounds were about half that of alanine. The C_5 compound reacted more slowly than the C_4 and C_6 compounds, and the C_8 compound was very slowly oxidized. The higher homologues did not react.

Branched-chain aliphatic α -aminomonocarboxylic acids. Valine was oxidized rather faster than its

Table 1. *Oxidation of amino-acids by D-amino-acid oxidase of sheep kidney*

(For conditions see text. All data are corrected for the blank O₂ absorption of the enzyme solution. $q_{O_2} = (\mu\text{l. of O}_2 \text{ absorbed})/(\text{mg. of dried kidney in enzyme solution} \times \text{hr.})$. q_{O_2} was calculated from the O₂ uptake during the first 10 min.)

Amino-acid	Volume of enzyme solution (ml.)	O ₂ absorbed (μl.) after				<i>q</i> _{O₂}
		5 min.	10 min.	20 min.	30 min.	
Straight chain α-aminomonocarboxylic acids:						
Alanine	0.2	20	43	93	132	64
α-Aminobutyric acid	0.5	29	53	103	135	31
α-Aminovaleric acid	0.5	15	34	74	106	20
α-Aminocaproic acid	0.3	15	37	74	109	36
α-Aminocaprylic acid	4.0	18	26	48	56	1.9
α-Aminohendecanoic acid	4.0	Nil	Nil	Nil	Nil	Nil
α-Aminolauric acid	4.0	Nil	Nil	Nil	Nil	Nil
α-Aminostearic acid	4.0	Nil	Nil	Nil	Nil	Nil
Branched chain aliphatic α-aminomonocarboxylic acids:						
Valine	0.5	27	60	127	180	35
Leucine	1.0	20	47	92	127	13.9
Isoleucine	0.5	21	37	71	101	22
Aliphatic α-aminodicarboxylic acids:						
Aspartic acid	4.0	6	19	38	57	1.4
Glutamic acid	4.0	Nil	Nil	Nil	Nil	Nil
α-Aminoadipic acid	4.0	Nil	Nil	Nil	Nil	Nil
α-Aminopimelic acid	4.0	Nil	Nil	Nil	Nil	Nil
1-Aminohendecane-1:11-dicarboxylic acid	0.5	32	70	126	162	41
α-Amino-acids with cyclic substituents:						
Phenylalanine	0.5	29	44	67	74	26
Tyrosine	0.1	37	64	110	137	190
<i>p</i> -Aminophenylalanine	0.2	20	44	82	112	65
<i>p</i> -Dimethylaminophenylalanine	0.3	12	33	106	170	32
<i>p</i> -(β-Aminoethyl)phenylalanine	1.0	25	55	96	130	16
<i>p</i> -Guanidinomethylphenylalanine	0.5	28	56	102	135	33
β-Quinolyl(4)alanine	1.0	12	28	60	82	8.3
β-Quinolyl(2)alanine	0.1	10	20	32	44	59
β-6-Methoxyquinolyl(4)alanine	0.2	5	18	42	57	27
β-Pyridyl(4)alanine	0.2	36	64	111	139	95
β-Furyl(2)alanine	1.0	18	40	107	—	11.9
ε- <i>N</i> -Sulphanilyl-lysine	4.0	Nil	Nil	Nil	Nil	Nil
Histidine	1.0	13	21	38	47	6.2
Tryptophan	0.2	8	25	52	80	37
Diaminomonocarboxylic acids:						
Lysine	4.0	2	8	12	19	0.6
Ornithine	2.0	10	21	43	56	3.1
Unclassified α-amino-acids:						
Serine	0.5	32	71	127	171	42
Threonine	4.0	11	28	55	76	2.1
Proline	0.1	24	50	90	115	148
Piperidine-α-carboxylic acid	2.0	7	18	22	30	2.6
Methionine	0.2	24	54	107	146	80
Cystine	2.0	6	13	26	37	1.9

straight-chain isomer. In the case of the amino-caproic acids on the other hand, the two branched-chain isomers, leucine and isoleucine, were oxidized at lower rates than the straight-chain isomer.

Aliphatic α -aminodicarboxylic acids. Aspartic acid was very slowly oxidized, whilst glutamic acid, aminoadipic and aminopimelic acids were not attacked. The homologous C₁₃ compound, in contrast, was oxidized at a high rate.

Amino-acids with cyclic substituents. Tyrosine showed the highest rate of oxidation of all the substrates tested. The substitution of the *p*-hydroxyl group of tyrosine by an amino group considerably reduced the rate of oxidation. Still lower rates were obtained with *p*-dimethyl-aminophenylalanine, *p*-(β -aminoethyl)phenylalanine, and *p*-guanidinomethylphenylalanine. β -Quinolyl(4)alanine reacted slowly in contrast to β -quinolyl(2)alanine which gave

a high rate; the rate of oxidation of β -6-methoxy-quinolyl(4)alanine was between these two values. β -Pyridyl(4)alanine was oxidized rapidly and β -furyl(2)alanine slowly, but ϵ -*N*-sulphanilylsine was not attacked. Tryptophan was oxidized at about half the rate of alanine and histidine was only slowly attacked. The products of oxidation of β -quinolyl(4)alanine, β -quinolyl(2)alanine and of β -6-methoxy-quinolyl(4)alanine formed bright yellow solutions.

Total oxygen uptake

The total oxygen uptake did not exceed the theoretical value of one molecule of oxygen for four molecules of DL-amino-acid, except in the case of β -*p*-dimethylaminophenylalanine. This substance, on addition to kidney extract, absorbed four times the amount of oxygen expected, namely one molecule of oxygen per molecule of the DL-form.

For example, 2.25×10^{-5} g.mol. of the DL-amino-acid absorbed 503 μ l. (2.25×10^{-5} g.mol.) of O_2 in 270 min. The greater part of this (410 μ l., equiv. to 3.25 atoms of O_2) was absorbed in the first 90 min. The amount of O_2 consumed in successive 30 min. periods was 183, 167, 60, 25, 20, 15, 10 and 10 μ l. respectively. The amount of NH_3 produced during the incubation was equivalent to 0.5 molecule/molecule of DL-compound added. E.g. 9.35 mg. of substrate yielded 0.355 mg. of NH_3 . In an experiment where the reaction was stopped when one atom of oxygen had been absorbed for each molecule of substrate the formation of NH_3 had already reached the maximum value. These yields are in accordance with the assumption that the D-form only is deaminated.

No carbon dioxide was produced from *p*-dimethylaminophenylalanine. The reaction product was soluble and brown coloured. It has not been identified.

Neither tyrosine nor phenylalanine, nor *p*-aminophenylalanine absorbed more than the expected amount of oxygen under the same conditions. Dimethylaniline, *p*-dimethylaminoazobenzene and *p*-dimethylaminobenzaldehyde were not attacked, but *p*-dimethylaminophenylacetate was rapidly oxidized. This substance is autoxidizable in pyrophosphate buffer, pH 8.4, in the absence of kidney extract, but the rate of oxidation is much increased by addition of 'D-amino-acid oxidase'. Boiled enzyme has no effect. This phenomenon is under further investigation.

Oxidation of mixtures of amino-acids. It was thought that amino-acids which are not oxidized by the enzyme might, nevertheless, combine with it and inhibit the oxidation of other amino-acids. However, no inhibitions were observed when α -amino- α -ethylbutyric, α -amino- β -*n*-octylsuccinic, α -aminopimelic or α -aminoadipic acids were added together with alanine. Nor had α -aminopimelic acid any effect on the rate of oxidation of 1-aminohexadecane-1:11-dicarboxylic acid.

EXPERIMENTS WITH L-AMINO-ACID OXIDASE OF COBRA VENOM

Procedure

The source of this enzyme was dried cobra venom kindly provided by Dr C. H. Kellaway, F.R.S. The conditions of the test were similar to those used by Zeller & Maritz (1945). The main compartment of the Warburg cup contained 1 mg. of cobra venom freshly dissolved in 4 ml. of 0.067M-phosphate buffer, pH 7.2, and 1 drop of octanol. To decompose the H_2O_2 formed during the oxidation, 0.1 ml. of catalase in the form of a liver homogenate (1 part rat liver : 10 parts water) was added. The substrate (4.5×10^{-5} g.mol. of the DL-compound except in the case of arginine when half this amount of the L-compound was used) was weighed into the side arm and moistened with 0.5 ml. of water or the equivalent amount of NaOH; 2N-NaOH (0.2 ml.) was placed in the centre well. The gas space contained O_2 . The temperature was 40°. On addition of 0.5 ml. of water from the side arm to the enzyme solution a positive pressure of about 4 mm. was observed, and all the readings were corrected accordingly. No explanation can be offered for this effect.

Results

Rate of oxidation of various amino-acids

The results of the measurements are shown in Table 2. As the cup contained 1 mg. of dry enzyme, the 60 min. value is comparable to the q_{O_2} value given in the last vertical column of Table 1.

Straight chain α -aminomonocarboxylic acids. The C_3 , C_4 , C_{11} , C_{12} and C_{18} compounds did not react, whilst the C_5 , C_6 and C_8 compounds were oxidized. The highest rate was shown by the C_6 compound.

Branched-chain aliphatic α -aminomonocarboxylic acids. The only member of this group to react was leucine, the rate of oxidation being similar to that of its straight-chain isomer.

Aliphatic α -aminodicarboxylic acids. Of the five α -amino-acids of the group tested only the C_{11} compound was attacked, and the rate of oxidation was slow. The C_4 , C_5 , C_6 and C_7 homologues were not oxidized.

Amino-acids with cyclic substituents. Tyrosine gave the highest rate of oxidation and phenylalanine the second highest. Of the twelve other substances tested ten were oxidized. Among the two substances which did not react was histidine. The oxidation product of β -quinolyl(4)alanine, β -quinolyl(2)alanine and of β -6-methoxyquinolyl(4)alanine showed the same bright yellow colour noted in the experiments with D-amino-acid oxidase.

Diaminomonocarboxylic acids. Ornithine and lysine were not attacked.

Unclassified amino-acids. Methionine was slowly oxidized, but serine, threonine, proline and cystine did not react.

Total oxygen uptake

In some cases the rate of reaction was too slow to reach the theoretical value of 252 μ l. of oxygen

Table 2. Oxidation of amino-acids by L-amino-acid oxidase of cobra venom

(For conditions see text. All data corrected for initial gas output of 6 μ l.)

Amino-acid	O ₂ absorbed (μ l.) after								
	10 min.	20 min.	30 min.	1 hr.	2 hr.	4 hr.	5 hr.	6 hr.	22 hr.
Straight-chain α-aminomonocarboxylic acids:									
Alanine; α -aminobutyric acid; α -aminohendecanoic acid; α -aminolauric acid and α -aminostearic acid	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
α -Aminovaleric acid	6	12	18	31	51	92	—	125	239
α -Aminocaproic acid	21	33	44	74	117	192	—	227	261
α -Aminocaprylic acid	7	17	25	50	81	140	—	176	213
Branched-chain aliphatic α-aminomonocarboxylic acids:									
Valine; isoleucine	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Leucine	14	28	41	77	132	203	—	134	256
Aliphatic α-aminodicarboxylic acids:									
Aspartic acid; glutamic acid; α -aminoadipic acid; α -aminopimelic acid	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
1-Aminohendecane-1:11-dicarboxylic acid	—	6	9	18	41	76	—	104	168
α-Amino-acids with cyclic substituents:									
Histidine; ϵ -N-sulphanilyllysine	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Phenylalanine	19	39	51	111	204	274	—	—	—
Tyrosine	35	86	136	246	306	346	—	388	477
<i>p</i> -Aminophenylalanine	13	29	51	113	256	489	—	582	766
<i>p</i> -Dimethylaminophenylalanine	5	17	32	86	189	338	372	—	—
<i>p</i> -(β -Aminoethyl)phenylalanine	—	—	—	12	22	44	—	60	149
<i>p</i> -Guanidinomethylphenylalanine	—	11	14	30	50	89	99	—	—
β -Quinolyl(4)alanine	—	20	27	47	83	129	—	169	236
β -Quinolyl(2)alanine	—	—	9	15	23	37	—	51	—
β -6-Methoxyquinolyl(4)alanine	—	8	13	28	50	84	—	106	149
β -Pyridyl(4)alanine	7	16	26	58	108	184	—	230	282
β -Furyl(2)alanine	12	30	45	85	152	248	271	—	—
Tryptophan	10	26	38	76	156	266	—	356	—
Diaminomonocarboxylic acids:									
Ornithine; lysine	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Unclassified α-amino acids:									
Serine; threonine; proline; piperidine- α -carboxylic acid; cystine	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Methionine	18	36	52	94	156	230	—	272	313
Arginine	7	12	14	23	38	—	—	—	—

within the time of the experiment. In the others the final value was of the expected order except in four cases where the oxygen uptake exceeded the theoretical figure. In the experiment recorded in Table 2 tyrosine absorbed 1.9, tryptophan 1.4, and *p*-aminophenylalanine 3.05 atoms of oxygen. In the case of β -furyl(2)alanine and of *p*-dimethylaminophenylalanine the oxygen uptake had not stopped at the end of the experiment, and to measure the final value for these substances further experiments were set up with larger quantities of enzyme (4 mg.) and smaller quantities of substrate (2.25×10^{-5} g.mol.). Under these conditions β -furyl(2)alanine absorbed 2.2 atoms of oxygen and *p*-dimethylaminophenylalanine 3.5 atoms. The solutions in which *p*-dimethylaminophenylalanine and *p*-aminophenylalanine had undergone oxidation were dark brown.

EXPERIMENTS WITH THE L-AMINO-ACID OXIDASE OF *NEUROSPORA CRASSA*

Procedure

It was found in the course of the study, as already reported elsewhere (Bender, Krebs & Horowitz, 1949), that some strains of *N. crassa* produce an L-amino-acid oxidase, others a D-amino-acid oxidase. The experiments were carried out with the strain 3411 of the National Collection of Type Cultures. It was grown on a medium similar to that used by Horowitz & Beadle (1943), which contained (w/v) 0.5% ammonium tartrate, 0.1% NH_4NO_3 , 0.1% KH_2PO_4 , 0.01% NaCl , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01% CaCl_2 , 2% sucrose, 0.3% (dry) malt extract and 0.01% (dry) yeast extract. In addition, the following quantities of trace elements were added per litre: $\text{Na}_2\text{B}_4\text{O}_7$, 0.05 mg.; Na_2MoO_4 , 0.04 mg.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1 mg.; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.4 mg.; $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 mg. and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 9 mg. The medium (150 ml.) was filled into 700 ml. conical Roux bottles, and autoclaved at

15 lb. pressure for 15 min. At an early stage of the work, the organism lost its ability to sporulate; it was propagated by transferring small pieces of mycelium to the new medium.

The cultures were incubated at 25° for 14–18 days. This period was found to give the maximum enzymic activity. The yield of mycelium per bottle averaged 3.5–4.0 g. wet wt. The harvested mycelia were briefly rinsed with distilled water, squeezed dry through muslin, and disintegrated in a Waring blender with 2 ml. of 0.0167M-pyrophosphate buffer pH 8.4 per g. wet wt. of mycelium. The material was centrifuged and the supernatant, after dilution with 0.25 vol. of

0.2M-pyrophosphate buffer, pH 8.4, was used as a source of the oxidase.

The O_2 uptake on addition of the amino-acids was measured in Warburg flasks at 30°. The main compartment contained 2 ml. of the mycelium extract and 2 ml. of 0.1M-pyrophosphate buffer, pH 8.4. 2N-NaOH (0.2 ml.) was used in the inner cup; the gas space contained O_2 . Of the DL-amino-acids 4.5×10^{-5} g.mol. were weighed into the side arm and moistened with 0.5 ml. of 0.1M-pyrophosphate buffer, or, in the case of acidic substrates, with 0.5 ml. of NaOH solution of sufficient strength to neutralize the acid.

Table 3. *Oxidation of amino-acids by the L-amino-acid oxidase of Neurospora crassa (Strain 3411 N.C.T.C.)*

(For conditions and the calculation of q_{O_2} see text. All data corrected for the blank O_2 absorption of the enzyme solution.)

Amino-acid	O ₂ absorbed (μl.) after							q _{O₂}
	5 min.	10 min.	20 min.	30 min.	60 min.	120 min.	180 min.	
Straight-chain α-aminomonocarboxylic acids:								
Alanine	26	65	144	216	—	—	—	41
α-Aminobutyric acid	52	113	238	—	—	—	—	64
α-Aminovaleric acid	26	97	228	—	—	—	—	75
α-Aminocaproic acid	16	73	182	232	—	—	—	60
α-Aminocaprylic acid	15	31	52	78	104	124	139	17
α-Aminohendecanoic acid	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
α-Aminolauric acid	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
α-Aminostearic acid	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Branched-chain aliphatic α-aminomonocarboxylic acids:								
Valine	5	11	22	42	72	165	—	6
Leucine	28	102	223	—	—	—	—	78
Isoleucine	14	45	91	135	191	213	—	33
Aliphatic α-aminodicarboxylic acids:								
Aspartic acid	—	4	9	14	22	30	31	5
Glutamic acid	1	10	22	45	81	135	176	9
α-Aminoadipic acid	25	83	160	213	251	—	—	61
α-Aminopimelic acid	48	108	214	—	—	—	—	63
1-Aminohendecane-1:11-dicarboxylic acid	2	21	52	83	244	—	—	20
α-Amino-acids with cyclic substituents:								
Phenylalanine	21	70	168	276	—	—	—	41
Tyrosine	—	20	85	168	220	448	672	24
p-Aminophenylalanine	14	42	104	193	293	341	390	29
p-Dimethylaminophenylalanine	26	80	181	313	464	580	612	57
p-(β-Aminoethyl)phenylalanine	1	5	35	61	140	—	—	16
p-Guanidinomethylphenylalanine	2	19	45	83	224	—	—	18
β-Quinolyl(4)alanine	50	94	220	255	—	—	—	66
β-Quinolyl(2)alanine	43	100	200	214	—	—	—	60
β-6-Methoxyquinolyl(4)alanine	21	70	190	—	—	—	—	41
β-Pyridyl(4)alanine	43	95	212	247	—	—	—	55
β-Furyl(2)alanine	32	77	189	250	—	—	—	58
ε-N-Sulphanilyl-lysine	—	16	34	60	132	423	444	9
Histidine	24	94	207	215	—	—	—	37
Tryptophan	8	34	84	149	167	180	205	27
Diaminomonocarboxylic acids:								
Lysine	8	17	44	86	198	209	215	14
Ornithine	29	73	169	175	—	—	—	51
Unclassified α-amino-acids:								
Serine	5	13	28	50	84	115	136	8
Threonine	1	3	4	6	12	20	24	2
Proline	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Piperidine-α-carboxylic acid	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Methionine	14	52	128	221	254	—	—	40
Cystine	18	72	178	304	484	558	610	56

The amino-acid oxidase produced by this organism was found to be specific for L-amino acids. The enzyme was present in the clear medium as well as in the mycelium. Both the mycelium extract and the culture fluid contained a powerful catalase so that H_2O_2 did not accumulate.

Results

Rate of oxidation of various amino-acids

Variations were found in the potency of the enzyme prepared from different batches, and there was a slight fall in the potency of the crude extract on storage even at 0°. All the amino-acids were therefore tested on the same day with the same enzyme preparation. The enzyme solution used contained 0.57% dry matter (excluding buffer) and gave a blank oxygen absorption of 4 μ l./ml. in 5 min. and 8 μ l./ml. in 10 min.

The data obtained are shown in Table 3. The figures in the last column indicate the relative maximum rates of oxidation; they were calculated from the difference between either the 5 and 10 min. readings or the 10 and 20 min. readings whichever gave the higher value. This procedure was prompted by the existence of a lag period which delayed the development of the maximum rates.

Straight-chain α -aminomonocarboxylic acids. The data show that in the homologous series the C_4 and C_5 acids were oxidized more rapidly, and the C_3 acid more slowly than alanine; the higher homologues, from C_{11} upwards, were not attacked.

Branched-chain α -aminomonocarboxylic acids. Valine was oxidized very much more slowly than its straight-chain isomer. Leucine was oxidized more readily and isoleucine more slowly, than the straight-chain isomer.

Aliphatic α -aminodicarboxylic acids. Amino-adipic and aminopimelic acids were much more rapidly oxidized than aspartic and glutamic acids and the C_{13} member of this series.

Amino-acids with cyclic substituents. Tyrosine showed a lower rate of oxidation than phenylalanine. All of the amino-acids in this group were oxidized including ϵ -N-sulphanilyllysine. β -Quinolyl(4)-alanine was attacked at approximately the same rate as β -quinolyl(2)alanine.

Diaminomonocarboxylic acids. Both amino-acids tested were attacked rather slowly, ornithine reacting more rapidly than lysine.

Unclassified amino-acids. The β -hydroxy- α -amino-acids, serine and threonine, were slowly oxidized. Methionine and cystine were oxidized at a moderate rate, but proline and piperidine- α -carboxylic acid were not attacked.

Total oxygen uptake

Several of the amino-acids with a cyclic substituent consumed oxygen in excess of the theoretical

figure of 252 μ l. Under the conditions of the experiment recorded in Table 3 tyrosine consumed 2.7, *p*-aminophenylalanine 1.5, *p*-dimethylaminophenylalanine 2.4 and ϵ -N-sulphanilyllysine 1.8 atoms of oxygen. In none of these cases had the oxygen consumption ceased at the end of the period of observation. In another experiment the oxygen absorption of *p*-aminophenylalanine ceased after 2 atoms of oxygen had been absorbed. In the case of tyrosine the final solution was dark brown.

Cystine also absorbed more than the expected amount of oxygen (more than 2.4 atoms per molecule).

EXPERIMENTS WITH THE D-AMINO-ACID OXIDASE OF *NEUROSPORA CRASSA*

Procedure

The strain 'Beadle 25a', supplied by Dr N. H. Horowitz, was used as a source of this enzyme, which was found to be specific for D-amino-acids. The growth medium and the procedure generally was essentially the same as that of Horowitz (1944). The incubation period was 7 days, when the maximum enzyme activity was reached. Of the mycelium extract, 4 ml. were used per manometer cup.

Results

Rate of oxidation of various amino-acids

The data obtained are recorded in Table 4. All figures are corrected for the oxygen consumption of the blank control which was 20 μ l. for the first 20 min./4 ml. of extract. The enzyme solution contained 2.75% dry matter excluding the buffer. The figures in the last column of Table 4, indicating the relative maximum rates of oxidation, were calculated from the oxygen uptake values for the second or third 10 min. periods, whichever gave the higher value. In general, the relative activity of the D-amino-acid oxidase is much weaker than that of the L-enzyme, as shown by the comparison of the last columns of Tables 3 and 4. In many cases q_{O_2} for the same amino-acid is 10–50 times greater in the case of the L-enzyme.

Straight-chain α -aminomonocarboxylic acids. The C_4 homologue showed the highest rate of oxidation; the C_8 , C_{11} and C_{13} compounds were not attacked while the C_{18} compound, after a lag period, was slowly oxidized.

Branched-chain α -aminomonocarboxylic acids. Valine and isoleucine were oxidized more slowly and leucine more rapidly than their straight-chain isomers.

Aliphatic α -aminodicarboxylic acids. Glutamic acid showed the highest rate. It is further noteworthy that the C_7 acid reacted more rapidly than the C_6 acid. In the case of aspartic acid there was a lag period of about 30 min. before the rate became appreciable.

Table 4. *Oxidation of amino-acids by the D-amino-acid oxidase of Neurospora crassa (Strain Beadle 25a)*
(For conditions and calculations of q_{O_2} see text. All data are corrected for the blank O_2 absorption of the enzyme solution.)

Amino-acid	O ₂ absorbed (μl.) after					q _{O₂}
	10 min.	20 min.	30 min.	60 min.	120 min.	
Straight-chain α-aminomonocarboxylic acids:						
Alanine	7	21	33	67	103	0.8
α-Aminobutyric acid	40	94	139	192	—	3.0
α-Aminovaleric acid	35	75	113	233	—	2.2
α-Aminocaproic acid	4	17	29	82	179	0.8
α-Aminocaprylic acid	Nil	Nil	Nil	Nil	Nil	Nil
α-Aminohendecanoic acid	Nil	Nil	Nil	Nil	Nil	Nil
α-Aminolauric acid	Nil	Nil	Nil	Nil	Nil	Nil
α-Aminostearic acid	0	2	3	11	35	0.1
Branched-chain aliphatic α-aminomonocarboxylic acids:						
Valine	0	4	7	21	45	0.2
Leucine	27	55	81	167	205	1.5
Isoleucine	5	8	9	27	59	0.2
Aliphatic α-aminodicarboxylic acids:						
Aspartic acid	0	0	5	29	57	0.3
Glutamic acid	10	23	36	78	143	0.7
α-Aminoadipic acid	0	1	5	12	22	0.2
α-Aminopimelic acid	5	14	20	36	55	0.5
1-Aminohendecane-1:11-dicarboxylic acid	Nil	Nil	Nil	Nil	Nil	Nil
α-Amino-acids with cyclic substituents:						
Phenylalanine	26	53	76	167	238	1.2
Tyrosine	0	6	8	27	55	0.3
p-Aminophenylalanine	0	0	0	17	31	—
p-Dimethylaminophenylalanine	0	12	24	81	173	—
p-(β-Aminoethyl)phenylalanine	59	124	179	208	286	3.5
p-Guanidino-methylphenylalanine	17	40	65	133	188	1.3
β-Quinolyl(4)alanine	Nil	Nil	Nil	Nil	Nil	Nil
β-Quinolyl(2)alanine	Nil	Nil	Nil	Nil	Nil	Nil
β-6-Methoxyquinolyl(4)alanine	10	17	25	48	93	0.4
β-Pyridyl(4)alanine	43	95	147	222	298	2.8
β-Furyl(2)alanine	52	113	176	247	—	3.4
ε-N-Sulphanilyllysine	Nil	Nil	Nil	Nil	Nil	Nil
Histidine	0	1	10	47	87	0.5
Tryptophan	Nil	Nil	Nil	Nil	Nil	Nil
Diaminomonocarboxylic acids:						
Lysine; ornithine	Nil	Nil	Nil	Nil	Nil	Nil
Unclassified α-amino-acids:						
Serine; threonine; proline; piperidine-α-carboxylic acid and cystine	Nil	Nil	Nil	Nil	Nil	Nil
Methionine	46	101	155	246	—	3.0

Amino-acids with cyclic substituents. Several of these substances were rapidly oxidized. Considerable lag periods were noted in the case of tyrosine, p-aminophenylalanine, and histidine. In some cases the total oxygen uptake exceeded the calculated amount, and this was further examined in additional experiments where the oxygen uptake was measured over a period of 4 hr. An excess oxygen absorption was found with p-(β-aminoethyl)phenylalanine, p-aminophenylalanine, and p-dimethylaminophenylalanine, pyridyl(4)alanine and tyrosine.

Diaminomonocarboxylic acids. Neither of two substances tested was attacked.

Unclassified amino-acids. Methionine was more rapidly oxidized than any of the other naturally occurring amino-acids, as already noted by Horowitz (1944). Serine, threonine, cystine and proline were not attacked.

witz (1944). Serine, threonine, cystine and proline were not attacked.

DISCUSSION

Comparison of the specificity of the four amino-acid oxidases. Data suitable for the comparison of the activities of the four enzymes studied are those given in the last column of Tables 1, 3 and 4 and the 60 min. values in Table 2. These figures all represent the oxygen uptake per mg. dry wt. of enzyme preparation per hour. Some comparative data are listed in Table 5. The relative rates at which the different amino-acids undergo oxidation vary considerably from enzyme to enzyme. For example, of the five monoaminodicarboxylic acids tested only the C₁₃ compound is attacked by the cobra-venom

Table 5. Comparison of the reactivity of amino-acids with the four different amino-acid oxidases

Type of amino-acid	D-Amino-acid oxidase of sheep kidney	L-Amino-acid oxidase of cobra venom	L-Amino-acid oxidase of <i>N. crassa</i>	D-Amino-acid oxidase of <i>N. crassa</i>
Straight-chain aliphatic mono- aminomonocarboxylic acids	$C_3 > C_6 > C_4 > C_5 > C_8$ C_{11}, C_{12}, C_{13} not attacked	$C_6 > C_8 > C_5$ $C_3, C_4, C_{11}, C_{12}, C_{13}$ not attacked	$C_4 > C_5 > C_6 > C_3 > C_8$ C_{11}, C_{12}, C_{13} not attacked	$C_4 > C_5 > C_6, C_8 > C_{13}$ C_8, C_{11}, C_{12} not attacked
Branched-chain monoamino- monocarboxylic acids	Valine > isoleucine > leucine	Only leucine oxidized	Leucine > isoleucine > valine	Leucine > isoleucine, valine
Aliphatic monoaminodicarbo- xylic acids	C_{13} rapidly oxidized C_4 slowly oxidized C_5, C_6 and C_7 not attacked	Only C_{13} oxidized — —	$C_7 > C_8 > C_{13} > C_5 > C_4$ All oxidized	$C_5 > C_7 > C_4 > C_6$ C_{13} not attacked
Diaminomonocarboxylic acids	Ornithine > lysine	Not attacked	Ornithine > lysine	Not attacked
Amino-acids with cyclic sub- stituent	Tyrosine > aminophenylalanine > dimethylaminophenyl- alanine > phenylalanine Dimethylaminophenylalanine consumed > 1 atom O ϵ -N-Sulphanillyllysine not attacked β -Quinolyl(2)alanine > β -quino- lyl(4)alanine	Tyrosine > aminophenylalanine and phenylalanine > dimethyl- aminophenylalanine Tyrosine, aminophenylalanine, dimethylaminophenylalanine, tryptophane and β -furyl(2)- alanine consumed > 1 atom O Histidine and ϵ -N-sulphanillyl- lysine not attacked β -Quinolyl(4)alanine > β -quino- lyl(2)alanine	Dimethylaminophenylalanine > phenylalanine > amino- phenylalanine > tyrosine Tyrosine, aminophenylalanine, dimethylaminophenylalanine, ϵ -N-sulphanillyllysine con- sumed > 1 atom O —	<i>p</i> -Aminophenylalanine > β -pyri- dyl(4)alanine > phenylalanine > tyrosine <i>p</i> -(β -Aminoethyl)phenylalanine, <i>p</i> -aminophenylalanine, <i>p</i> -dime- thylaminophenylalanine, pyridyl(4)alanine, tyrosine consumed > 1 atom of O
Unclassified amino-acids	Proline > methionine > serine > threonine > cystine	Only methionine oxidized	Serine > cystine > methionine > threonine Proline and piperidine- α -car- boxylic acid not attacked Cystine consumed > 1 atom O	Only methionine attacked
Total number of amino-acids oxidized out of 38 tested	31	19	33	23

oxidase, whilst the D-amino-acid oxidase of kidney attacks the C₄ and C₁₃ compounds but not the C₅, C₆ and C₇ homologues. The L-enzyme of *Neurospora* oxidizes all five substances with the maximum rate for the C₅ compound; the D-enzyme of *Neurospora* oxidizes the C₄, C₅, C₆ and C₇ but not the C₁₃ compounds with the maximum rate for the C₅ compound.

Proline and piperidine- α -carboxylic acid are attacked by the kidney D-amino-acid oxidase only, and ϵ -N-sulphanilyllysine by the L-enzyme of *Neurospora* only. Another example of the different behaviour of the four enzymes is given by the relative reactivity of β -quinolyl(4)alanine and β -quinolyl(2)alanine. The kidney D-amino-acid oxidase oxidizes the latter much more rapidly than the former. The L-enzyme of *Neurospora* oxidizes both compounds rapidly at about the same rate, but the D-enzyme of *Neurospora* does not attack these substances. Similar differences could be enumerated for other series of amino-acids. It might be especially emphasized that the D- and L-enzymes of *Neurospora* differ not only in their optical specificity but also in many other properties. Common to all four enzymes is the inability to attack α -amino-acids with a tertiary α -carbon and β -substituted aspartic acids.

The findings lead to the general conclusion that among the factors which determine the oxidizability of α -amino-acids the specific properties of the enzyme are no less important than the structure of the amino-acid molecules.

Lag periods. The comparison of the reaction rates is in many cases complicated by the occurrence of a lag period. With kidney D-amino oxidases this was usually of the order of a few minutes; with the two *Neurospora* enzymes it varied between a few minutes and about 1 hr. Lag periods of this kind are generally ascribed to the formation, by the initial process, of a new reactive substance, either from the substrate or from the catalyst. A more specific hypothesis to explain the lag period for the enzymes under discussion is so far lacking.

Comparison with previous observations. Considering differences in experimental conditions the data reported in this paper may be regarded as agreeing essentially with those recorded in the

literature for the naturally occurring amino-acid (for kidney D-amino-acid oxidase see Krebs, 1935, 1948; Felix & Zorn, 1939; Felix, 1947; Kögl, Herken & Erxleben, 1940; Klein & Handler, 1941; for cobra-venom L-amino-acid oxidase see Zeller, 1948; for *Neurospora* D-amino-acid oxidase see Horowitz, 1944). A few discrepancies occur in cases where the rate of oxidation, and therefore the accuracy of the measurements, is low.

SUMMARY

1. The rate of oxidation was examined of forty-eight α -amino-acids, among them twenty-seven synthetic compounds not previously tested, in the presence of crude preparations of the D-amino-acid oxidase of sheep kidney, of the L-amino-acid oxidase of cobra venom and the L- and D-amino-acid oxidases of *Neurospora crassa*. Data are given for the rates of oxidation.

2. None of the enzymes attacked α -amino-acids with a tertiary α -carbon atom, or β -substituted aspartic acids. Of the remaining thirty-eight amino-acids tested the kidney D-amino acid oxidase attacked thirty-one compounds, the cobra-venom L-enzyme nineteen, the *Neurospora* L-enzyme thirty-three, and the *Neurospora* D-enzyme twenty-three.

3. The specificity of the four enzymes showed many differences. The D- and L-enzymes of *Neurospora* differed not only in the optical specificity but also in many other properties.

4. Lag periods during which the rate of reaction increased were observed in the case of the kidney D-amino-acid oxidase and of the two *Neurospora* enzymes.

5. A number of amino-acids with cyclic substituents absorbed more oxygen than required for the conversion into the keto acid. This applied to *p*-dimethylaminophenylalanine with all four enzyme preparations, to tyrosine and *p*-aminophenylalanine with the cobra enzyme and the two *Neurospora* enzymes, to tryptophan and β -furyl(2)alanine with the cobra enzyme, ϵ -N-sulphanilyllysine with the *Neurospora* L-enzyme, to *p*-(β -aminoethyl)-phenylalanine and β -pyridyl(4)alanine with the *Neurospora* D-enzyme.

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