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METABOLISM OF γ-GUANIDOBUTYRIC ACID IN FRUIT-BODIES OF *PANUS TIGRINUS* (FR.) SING. (TRICHOLOMATACEAE)

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Abstract—Fruit-bodies of *Panus tigrinus* (Fr.) Sing. (Tricholomataceae) were fed with L-arginine-amidine-^{14}C and L-arginine-U-^{14}C. There was a high incorporation of the radiocarbon into γ-guanidobutyrate. When γ-aminobutyric acid-U-^{14}C was fed together with non-labelled L-arginine, a high proportion of the radioactivity was incorporated into γ-guanidobutyric acid, and alkaline hydrolysis showed that most of the radiocarbon was located in the γ-aminobutyric acid moiety of the guanidine compound, suggesting the operation of an amidino transferase reaction. Uniformly labelled γ-guanidobutyrate-^{14}C which was prepared by the action of purified L-amino acid oxidase of snake venom (*Agkistrodon piscivorus*) on L-arginine-U-^{14}C was effectively degraded to γ-aminobutyrate and urea (the latter being rapidly hydrolysed by means of an active urease present), suggesting some mode of amidinase action. Amidino transferase and amidinase activities have been demonstrated using crude enzyme preparations obtained by different methods from acetone-dried fruit-bodies of *P. tigrinus*. L- Arginine, L-canavanine, γ-guanidobutyrate, and hydroxyguanidine function as active donor compounds in transamidination; L-ornithine, L-canaline, γ-aminobutyrate, and agmatine act as acceptor compounds in amidine group transfer. At the present stage of purification the amidino transferase activity of the enzyme preparation is, however, low. A crude amidinase was prepared from acetone-dried fruit-bodies of *P. tigrinus* that preferentially decomposes γ-guanidobutyric and β-guanidopropionic acids. Of nine other guanidine derivatives examined, only L-arginine, L-arginic acid and L-canavanine were slightly attacked. It is not clear, however, whether there is some arginase activity, too. From the preliminary data, the enzyme may be classified being a γ-guanidobutyrase or heteroarginase. As a result of the *in vivo* and *in vitro* studies, a new path of arginine catabolism is suggested involving transamidinase and γ-guanidobutyrase. This is, as we are aware, the first report concerning the occurrence of these enzymes in plants. Interestingly, arginine: NH_2—R amidinotransferase obviously functions here in a catabolic rather than a biosynthetic pathway.

INTRODUCTION

γ-GUANIDOBUTYRIC acid appears to be widespread in nature. The compound has been found in a number of both animal and plant tissues. Recently, the guanidine derivative has been reported in fruit-bodies of *Lycoperdon* spp.[1] and *Panus tigrinus*.[2] In *L. pyriforme*, γ-guanidobutyrate is present both in the mycelium and in isolated ripe spores.[2] Fruit-bodies of *P. tigrinus* (Fr.) Sing., which were cultivated under sterile and controlled conditions on a complex malt-agar medium, contain at least four different compounds which gave a positive reaction with Sakaguchi spray reagent. Two of these guanidine derivatives have been isolated and identified as L-arginine and γ-guanidobutyrate.[2] The two other guanidines have been tentatively identified by paper chromatographic analysis as agmatine and arcaine (diguanido putrescine).

Relatively little is known on γ-guanidobutyrate metabolism in plant tissues.[3] When arginine uniformly labelled with ^{14}C was fed to various tissues of pines, a large amount

[1] H. REINBOTHE, *Phytochem.* 3, 327 (1964).
[2] J. MIERSCH and H. REINBOTHE, *Flora* **156**, 543 (1966).
[3] J. MIERSCH, *Biol. Rdsch.* (In press).

of arginine was converted to γ-guanidobutyrate.[4] The apparent route of formation of the compound in pine tissues might be the oxidative pathway via α-keto-δ-guanidovaleric acid. Such a pathway has been reported in marine invertebrates[5] and has been presumed to be the path of γ-guanidobutyric acid biosynthesis in fruit-bodies of *Lycoperdon*.[1] A second possible pathway of γ-guanidobutyrate formation, is the transamidination reaction between arginine and γ-aminobutyrate, known to take place in some animal tissues,[6] although apparently not in higher plants.[3] There is little evidence coming from tracer experiments only for the participation of transamidination in the biosynthesis of galegine in *Galega officinalis*.[7, 8] A third different path of γ-guanidobutyric acid biosynthesis has been reported in *Streptomyces griseus*.[9] Decarboxylative oxidation of L-arginine leads to γ-guanidobutyramide that is degraded to γ-guanidobutyric acid by means of a new amidinase being obviously specific for γ-guanidobutyrate breakdown. The reactions represent steps of a catabolic pathway of arginine leading directly to succinic semialdehyde and to the compounds of the tricarboxylic acid cycle. For γ-guanidobutyric acid is degraded to γ-aminobutyric acid and urea; the former is involved in a transamination reaction yielding succinic semialdehyde.

A γ-guanidobutyrase (γ-guanidobutyrate urea hydrolase) of broader specificity ("hetero-arginase") has been reported in liver and kidney of *Raia clavata*[10] and has then been found in the kidneys of several fishes.[11] A deamination of γ-guanidobutyrate has also been described in liver and kidney of some birds, the pig, and in the hepatopancreas of mussels.[12] In fruit-bodies of *L. pyriforme* γ-guanidobutyrate-U-^{14}C is degraded to γ-aminobutyric acid and urea.[13] The enzyme concerned with this reaction has not been investigated.

Biosynthesis and degradation of γ-guanidobutyric acid in fruit bodies of *P. tigrinus* (Fr.) Sing. and by crude enzymes prepared from them is the subject of the present communication.

RESULTS AND DISCUSSION

Fruit-bodies of *P. tigrinus* transform supplied L-arginine to γ-guanidobutyrate. In short-term experiments using L-arginine-amidine-^{14}C, radioactivity was exclusively introduced into the amidine carbon atom of γ-guanidobutyric acid, suggesting transamidinase activity.[2] To prove this hypothesis, and to study metabolic relationships of arginine in this fungus the following compounds were fed through the stipe to freshly harvested fruit-bodies for a period of 2 hr: DL-ornithine-2-^{14}C, L-citrulline-ureido-^{14}C, L-arginine-amidine-^{14}C, L-arginine-U-^{14}C, γ-guanidobutyric acid-U-^{14}C, γ-guanidobutyric acid-1,2,3,4-^{14}C, γ-aminobutyric acid-U-^{14}C, and DL-glutamic acid-3,4-^{14}C. The incorporation of radioactivity into components of the 70% ethanol extracts was determined (Tables 1 and 2).

It can be seen that DL-ornithine-2-^{14}C is only partly assimilated in the feeding time of 2 hr, and is incorporated into aspartic and glutamic acids, proline, citrulline and arginine. L-Citrulline-ureido-^{14}C is also only slowly metabolized mainly into arginine. L-^{14}C-amidine arginine is strongly incorporated into γ-guanidobutyric acid which confirms earlier

[4] R. L. BARNES, *Nature* **193**, 781 (1962).
[5] N. THOAI, J. ROCHE and Y. ROBIN, *Compt. Rend.* **235**, 832 (1952).
[6] J. J. PISANO, C. MITOMA and S. UDENFRIEND, *Nature* **180**, 1125 (1957).
[7] G. REUTER, *Arch. Pharm.* **296**, 516 (1963).
[8] G. REUTER, Flora **154**, 136 (1964).
[9] N. THOAI, In *Comparative Biochemistry of Arginine and Derivatives* (Edited by J. ROCHE). London (1965).
[10] R. BARET, M. MOURGUE and A. BROC, *Compt. Rend. Soc. Biol.* **155**, 857 (1961).
[11] R. BARET, M. MOURGUE and A. BROC, *Compt. Rend. Soc. Biol.* **156**, 1117 (1962).
[12] R. BARET, M. MOURGUE and A. BROC, *Compt. Rend. Soc. Biol.* **159**. 1184 (1965).
[13] H. REINBOTHE, Unpublished.

TABLE 1. METABOLISM OF ORNITHINE CYCLE INTERMEDIATES AND COMPOUNDS THAT ARE METABOLICALLY RELATED TO γ-AMINOBUTYRATE IN FRUIT-BODIES OF *Panus tigrinus* (Fr.) Sing.

Compounds were fed through the stipe for 2 hr

^{14}C-compound fed†	Substrate conc. (μmoles)	^{14}C added (μc)	Total radioactivity c/min 10^{-4}	% radioactivity in 70% EtOH extract†								
				orn	cit	arg	AB	GB	asp	glu*	pro	unk.
L-arg-amidine	3·0	5·0	0·103	—	—	63	—	13	—	—	—	24
L-arg-U	3·0	10·0	0·660	13	—	57	2	15	1	2	—	10
γ-AB-U	3·0	5·0	0·384	—	—	tr.	67	14	12	5	—	2
γ-AB-U+L-arg‡	1·74	5·4	0·605	—	—	6	5	79	—	—	—	10
DL-orn-2	3·0	4·4	0·577	79	1	1	tr.	—	8	6	2	2
L-cit-ureido	3·0	4·0	0·428	—	76	9	—	4	—	—	—	11
DL-glu-3,4	3·0	10·0	0·977	—	—	tr.	tr.	—	—	tr.	94	5

* Sum glutamic acid and glutamine.
† orn, ornithine; cit, citrulline; arg, arginine; AB and GB, amino- and guanido-butyric acid; glu, glutamic acid; pro, proline; unk, unknown.
‡ 1·45 μmoles γ-aminobutyric acid-U-^{14}C+0·29 μmole L-arginine.

TABLE 2. METABOLISM OF γ-GUANIDOBUTYRIC ACID-U-^{14}C AND OF ARGININE-^{14}C IN DEPENDENCE ON SUBSTRATE CONCENTRATION IN FRUIT-BODIES OF *Panus tigrinus* (Fr.) Sing.

^{14}C-compound fed*	μmoles	μc	Total radioactivity c/min 10^{-4}	% radioactivity in 70% EtOH extract*							
				orn	arg	AB	GB	asp	glu	pro	unk.
L-arg-amidine	3·0	5·0	0·103	—	63	—	13	—	—	—	24
L-arg-amidine	0·3	5·0	0·295	—	58	—	38	—	—	—	4
L-arg-U	3·0	10·0	0·660	13	57	2	15	1	2	—	10
L-arg-U	0·3	10·0	0·548	—	49	8	40	tr.	tr.	—	2
γ-GB-U	45·0	5·4	0·384	—	—	34	tr.	43	14	—	10

* For abbreviations, see Table 1.

results.[2] γ-Guanidobutyrate formation is high also with L-arginine-U-^{14}C. Only part of the supplied arginine-U-^{14}C gives rise to γ-aminobutyrate-^{14}C. The mode of formation of this latter compound from arginine is speculative and presumably occurs via ornithine or agmatine. γ-Guanidobutyric acid is degraded to γ-aminobutyrate and urea as is shown by Table 2. The latter compound is immediately hydrolyzed by means of an active urease that is present in *Panus*.[14]

By lowering the supplied arginine from 3·0 to 0·3 μmoles per gram fresh material, arginine-^{14}C feeding results in a relatively higher incorporation of radiocarbon into γ-guanidobutyrate. Presumably, the amidinase hydrolysing γ-guanidobutyric acid requires relatively high substrate concentrations. The large amount of carbon-14 converted in γ-guanidobutyrate-U-^{14}C feedings to aspartic and glutamic acids and glutamine is noticeable. This might be the result of randomization of radioactivity resulting from urea-^{14}C hydrolysis. When urea-^{14}C was fed to fruit-bodies of *Panus*, there is, indeed, a high incorporation of the radiocarbon into aspartic acid, glutamic acid and glutamine. Aspartic acid labelling might be, however, a result of γ-guanidobutyric acid breakdown to γ-aminobutyrate that is presumably catabolized via succinic semialdehyde and the reactions of the tricarboxylic acid cycle. As can be seen from Table 1, there is a remarkable incorporation of radiocarbon from γ-aminobutyric acid-U-^{14}C into aspartic acid. The main product of γ-aminobutyric acid metabolism is, however, γ-guanidobutyrate. The proportion of radioactivity in the 70% EtOH extract appearing in γ-guanidobutyrate is markedly increased when γ-aminobutyrate-U-^{14}C was fed together with unlabelled L-arginine (Table 1). Chemical degradation of the isolated γ-guanidobutyrate-^{14}C shows that by far most of the ^{14}C (85%) is located in the γ-aminobutyric acid moiety of the molecule. It seems possible that the alkaline hydrolysis used also attacks the carboxyl group of γ-guanidobutyrate. In this case, all the radioactivity would be located in the γ-aminobutyrate part of the compound. The results suggest a transamidination reaction in γ-guanidobutyric acid formation in which L-arginine acts as the donor compound, and γ-aminobutyric acid as the acceptor compound. γ-Guanidobutyrate might be split by the action of an amidinase, thus yielding γ-aminobutyrate and urea. γ-Aminobutyric acid is evidently not the product of glutamic acid decarboxylation, for DL-glutamic acid-3,4-^{14}C mainly gives rise to labelled proline. Only traces of the radiocarbon appear in γ-aminobutyric acid (Table 1).

Our working hypothesis concerning γ-guanidobutyric acid formation and degradation was confirmed by preparing crude enzymes exhibiting transamidinase and γ-guanidobutyrase ("heteroarginase") activities. Acetone-dried fruit-bodies of *P. tigrinus* were extracted with phosphate buffer, pH 7·5, and centrifuged. The supernatant was dialysed and was then found free from soluble amino acids. The dialysed enzyme was used in transamidination studies. The complete reaction mixture contained: enzyme solution, 1·0 ml; the respective donor (15 μmoles) and acceptor (20 μmoles) compounds, dissolved in 0·067 M phosphate buffer, pH 7·5, in a total volume of 2·0 ml. The mixture was incubated at 38° up to 4 hr. Aliquots were withdrawn after 1, 2 and 4 hr for thin-layer and paper chromatographic analyses. Different donor–acceptor combinations were examined (Table 3).

As can be seen, the enzyme source is capable of catalysing amidine group transfer from L-arginine to γ-aminobutyrate. L-Canavanine can substitute for L-arginine as a donor compound in γ-guanidobutyrate formation. The reactions are freely reversible; for, γ-guanidobutyric acid reacts with L-ornithine to give arginine and γ-aminobutyric acid, and with L-canaline to yield canavanine and γ-aminobutyrate. With the help of ^{14}C

[14] J. MIERSCH, Unpublished.

labelled compounds, the following exchange reactions have been demonstrated: arginine + ornithine-^{14}C ⟶ arginine-^{14}C (+ ornithine); γ-guanidobutyrate + γ-aminobutyrate-^{14}C ⟶ γ-guanidobutyrate-^{14}C (+ γ-aminobutyrate).

TABLE 3. TRANSAMIDINASE ASSAY IN A CRUDE ENZYME PREPARATION OBTAINED FROM FRUIT-BODIES OF *Panus tigrinus* (Fr.) Sing.

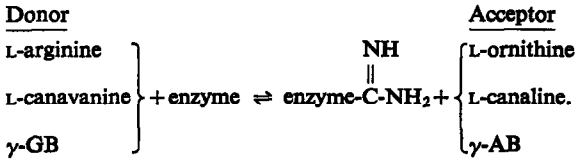
Compounds added*		
Donor	Acceptor	Reaction products formed*
L-arginine	γ-AB	γ-GB,† ornithine
L-arg-amidine-^{14}C	γ-AB	γ-GB-^{14}C,† ornithine
L-arginine	DL-ornithine-2-^{14}C	arginine-^{14}C
L-arginine	L-canaline	canavanine, ornithine
L-arginine	agmatine	arcaine, ornithine
L-arginine	NH_2OH	none
L-canavanine	L-ornithine	arginine, canaline
L-canavanine	γ-AB	γ-GB, canaline
L-canavanine	NH_2OH	canaline (non-enzymatic?)
γ-GB	L-ornithine	arginine, γ-AB
γ-GB	γ-AB-U-^{14}C	γ-GB-^{14}C
γ-GB	L-canaline	canavanine, γ-AB
γ-GB	NH_2OH	none
hydroxyguanidine	L-ornithine	arginine
hydroxyguanidine	γ-AB	γ-GB
hydroxyguanidine	L-canaline	canavanine

* For abbreviations see Table 1.

† The enzyme preparation contain some amidinase and urease activities. Resulting γ-guanidobutyrate is thus partly degraded to γ-aminobutyrate, ammonia and carbon dioxide.

With hydroxyguanidine as the donor compound, transamidination occurs with L-ornithine, γ-aminobutyrate and L-canaline. Surprisingly, no transamidination occurs using hydroxylamine as amidine acceptor.

Further experiments are, however, required to answer this question. Interestingly, L-arginine can transfer its amidine group to agmatine yielding arcaine (diguanidine putrescine). Preliminary paper chromatographic analysis suggests the occurrence of agmatine and arcaine in the soluble nitrogen fraction of *P. tigrinus* fruit-bodies. Evidently, fruit-bodies of *P. tigrinus* contain transamidinase activity. L-Arginine, L-canaline, and γ-guanidobutyric acid function as active donor compounds, L-ornithine, L-canaline, and γ-aminobutyric acid are suited acceptor compounds:



At the present stage of purification, amidino transferase activity is, however, low. Only 4 per cent of the labelling added as L-arginine-amidine-^{14}C was introduced into γ-guanidobutyric acid in the incubation time of 4 hr. Further purification of the enzyme is planned and transamidination can be quantitatively followed by means of the usual colorimetric methods.

An enzyme solution was prepared from acetone-dried fruit-bodies of *P. tigrinus* by extraction with dilute manganese chloride and subsequent dialysis of the supernatant, obviously exhibiting γ-guanidobutyrase ("heteroarginase") activity (Table 4).

TABLE 4. DETECTION OF γ-GUANIDOBUTYRASE ("HETEROARGINASE") IN A CRUDE ENZYME PREPARATION OBTAINED FROM ACETONE-DRIED FRUIT-BODIES OF *Panus tigrinus* (Fr.) Sing.

Compound added*	Amount (μmoles)	Number of C-atoms	Reaction products*	Splitting
L-homoarginine	50	7	none	—
L-arginine-U-^{14}C	100	6	(orn-^{14}C, urea-^{14}C)†	weak
D-arginine	100	6	none	—
L-arginic acid	50	6	(unknown, urea)†	weak
L-canavanine	50	6	(canaline, urea)†	weak
γ-GB-1,2,3,4-^{14}C‡	100	5	γ-AB-^{14}C, urea	strong
α-guanylserine	100	4	none	—
α-guanidopropionic acid	100	4	none	—
β-guanidopropionic acid	100	4	β-alanine, urea	strong
α-amino-β-guanidopropionic acid	100	4	none	—
glycocyamine	100	3	none	—

* For abbreviations, see Table 1.

† Only traces of reaction products. The unknown reaction product formed after application of L-arginic acid (α-hydroxy-δ-guanidovaleric acid) might be hydroxyornithine.

‡ Thirty per cent of the radioactivity of γ-GB-^{14}C was transferred to γ-AB after 6 hr.

In a typical experiment to detect γ-guanidobutyrase activity, the incubation mixture contained: enzyme solution, 1·0 ml; the respective guanidine derivative (50 or 100 μmoles) dissolved in pyrosphosphate buffer, pH 9·8, 1·0 ml; 8% sodium pyrophosphate buffer, pH 9·8, 1·0 ml, in a total volume of 3·0 ml. The mixture was incubated at 38° and aliquots were withdrawn after 1, 6 and 10 hr for thin-layer chromatographic analysis. Amino acids formed were located by a ninhydrin spray, and urea by the Ehrlich spray reagent. Radioactive reaction products were detected by paper radioautography. Under the experimental conditions, no transamidinase activity was detectable, but some urease activity was present which is completely inhibited by means of $5 \cdot 10^{-5}$ M formamidinedisulphide (FADS). FADS has no effect upon amidinase ("heteroarginase") and arginase activity (commercial product)

As is seen from Table 4, only γ-guanidobutyric and β-guanidopropionic acids were markedly attacked by the enzyme in the incubation period of 1 hr. γ-Aminobutyric acid and β-alanine have been identified as products of deamination of γ-guanidobutyrate and β-guanidopropionate, respectively. Only traces of urea are detectable without addition of FADS. L-Arginine, L-canavanine and L-arginic acid as the classical substrates of arginase action were only slowly deaminated by the enzyme preparation. It is not clear, however, whether there is a separate arginase present. Without heat treatment of the preparation, the relative hydrolysis rate of arginine compared to γ-guanidobutyrate is slightly increased, but without heat treatment, the degree of splitting of all the substrates is much lower. Experiments are now being planned to purify the enzyme under study.

From our results, we can suggest a new catabolic path of arginine by the consecutive action of amidino transferase and "heteroarginase" (Fig. 1). Arginine which might be formed from ornithine by reactions of the Krebs–Henseleit cycle is subjected to a transami-

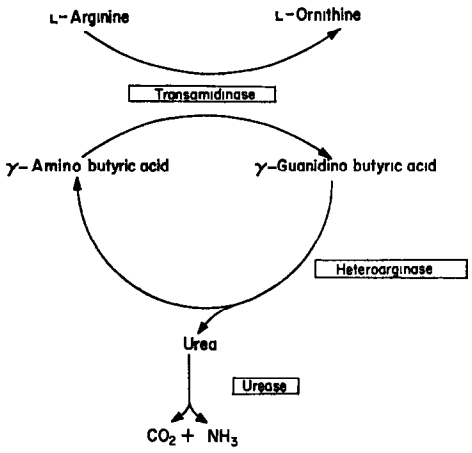


FIG. 1.

dination reaction, γ-aminobutyric acid being the amidine acceptor compound. γ-Guanidobutyric acid arises which is further degraded by γ-guanidobutyrase ("heteroarginase") thus yielding γ-aminobutyrate again and urea. The latter compound is rapidly catabolized, however, by means of an active urease. γ-Aminobutyrate might arise from arginine in an unknown manner. Preliminary studies suggest the existence of a putrescine[15] or arcaine pathway of γ-aminobutyrate formation from arginine in this fungus. γ-Aminobutyrate once formed in continuously regenerated from γ-guanidobutyrate breakdown. But part of the γ-aminobutyrate might participate in a transamination reaction yielding succinic semialdehyde which is then converted to succinic acid to undergo final oxidation through the tricarboxylic acid cycle. But this has not been proved as yet. The stated high introduction of the radiocarbon from γ-amino- or guanidino butyrate-U-^{14}C into aspartic acid might be explained by the following reaction sequence:

γ-GB⟶γ-AB⟶succinic semialdehyde⟶succinic acid⟶intermediates of the tricarboxylic acid cycle⟶oxaloacetic acid⟶aspartic acid.

The reaction sequence suggested (Fig. 1) would represent a new path of arginine breakdown and urea formation from arginine, by-passing the classical arginase-catalysed arginine degradation. In contrast to the stated function of transamidinase in biosyntheses,[16] amidino transferase of *P. tigrinus* fruit-bodies would have a catabolic role. The system suggested might function as a "overflow" mechanism to overcome high arginine concentrations.

Many questions remain to be answered concerning arginine metabolism and its metabolic control in *P. tigrinus*. Experiments are now being planned to study the enzymes concerned with arginine catabolism in this fungus in more detail.

EXPERIMENTAL

Cultivation of Panus tigrinus *and Feeding Technique*

Mycelium of *P. tigrinus* was cultivated under sterile and controlled conditions on a complex malt-agar medium that was high in carbohydrates and poor in arginine but contained a number of soluble amino acids as revealed by means of paper chromatography.

[15] W. JACOBY and J. FREDERICKS, *J. Biol. Chem.* **234**, 2145 (1959).
[16] J. B. WALKER, In *Comparative Biochemistry of Arginine and Derivatives* (Edited by J. ROCHE). London (1965).

Under the conditions employed (20–22°, 12 hr light and 12 hr dark), the fungus fructificed after 21–24 days[16a] and was harvested at this stage of development. Fruit-bodies freed from the mycelium were fed through the stipe with one of the following radioactive compounds: DL-Ornithine-2-^{14}C-dihydrochloride (Calbiochem); L-citrulline-ureido-^{14}C (Calbiochem); L-arginine-amidine-^{14}C-HCl (Radiochemical Centre, Amersham); L-arginine-U-^{14}C-HCl (Radiochemical Centre, Amersham); γ-aminobutyric acid-U-^{14}C (Calbiochem); DL-glutamic acid-3,4-^{14}C (CEA, France); γ-guanidobutyric acid-U-^{14}C (prepared from L-arginine-U-^{14}C); γ-guanidobutyric acid-1,2,3,4-^{14}C (prepared from γ-aminobutyrate-U-^{14}C).

Paper and Thin-layer Chromatography, Radioactive Measurements

Extraction of plant material, paper chromatography and radioautography was carried out as previously described.[1, 17] Radioactivities as detected by radioautography were determined by counting directly on the paper by means of a thin end-window Geiger-Müller tube (VA-Z 310, VEB Vakutronik-Dresden, with counting equipment VA-M-16 D). Data are expressed as a percentage of the total radioactivity of all the areas on paper chromatograms. Radioactivities of the compounds of the chemical degradation of γ-guanidobutyric acid-^{14}C were counted in infinite thickness of polystyrene planchettes by means of a methane-counter (Frieseke & Hoepfner FH 49). Thin-layer chromatography was performed as previously described.[2]

Enzyme Preparations

Acetone powders of *P. tigrinus* fruit-bodies were prepared as has been previously reported.[1]

Transamidinase. Crude enzyme preparations exhibiting transamidinase activity were obtained by extracting 1·0 g acetone powder for 10 min at 2° with 12 ml m/15 phosphate buffer, pH 7·5, containing 5 mg EDTA and 5 μmoles GSH, under continuous stirring. The suspension was centrifuged at 10·000 *g* for 10 min at −2°. The supernatant was dialysed against 0·006 M phosphate buffer, pH 7·5. The dialysed enzyme solution was used in transamidinase assay.

γ-Guanidobutyrase ("*heteroarginase*"). Crude enzyme preparations possessing amidinase activity were obtained by homogenizing 1·0 g acetone powder at 2° with 12·0 ml 0·033% $MnCl_2$ solution. The suspension was centrifuged at 10·000 *g* for 10 min at −2°. The supernatant was subjected to heat treatment at 60° for 15 min. Precipitated protein was centrifuged off (10 min at 10·000 *g* at −4°) and discarded. The supernatant was dialysed against 0·05% $MnCl_2$ solution at 2° for 24 hr. The dialysed enzyme solution was used in amidinase assay. Enzyme assays were carried as described in the text. Reactions were stopped by short heating at 100°.

Chemical Preparations

γ-Guanidobutyric acid-1,2,3,4-^{14}C was prepared from γ-aminobutyric acid-U-^{14}C and S-methylisothiourea.[18, 19] Hydroxyguanidine was prepared according to Walker.[20] Some other guanidines were synthesized by conventional methods.[18, 19]

γ-Guanidobutyric acid-U-^{14}C was enzymatically prepared by the action of purified

[16a] H.-H. HANDKE, Unpublished.
[17] D. SCHLEE and H. REINBOTHE, *Phytochem.* **4**, 311 (1965).
[18] E. SCHÜTTE, *Z. Physiol. Chem.* **279**, 52 (1943).
[19] R. PANT, *Hoppe-Seylers Z. Physiol. Chem.* **335**, 272 (1964).
[20] J. B. WALKER and M. S. WALKER, *J. Biol. Chem.* **234**, 1481 (1959).

L-amino acid oxidase (purified according to Meister[21]) of dried *Agkistrodon piscivorus* venom on uniformly labelled L-arginine-^{14}C, and hydrogen peroxide was added to the reaction mixture to convert the keto acid formed quantitatively to γ-guanidobutyrate. L-Canaline-dipicrate was freed from picrinic acid by means of a cation exchange resin.

Degradation of γ-Guanidobutyrate

γ-Guanidobutyric acid-^{14}C formed from γ-aminobutyric acid-U-^{14}C and L-arginine by the transamidinase was precipitated by adding appropriate amounts of the inactive carrier with flavianic acid. Flavianate was precipitated with $Ba(OH)_2$. The clear supernatant was passed through a column of Dowex 50-X-4 (H^+, 200–400 mesh) resin. γ-Guanidobutyrate was eluted by means of 10% ammonia solution. The eluate was evaporated to dryness, the residue was redissolved in a minimum of water, and after clearing with charcoal conc. ammonia was added. The crystalline precipitate was recrystallized to constant specific activity. The pure compound thus obtained was identified by its melting point and by co-chromatography in seven different solvent systems in comparison to the authentic compound. γ-Guanidobutyrate-^{14}C was subjected to alkaline hydrolysis following the procedure of Bell.[22] The alkaline hydrolysate was passed through a column of Dowex 50-X-4 (NH_4^+, 200–400 mesh) resin. After evaporation to dryness, the dry residue of the effluate was subjected to thin-layer chromatography. The eluted areas of the degradation products (γ-aminobutyric acid and urea) were measured as described above. Resulting urea was degraded by means of commercial urease; carbon dioxide-^{14}C was trapped in 2 M KOH in Conway units. The following abbreviations have been used:

γ-AB = γ-aminobutyric acid; cit = citrulline; EDTA = ethylene diamine tetraacetic acid (Chelaplex III); FADS = formamidinedisulphide; γ-GB = γ-guanidobutyric acid; GSH = reduced glutathione; unkn. = not identified radioactive compounds.

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