METABOLISM OF DL-α-AMINOBUTYRATE-3-^{14}C AND DL-NORLEUCINE-3-^{14}C*

by

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The metabolism of α-aminobutyric acid in the animal body is of interest because of its general distribution in body fluids. Precursors of this amino acid in the animal organism are threonine[1,2] and methionine[3,4]. α-Aminobutyric acid can substitute for pyrimidines in supporting the growth of certain pyrimidine-requiring mutants of *Neurospora crassa*[5], and it has been reported to be the precursor of propanol in fermenting grape juice[6]. So far, it has not been found to occur in any protein.

Norleucine has not been discovered in natural material. Since it is readily utilized by the mammal[7], the pattern of its metabolism assumes significance. The pathways of the catabolism of α-aminobutyric acid and norleucine converge in that both yield propionic acid. This explains their glucogenic and antiketogenic properties. α-Aminobutyric acid was shown to be antiketogenic by COHEN[8] and the glucogenic character of norleucine was reported by GREENWALD[9] and by BUTTS and co-workers[10].

The L-forms of both of these amino acids are transaminated by heart muscle[11] and the D-forms are oxidatively deaminated by D-amino acid oxidase[12].

In addition to the inherent interest in the metabolism of α-aminobutyric acid and norleucine, the investigation of their catabolism, which is presented in this paper, was undertaken in order to gather evidence for a general mechanism for the catabolism of straight-chain α-amino acids.

RESULTS AND DISCUSSION

Catabolism of α-aminobutyric acid§

This was studied by incubating DL-α-aminobutyrate-3-^{14}C (5 mg, 4.3 uc/mg) with rat liver homogenate for 2 hours at 38° and chromatographing the deproteinized aqueous extract on a silica gel column. Isolation and identification of the radioactive intermediates, α-ketobutyric acid§§ and its decarboxylation product, propionic acid, were

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§ The radioactive amino acids were prepared in the Bioorganic Group, Radiation Laboratory, University of California, by R. OSTWALD, P. T. ADAMS, and B. TOLBERT. Details of synthesis will be published elsewhere. The preparations were tested for radioactive purity by column chromatography and by radioautography on paper.

§§ Unlabeled α-ketobutyric acid and α-ketocaproic acid, employed as reference standards, were kindly prepared by Mr. DONALD MORRISON in our laboratory.

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accomplished by silica gel column chromatography with the benzene-ethyl ether mixture (Fig. 1) and, separately, with the chloroform-*tert.* amyl alcohol mixture (Fig. 2) regularly

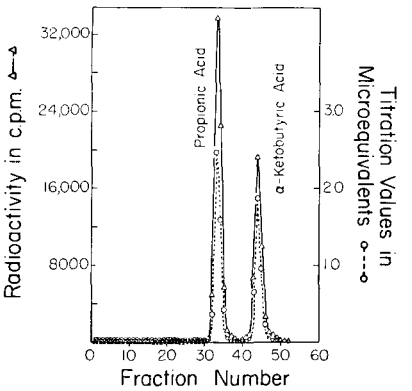


Fig. 1. Chromatographic identification of α-ketobutyric acid and propionic acid from the incubation of DL-α-aminobutyrate-3-^{14}C with rat liver homogenate. Silica gel column chromatography with a benzene-ether solvent system was used.

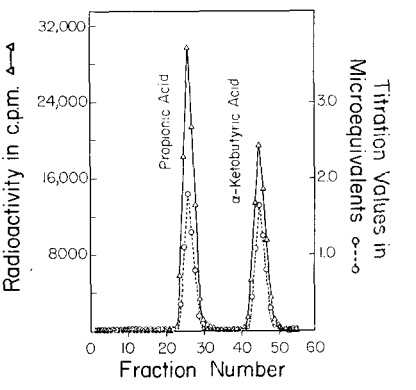
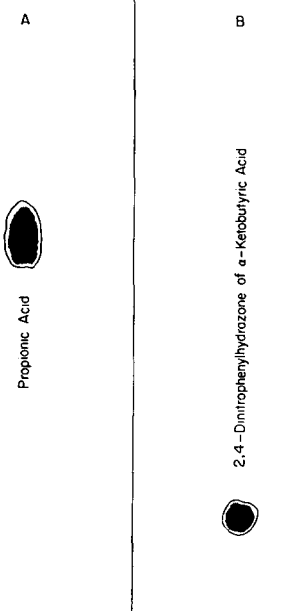


Fig. 2. Chromatographic identification of α-ketobutyric acid and propionic acid from the incubation of DL-α-aminobutyrate-3-^{14}C with rat liver homogenate. Silica gel column chromatography with a chloroform-*tert.* amyl alcohol solvent system was used.



employed by us as the solvent systems. The identities of α-ketobutyric acid, as its 2,4-dinitrophenylhydrazone derivative, and of propionic acid were further established by paper chromatography and radioautography (Fig. 3).

Fig. 3. Paper chromatograms and radioautograms of radioactive fractions isolated by silica gel column chromatography from the incubation of DL-α-aminobutyrate-3-^{14}C. The black spots indicate radioactivity, and the areas enclosed by the outlines show the location of the corresponding, unlabeled compounds, which were used for comparison. A. Propionic acid. The ammonium salt of the acid was chromatographed by the descending method using an *n*-butanol-ammonium hydroxide solvent system[13]. B. 2,4-Dinitrophenylhydrazone of α-ketobutyric acid. The dry hydrazone was dissolved in 0.2 ml of ethanol and neutralized with 0.3 ml of 0.1 *M* phosphate buffer, pH 7.2. Aliquots of this solution were chromatographed by the descending method using a mixture of *tert.* amyl alcohol, ethyl alcohol, and water in the ratio 50:10:40 (v/v)[18].

Catabolism of norleucine[§]

This was investigated by incubating DL-norleucine-3-^{14}C (2.5 mg, 21 uc/mg) in the same manner as α-aminobutyric acid.

* The norleucine chromatogram, developed with the benzene-ether solvent system, exhibited peaks of radioactivity in the regions of the appearance of acetic acid and also propionic acid. In both instances there was poor coincidence of the radioactivity with the titration peaks of the added carrier acids. Paper chromatograms were run on the ammonium salts of these acid fractions with added carriers[13] to seek an explanation for the observed discrepancies. The acetate spot on the paper (R_F 0.24) showed considerable radioactivity, but a second spot remained at the origin, which may be the sodium salt of acetic acid. No radioactivity was found in the propionate spot (R_F 0.32), but radioactivity was found in a region on the paper at R_F 0.55, which was negative to the indicator. We are greatly indebted to Dr. A. ICHIHARA for performing these tests.

§ See footnote on page 561.

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The radioactive products obtained from the catabolism of the labeled norleucine were α-ketocaproic acid, valeric acid, and β-hydroxyvaleric acid. A good separation of these intermediates was obtained with the benzene-ether solvent mixture (Fig. 4); the chloroform-*tert.* amyl alcohol mixture gave only an incomplete but noticeable separation of valeric acid and α-ketocaproic acid. The chromatographically isolated α-ketocaproic acid was further characterized by converting it to the 2,4-dinitrophenylhydrazone and subjecting the hydrazone derivative to paper chromatography and radioautography (Fig. 5A). Additional verification of the identities of valeric acid and β-hydroxyvaleric acid was also made by paper chromatography and radioautography (Fig. 5, B and C).

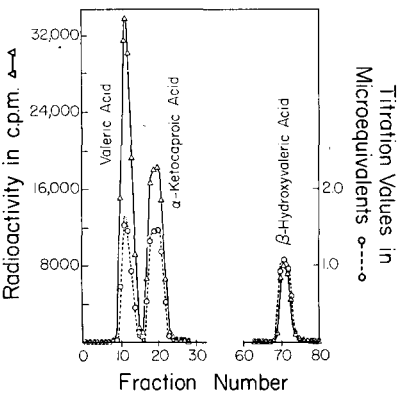

Fig. 4. Chromatographic identification of α-ketocaproic, valeric, and β-hydroxyvaleric acids from the incubation of DL-norleucine-3-^{14}C with rat liver homogenate. Silica gel column chromatography with a benzene-ether solvent system was used.

It appears that the oxidation of the intermediate valeric acid is initiated by β-oxidation, as indicated by the formation of β-hydroxyvaleric acid, and that the further oxidation of β-hydroxyvaleric acid results in the formation of acetic and propionic acids, as demonstrated by the *in vitro* experiments of ATCHLEY[14] and the *in vivo* studies of SIEGEL AND LORBER[15]. In the present investigation with norleucine-3-^{14}C, the intermediate propionic acid formed should be devoid of a label, since it would be derived from carbon atoms 4, 5, and 6 of norleucine, whereas the intermediate acetic acid, arising from carbon atoms 2 and 3 of the amino acid, would be expected to be radioactive. Radioactivity was found in the acetic acid peak of the chromatograms with both of the solvent pairs used*.

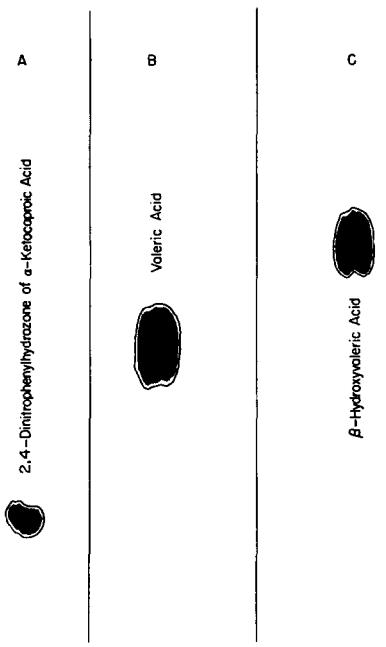

Fig. 5. Paper chromatograms and radioautograms of radioactive fractions isolated by silica gel column chromatography from the incubation of DL-norleucine-3-^{14}C with rat liver homogenate. The black spots indicate radioactivity and the areas enclosed by the outlines show the location of the corresponding, unlabeled compounds, which were used for comparison. A. 2,4-Dinitrophenylhydrazone of α-ketocaproic acid. The same method was used as in 3B. B. Valeric acid. The same method was used as in 3A. C. β-Hydroxyvaleric acid. The same method was used as in 3A.

Mechanism of catabolism of straight-chain α-amino acids

The results obtained with the several amino acids that have now been studied[16] support the general scheme that the straight-chain α-amino acids are catabolized by being first transaminated or deaminated to the corresponding α-keto acid, which

* See footnote on page 562.

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is then decarboxylated to yield the monocarboxylic acid with one less carbon atom. The resulting monocarboxylic acid is further oxidized by the general β-oxidation pathway (for monocarboxylic acids). The terminal carbon atoms of even-carbon amino acids thus form propionic acid, and those of odd-carbon amino acids give rise to acetyl groups.

EXPERIMENTAL

The procedures for preparing the liver homogenates, carrying out the incubations, preparing the protein-free filtrates for chromatography, and counting the radioactive samples were the same as described in previous publications[16,17]. The method of preparation of the silica gel columns and the technique of chromatography with the solvent systems employed have also been published[18].

SUMMARY

The catabolism of DL-α-aminobutyric acid-3-^{14}C and of DL-norleucine-3-^{14}C have been studied by incubation in rat liver homogenates. Radioactive α-ketobutyric acid and propionic acid were isolated from the α-aminobutyric acid incubations. Radioactive α-ketocaproic acid, valeric acid, and β-hydroxyvaleric acid were isolated from the norleucine incubations.

The evidence obtained supports the scheme that the straight-chain α-amino acids are catabolized by conversion to the corresponding α-keto acid, oxidative decarboxylation, and the subsequent oxidation of the resulting monocarboxylic acid by the scheme of β-oxidation.

RÉSUMÉ

Le catabolisme de l'acide DL-3-^{14}C-α-aminobutyrique et de la DL-3-^{14}C-norleucine a été étudié par incubation avec des homogénats de foie de rat. L'acide α-cétobutyrique et l'acide propionique radioactifs ont été isolés après incubation en présence d'acide α-aminobutyrique. Les acides α-cétocaproïque, valérique, et β-hydroxyvalérique radioactifs ont été isolés après incubation en présence de norleucine.

Les résultats obtenus sont en accord avec un schéma selon lequel les acides α-aminés à chaîne droite sont catabolysés par transformation en l'acide α-cétonique correspondant, par décarboxylation oxydative et par oxydation ultérieure en β de l'acide monocarboxylique résultant.

ZUSAMMENFASSUNG

Durch Inkubation in Rattenleberbrei wurde der Katabolismus von DL-α-Aminobuttersäure-3-^{14}C und von DL-Norleucin-3-^{14}C untersucht. Radioaktive α-Ketobuttersäure und Propionsäure wurden aus α-Aminobuttersäureninkubationen isoliert. Radioaktive α-Ketokapronsäure, Valeriansäure und β-Hydroxyvaleriansäure wurden aus Norleucininkubationen isoliert.

Diese Ergebnisse sprechen zu Gunsten der Annahme, dass der Katabolismus von α-Aminosäuren mit gerader Kette Etappen laut folgendem Schema aufweist: Umwandlung in die entsprechenden α-Ketosäuren, oxydative Dekarboxylation und nachfolgende Oxydation der daraus entstandenen Monokarbonsäuren durch β-Oxydation.

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