ENZYMATIC CONVERSION OF HOMOISOCITRIC ACID INTO α-KETOADIPIC ACID[1]

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On the basis of isotopic tracer data, a pathway for lysine biosynthesis in yeast has been proposed, involving a condensation of acetyl CoA and α-ketoglutarate to yield β-carboxy-β-hydroxyadipic acid, called homocitric acid (Strassman and Weinhouse, 1953). This condensation reaction and the suggested isomerization of homocitric acid to α-hydroxy-β-carboxyadipic acid, called homoisocitric acid, followed by oxidative decarboxylation to α-ketoadipic acid, a known precursor of lysine, are reactions analogous to those involved in the conversion of citric acid to α-ketoglutaric acid via the citric acid cycle. Confirmation of the first step in this pathway, the formation of homocitrate from acetate and α-ketoglutarate, was shown in the previous paper (Strassman & Ceci, 1963) to be catalyzed by a crude yeast extract. The present communication presents evidence for another step, the conversion of homoisocitrate to α-ketoadipate, in the presence of the same type of yeast extract.

The yeast extract was prepared by disruption of fresh baker's yeast in 0.02 M phosphate buffer, pH 7.2, in a high speed centrifuge shaker as previously described (Strassman et al., 1960). The homoisocitric acid used in these experiments was synthesized by Dr. M. Yamashita (1958) by catalytic reduction of the triethyl 2-oxalglutarate

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formed by condensation of diethyl oxalate and diethyl glutarate. Enzyme reaction mixtures were incubated at 37° for 2 hours, deproteinized with tungstic acid and the 2,4-dinitrophenylhydrazones of the keto acids were prepared, extracted and chromatographed on paper as described (Strassman et al., 1960).

In preliminary experiments incubation of homoisocitrate with yeast extract in the presence of DPN and Mg^{++} ions gave a strong hydrazone spot not observed if enzyme or substrate was omitted from the incubation mixture. Comparison on paper chromatograms of the enzymatically produced hydrazone with synthetically prepared hydrazone of α-ketoadipic acid, in 8 different solvent systems, gave identical Rf values as shown in Table I. Catalytic reduction of the unknown hydrazone in the presence of platinum oxide gave an amino acid which proved to be identical with α-aminoadipic acid by comparison of Rf values in 6 solvent systems, also shown in Table I, thus further establishing the identity of the enzymatically formed keto acid with α-ketoadipic acid.

TABLE I

Rf VALUES OF SYNTHETIC AND ENZYME PRODUCTS

Solvent system	2,4-Dinitrophenyl-hydrazones		Amino acid from reduced hydrazone	
	Enzyme product	α-Keto-adipic acid	Enzyme product	α-Amino-adipic acid
EtOH, ammonia, H_2O (80:16:4)	0.46	0.46	----	----
n-BuOH, formic acid, H_2O (7:3:12)	0.92	0.91	0.07	0.07
n-BuOH, acetic acid, H_2O (4:1:1)	0.78	0.77	----	----
n-BuOH, pyridine, H_2O (3:2:1.5)	0.54	0.53	0.05	0.07
n-BuOH, satd. with 3% NH_4OH	0.09	0.12	----	----
phenol satd. with water	0.58	0.58	0.38	0.40
n-BuOH, EtOH, H_2O (4:1:5)	0.58	0.58	----	----
s-BuOH, acetic acid (95:5) satd. with water	0.84	0.84	0.17	0.17
EtOAc, formic acid, H_2O (10:2:3)	----	----	0.26	0.26
EtOAc, acetic acid, H_2O, NaOAc (2:1:1:0.05%)	----	----	0.73	0.72

The results of a typical study on the cofactor requirements for the enzymatic conversion of homoisocitrate to α-ketoadipate are given in Table II. The product yields, in micromoles, were obtained by elution of the α-ketoadipic acid hydrazone spots from paper chromatograms and measurement of their absorption at 510 mμ in 1N sodium hydroxide solutions as described previously (Strassman et al., 1960). The complete system containing DPN, magnesium ions, substrate and enzyme gave a good yield of α-ketoadipate, 0.48 micromoles. Omission of DPN, enzyme or substrate resulted in negligible yields of keto acid. Omission of Mg^{++} or substitution of TPN for DPN decreased the yield of product considerably. Addition of AMP to the complete system did not increase the yield of keto acid as would have been expected from the work of Kornberg and Pricer (1951) on yeast isocitric dehydrogenase.

TABLE II

ENZYMATIC CONVERSION OF HOMOISOCITRIC ACID INTO α-KETOADIPATE

Complete system contained: 50 μmoles K phosphate buffer (pH 7.2), 5 μmoles $MgCl_2$, 1 μmole DPN, 3 μmoles K homoisocitrate, 0.03 ml dialyzed yeast extract (2 mg of protein by ultraviolet absorption). Where indicated 0.6 μmoles AMP added to complete system and 1 μmole TPN substituted for DPN. Total volume 0.8 ml, incubation 37° for 2 hours.

Omitted	α-Ketoadipic formed
	μmoles
----	0.48
Mg^{++}	0.16
DPN	0.02
homoisocitrate	0.01
enzyme	0.01
complete + AMP	0.37
complete + TPN (no DPN)	0.10

The data presented in this communication demonstrate the conversion of homoisocitric acid into α-ketoadipic acid. Since α-ketoadipic acid is a known lysine precursor in yeast, homocitrate may also be consid-

ered an intermediate in lysine formation in yeast. In this conversion DPN is required and is probably reduced to DPNH according to the reaction:

$$\text{Homoisocitrate} + \text{DPN} \longrightarrow \alpha\text{-Ketoadipate} + \text{DPNH} + CO_2$$

Future studies on the kinetics of the reaction involving the formation of both DPNH and α-ketoadipic acid are planned.

REFERENCES

Kornberg, A. and Pricer, W.E., Jr., J. Biol. Chem., 189, 123 (1951).

Strassman, M. and Ceci, L.N., Biochem. and Biophys. Res. Comm., 14, 262 (1964)

Strassman, M. and Weinhouse, S., J. Am. Chem. Soc., 75, 1680 (1953).

Strassman, M., Shatton, J.B. and Weinhouse S., J. Biol. Chem., 235, 700 (1960).

Yamashita, M., J. Org. Chem., 23, 835 (1958).