THE DEGRADATION OF HYDROXYL-L-LYSINE IN LIVER VIA ITS PHOSPHATE ESTER

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SUMMARY: From an incubation mixture of rat liver extract and 5-hydroxy-L-lysine, two products were isolated and identified as O-phosphohydroxylysine and 2-aminoadipate. The formation of the phosphate ester of hydroxylysine was dependent upon the presence of ATP or GTP. O-phosphohydroxylysine was converted to 2-aminoadipate in the presence of pyridoxal-5-phosphate and NAD^+ or $NADP^+$. On the basis of these findings and supporting data from *in vivo* experiments, the liver degradation of hydroxy-L-lysine is postulated to proceed by a reduction at C_5 and oxidation at C_6 via a phosphate ester to give 2-aminoadipic semialdehyde and subsequent oxidation to 2-aminoadipate.

L-Hylys[1] derived from collagen turnover is degraded and not directly reutilized for collagen synthesis (1). Lindstedt *et al.* (2) observed that 5-hydroxypipecolate became labeled from D-Hylys-6-^{14}C in rat liver preparations. This intermediate was further metabolized to 2-amino-5-hydroxyadipate which in turn labeled 2-keto-5-hydroxyadipate and 2-hydroxyglutarate (3, 4). In contrast, the *in vivo* rat experiments of Polan et al. (5) using L-Hylys presented evidence to indicate the early loss of the hydroxyl group. This apparent conflict was clarified by Hammerstedt, Swan, and Henderson (6) when they showed that in the rat, most of the hydroxypipecolate is derived from allo-D-Hylys and D-Hylys. In liver perfusions and *in vivo*, L-Hylys was shown to be metabolized to CO_2 apparently via glutarate. The early reactions which remove the hydroxyl group required further investigation. This communication reports data to support the degradation scheme for L-Hylys as shown in Figure 1.

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[1] Abbreviations: Hylys refers to all possible isomers of 5-hydroxylysine whereas specific isomers are designated by a prefix, i.e. erythro-5-hydroxy-L-lysine is L-Hylys. 2-AAA is 2-aminoadipic acid.

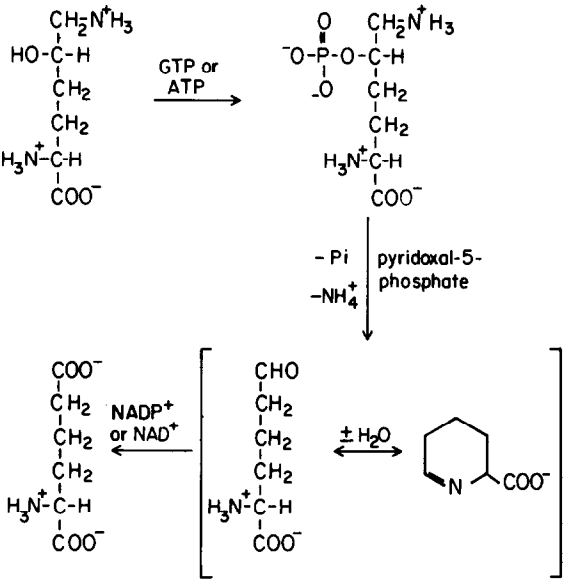

FIGURE 1. The Proposed Early Steps in the Degradation of 5-hydroxy-L-lysine.

EXPERIMENTAL. Male albino rats (200 to 300 g) were maintained on Purina laboratory chow. Vitamin B_6-deficiency was produced by placing 200 g rats on a diet containing 20% casein, 10% gelatin, 58% sucrose and other components as previously described (7) for a period of 8 to 12 weeks.

Homogenates were prepared from fresh rat livers and a 2:1 (v/w) ratio of 2 mM Tris·HCl (pH 8), 0.25 M sucrose buffer. For maximum enzyme activity, the extraction medium was made 10^{-3} M in glutathione and 10^{-5} M in ethyleneglycol-bis-(β-amino-ethyl ether) N,N'-tetraacetic acid. Unless otherwise stated, the supernatant fluid from a 10 min., 15,000 x g centrifugation was used as the enzyme source. Reactions were initiated by adding 2 ml of extract to the 2.6 ml solution of substrates and cofactors (see individual tables) and continued at 35° for 60 to 120 min. Six ml of absolute ethanol was added to stop the reactions and the protein was removed by centrifugation. The supernatant fluid was then placed on a 1.2 by 10 cm Dowex-1x8 acetate (200 to 400 mesh) column and washed with water to remove unreacted substrate. The products of the reaction were removed by elution with pH 5.2

pyridine-formate (0.1 M) buffer. Each 10 ml fraction was assayed for radioactivity by liquid scintillation counting.

The L-Hylys-6-^{14}C was prepared in this laboratory[2]. All other chemicals were obtained from commercial sources.

RESULTS. The incubation of rat liver extracts with the components shown in Table I resulted in the conversion of L-Hylys-6-^{14}C to products which were retained on a Dowex-1-acetate column. Elution with buffer removed two ^{14}C-compounds (I and II) with I present at a concentration about 10 times that of II.

TABLE I

THE INTRACELLULAR LOCALIZATION

OF ENZYMATIC ACTIVITIES[a]

Liver Fraction	Crude Extract (600 x g supernatant)	15,000 x g supernatant	15,000 x g Pellet
percent of recovered ^{14}C found in hydroxylysine phosphate (I)	9.7[b]	13.6	2.5
percent of recovered ^{14}C found in 2-aminoadipate (II)	0.9	1.1	0.3

[a] Each assay contained 5 μmoles of phosphocreatine, acetylphosphate, carbamylphosphate, GTP, ATP, and NAD^+, 3 μmoles of $NADP^+$ and 1 μmole of pyridoxal-5-phosphate all prepared in 0.1 M phosphate buffer (pH 7.4); 5 μmoles of $MgCl_2$, 10 μmoles of 2-ketoglutarate (pH 7.4), 0.4 μmoles tris·HCl (pH 8), 5-hydroxyl L-lysine-6-^{14}C (~ 2 μCi at 6.1 μCi/μmole) in a volume of 1.3 ml and 1.3 ml of 0.5 M sucrose 2mM tris·HCl (pH 8) buffer. Two ml of liver extract was added to this and the reaction continued for 2 hr at 38°. See text for product analysis.

[b] By omitting all potential phosphate donors, NAD^+, $NADP^+$ and pyridoxal-5-phosphate, only 0.9% of new product formed. With enzyme omitted, no conversion took place.

[2] Procedure to be published elsewhere.

Samples of radioactive I and II were purified on larger Dowex-1 acetate columns and used for identification. Compound I had the chromatographic and electrophoretic properties reported for isolated Hylys phosphate (8, 9). Acid hydrolysis of isolated Hylys phosphate into Hylys is only partially completed in 24 hr at 100° (10). Exposure of I to 6N HCl in a sealed tube at 110° resulted in 34% conversion to Hylys in 8 hr and 74% in 24 hr. Alkaline phosphatase treatment of I and Hylys phosphate (10) resulted in a complete conversion to Hylys. I was partially converted to Hylys by 1N KOH in a boiling water bath for 10 min. Under these conditions of hydrolysis, unknown II was stable. Compound II migrated similarly to authentic 2-AAA in three chromatography systems. Following the addition of carrier 2-AAA, II was crystallized to a constant specific activity.

As seen in Table I, a large part of the enzymatic activity for conversion of L-Hylys to L-Hylys phosphate and 2-AAA was present in the supernatant fluid and did not require the presence of the mitochondria.

TABLE II

PHOSPHATE DONORS FOR HYDROXYLYSINE KINASE

Reaction Mixtures	Percent Activity of Complete Mixture
Complete[a]	100 (31% conversion)
Enzyme, $MgCl_2$, 5 µmoles ATP	19
Enzyme, $MgCl_2$, 5 µmoles GTP	25
Enzyme, $MgCl_2$, 25 µmoles ATP	52
Enzyme, $MgCl_2$, 25 µmoles GTP	69
Enzyme, $MgCl_2$, 50 µmoles ATP[b]	70

[a] The reaction conditions were the same as in Table I except 0.2 µCi of hydroxy-L-lysine (~2 µCi/µmole) was used.

[b] ATP was added in two equal portions at 0 and 60 min.

Of the phosphate donors tested, only ATP or GTP would phosphorylate Hylys (Table II). However, maximum activity was obtained by also including creatine phosphate or carbamyl phosphate.

As shown in Table III, Hylys phosphate was converted into 2-AAA in the presence of liver extract and either NAD^+ or $NADP^+$. The addition of pyridoxal-5-phosphate was not stimulatory except when vitamin B_6-deficient livers or isonicotinic acid hydrazide treated liver extracts were used. No free Hylys-^{14}C was found following incubation of Hylys phosphate with liver extracts.

Table III

REQUIREMENTS FOR THE FORMATION OF 2-AMINOADIPATE FROM HYDROXYLYSINE PHOSPHATE

	Changes from Complete Mixture[a]	Percent Activity of Complete Mixture: Normal Rat	Vitamin B_6-deficient Rat
None		100 (18% conversion)	---
-	ATP, carbamyl phosphate, acetyl phosphate, phosphocreatin	133	100 (20% conversion)
-	NAD^+, $NADP^+$	0	---
-	pyridoxal-5-phosphate, 2-ketoglutarate	95	---
-	pyridoxal-5-phosphate, 2-ketoglutarate		
+	isonicotinic acid hydrazide	0	3
-	2-ketoglutarate	---	85
-	pyridoxal-5-phosphate	---	28
+	isonicotinic acid hydrazide[b]	116	---

[a] The reaction components were the same as in Table II except 10 μmole of ATP and no GTP was used and the substrate was hydroxylysine-^{14}C-phosphate.

[b] The substrate was hydroxylysine-^{14}C.

In order to test the intermediary role of 2-AAA in the in vivo degradation of L-Hylys, B_6-deficient rats were injected with L-Hylys-^{14}C and overloading amounts of 2-AAA as previously described (11). 4 percent of the urinary ^{14}C was found in 2-AAA.

DISCUSSION. Hylys phosphate has been reported to occur in the cellular fluid of several mammalian tissues (8-10, 12). Its synthesis in liver supernatant and its further conversion, probably via 2-aminoadipic semialdehyde (13-16) into 2-AAA has now been demonstrated. The best explanation for these enzymatic activities is that L-Hylys phosphate is an intermediate in the degradative pathway of L-Hylys between the hydroxyamino acid and 2-ketoadipate (5). In vivo experiments confirm the suspected intermediary role of 2-AAA in this pathway. The involvement of these two new intermediates appears to exclude the intermediary role of 5-hydroxypipecolate (2-4) in the degradation of naturally occuring L-Hylys by the liver.

Because of the crude nature of the enzyme preparation, it could not be demonstrated in a definitive manner if GTP was a better phosphate donor than ATP for the kinase reaction or simply less susceptible to degradation. However, the data in Table II show that GTP does not become limiting as readily as ATP. No requirement for additional cofactors was demonstrated although it is assumed a divalent metal also particpates in the phosphate transfer.

The further conversion of L-Hylys phosphate to 2-AAA acid was shown to require both pyridoxal-5-phosphate and either NAD^+ or $NADP^+$. These reactions are analogous to those by which O-phosphoethanolamine is converted to ammonia, inorganic phosphate, and acetate (17) proceeding with an initial β-elimination of the phosphate (18). The enzyme is thus a phospho-lyase rather than an ammonia lyase [O-phosphohydroxylysine phospho-lyase (adding water and deaminating) 4.2.99].

In vivo (11) and in vitro (13, 19) experiments have shown that 2-AAA is a metabolite of L-lysine in the rat. The results reported here show that under similar conditions, L-Hylys also forms 2-AAA. Although lysine degra-

dation via saccharopine to 2-AAA acid proceeds in the mitochondria (13, 19) and both the L-Hylys phosphate and 2-AAA forming enzymes are in the soluble fraction, it seems logical to conclude that 2-aminoadipic-semialdehyde (Δ'-piperidine-6-carboxylic acid) is the first possible common intermediate in the degradation of the two amino acids (13, 14, 20). Whether the oxidative conversion of the semialdehyde to 2-AAA is due to the action of a specific oxidoreductase as in a pseudomonad (21) or a general aldehyde dehydrogenase awaits further studies.

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