CORRINOID AND THIOL DEPENDENT THREONINE DECARBOXYLATION

Susan H. Ford and Herbert C. Friedmann

Department of Biochemistry, The University of Chicago
Chicago, Illinois 60637

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SUMMARY

A novel system for the decarboxylation of L-threonine requiring the presence of certain aquocorrinoids, preferably diaquocobyric acid, and of a thiol is described. The reaction shows a marked optimum at a pH value of about 8. Other L-amino acids, with the exception of L-serine, are much less reactive. Optical data point to a ligand attachment of L-threonine, or of a substance derived from L-threonine, to the corrinoid cobalt. It is proposed that such a ligand attachment precedes incorporation of (R)-1-amino-2-propanol in amide linkage into corrinoid at the "f" propionic acid side chain, and that the present system serves as a model for this part of the cobalamin biosynthetic pathway.

INTRODUCTION

The formation of the vitamin B_{12} precursor cobinamide in bacteria such as Propionibacterium shermanii involves the incorporation of (R)-1-amino-2-propanol derived from L-threonine after decarboxylation of the amino acid (1). Direct enzymatic decarboxylation of threonine has never been described, however, and although various schemes for indirect threonine decarboxylation have been proposed, none has yet been substantiated (reviewed in 2).

Subsequent to our recently reported study of an enzyme system from P. shermanii which utilizes threonine for in vitro cobinamide formation (3), we observed CO_2 release from threonine in a system containing a thiol and a corrinoid, both in the presence and, to a lesser extent, in the absence of enzyme. To our knowledge no such corrinoid- and thiol-dependent threonine decarboxylation, enzymatic or non-enzymatic, has heretofore been described. Since not only corrinoids, but presumably also thiols are present in abundance in cobalamin-synthesizing organisms, it is quite possible that the

non-enzymatic system might serve as a model to study and to understand threonine decarboxylation during cobalamin synthesis in vivo. This communication describes our initial studies of corrinoid- and thiol-dependent non-enzymatic threonine decarboxylation.

METHODS AND MATERIALS

After distillation under reduced pressure, β-ME[1] (Eastman Kodak) was stored in the refrigerator as a 1 M aqueous solution. Corrinoid sources were as follows: hydroxocobalamin, Mann; cyanocobalamin (vitamin B_{12}), Sigma; adenosylcobalamin, Pierrel S.p.A. (Milan, Italy); cyanoaquocobyric acid, a kind gift of Dr. L. Mervyn, Glaxo Laboratories, England. Cyanoaquocobinamide was prepared from cyanocobalamin by cerous hydroxide hydrolysis (4,5). Diaquocobinamide and diaquocobyric acid were prepared by aeration (6) of the corresponding reduced corrinoids (7).

L-[U-^{14}C]threonine, L-[U-^{14}C]ornithine, L-[U-^{14}C]serine, L-[U-^{14}C]-leucine, and [U-^{14}C]β-alanine were obtained from New England Nuclear, and L-[U-^{14}C]arginine, L-[U-^{14}C]glutamic acid, L-[U-^{14}C]histidine, L-[U-^{14}C]tyrosine, L-[U-^{14}C]alanine, and hyamine hydroxide from Amersham Searle. Scintillation counting was performed as described before (3) using a Nuclear Chicago Unilux Scintillation Counter.

The [^{14}C]CO_2-trapping assays were run in 2-dram capacity snap-cap disposable glass vials (Kimble No. 60975-L). Reaction mixtures of 100 µl final volume were made up by pipetting buffer, thiol, corrinoid, and ^{14}C-amino acid into the individual vials and mixing. Each vial was immediately sealed with a sleeved rubber stopper (Kontes No. K-882310) in which a polypropylene center well (Kontes No. K-882320) above the reaction mixture was held by its stem passing through the tight-fitting center hole of the stopper. After incubation for 1 to 2 hr, 100 µl 50% TCA was injected through the stopper into the reaction mixture and, to trap released [^{14}C]CO_2, 100 µl hyamine hydroxide was injected into the suspended center well. After the vial and attached well had stood 30-60 minutes, the rubber stopper was removed and the well and contents were dropped into 10 ml scintillation fluid for measurement of radioactivity. All assays were performed in duplicate; control mixtures which contained water instead of thiol or corrinoid were run in parallel with all assays.

Absorption spectra of reaction mixtures contained in 1 ml quartz cuvettes were scanned from 310-610 nm in a Bausch and Lomb Spectronic 600 spectrophotometer.

RESULTS AND DISCUSSION

pH Dependence. The effect of pH on the rate of threonine decarboxylation in the presence of aquocobalamin and of β-ME was surveyed between pH 4 and pH 9 in acetate, phosphate, and Tris-HCl buffers (Figure 1). A marked pH

[1] Abbreviations: β-ME, β-mercaptoethanol; TCA, trichloroacetic acid.

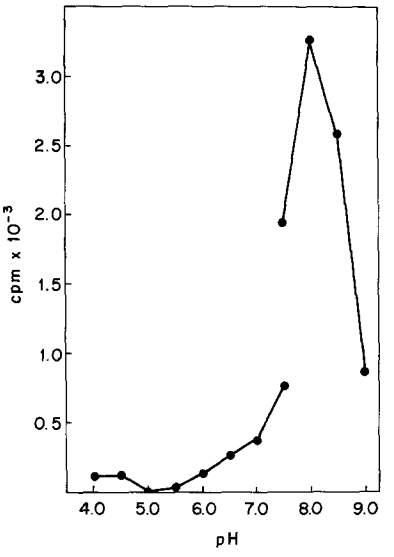

FIGURE 1: The effect of pH on threonine decarboxylation. Assay conditions: 0.1 M buffer of type noted below containing 10 mM β-ME, 0.041 mM threonine (0.5 μCi), 0.04 mM aquocobalamin; one hour incubation, 30 minute $[^{14}C]CO_2$ collection. Buffers used: pH 4.0-5.5, 0.1 M NaAc-HAc; pH 6.0-7.5, 0.1 M KH_2PO_4-NaOH; pH 7.5-9.0, 0.1 M Tris-HCl.

optimum was observed around pH 8. There was no appreciable reaction below pH 6 and a rapid fall in rate upon approaching pH 9. The reaction rate at pH 7.5 in Tris-HCl was more than twice that in phosphate. The basis for this behavior is not known. A comparable buffer effect was reported by Peel (8) in his studies on corrinoid-catalyzed oxidation of β-ME.

Comparison of the Effect of Various Corrinoids. The effects on the rates of threonine decarboxylation of changes in the types of corrinoids used are shown in Table I. The aquo- or diaquocorrinoids were the most reactive, while the cyano- or cyanoaquocorrinoids were very much less reactive. These results are analogous to those obtained for the oxidation of thiol and of CO in the presence of aquocobalamin and diaquocobinamide compared to cyanocobalamin and cyanoaquocobinamides (8,9).

Table I shows that unlike the weakly active cyanoaquocobinamide, cyanoaquocobyric acid maintains as much as 63.5% of the activity of diaquo-

Table I. Effect of Different Corrinoids and of Co^{2+} on Extent of Threonine Decarboxylation[a]

Corrinoid	$[^{14}C]CO_2$ cpm[b]	Relative Extent of Decarboxylation
---	357	---
Aquocobalamin[c]	20,916	75
Cyanocobalamin	593	0.86
β-adenosylcobalamin	689	1.2
Diaquocobinamide	21,837	79
Cyanoaquocobinamide[d]	3,017	9.7
Diaquocobyric Acid	27,658	100
Cyanoaquocobyric Acid	17,707	63.5
Cobalt chloride	612	0.93

[a] Assay conditions: 0.1 M Tris-HCl, pH 8.0, containing 25 mM β-ME, 0.065 mM corrinoid or Co^{2+}, 0.032 mM L-[U-^{14}C]threonine (0.5 μCi); incubation 1 1/2 hr; $[^{14}C]CO_2$ collection, 1 hr. [b] Average from duplicates; total threonine cpm per assay: 8.26×10^5 cpm, 2.07×10^5 cpm per carbon. [c] Probably a mixture of equal parts of aquo and hydroxo forms since these assays were run at pH 8, or at about the pK' of this tautomeric pair. [d] Contains approximately 1/3 dicyanocobinamide.

cobyric acid, and thus cobyric acid seems to exhibit a special behavior with regard to threonine decarboxylation in this system. From the viewpoint of vitamin B_{12} biosynthesis the greater effectiveness of cobyric acid, the immediate precursor of cobinamide, compared to other corrinoids in promoting threonine decarboxylation is unlikely to be coincidental. It is tempting to speculate that *in vivo* one and the same molecule of cobyric acid might function not only in threonine decarboxylation, but also as the recipient of the newly formed (R)-1-amino-2-propanol moiety.

Binding of Threonine to Corrinoid: Spectral Studies. Certain char-

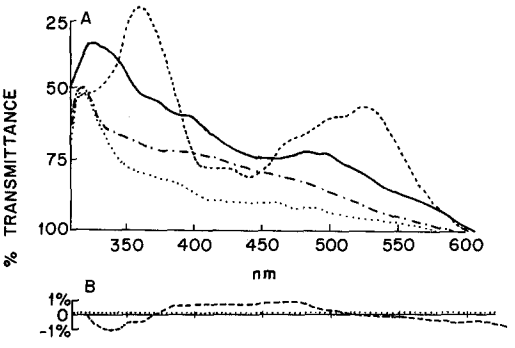

FIGURE 2A: Spectrum of aquocobalamin in the presence and absence of β-ME. Assay conditions: 0.1 M Tris-HCl, pH 8.0, containing 0.05 mM aquocobalamin and 77 mM β-ME in 1 ml; spectrum scanned at the following times after mixing: ———, 34 minutes; -•-•-•-, 5 days; •••••, 0.5 ml 50% TCA was added after 5 days, spectrum was scanned 20 hr later. -----, 0.05 mM aquocobalamin in 0.1 M Tris-HCl, pH 8.0.

FIGURE 2B: Difference spectrum of solutions containing buffer, aquocobalamin, and β-ME with and without threonine. Reaction conditions: duplicate solutions of 0.1 M Tris-HCl, pH 8.0, containing 0.05 mM aquocobalamin and 77 mM β-ME were prepared in 1 ml volume and allowed to react 30 minutes. To one solution threonine was added to 7.7 mM and to the other water was added. The difference spectrum was taken after 5 days with the solution containing threonine as the standard. -----, difference spectrum after 5 days; •••••, 0.5 ml 50% TCA was added after 5 days and the difference spectrum was taken 20 hours later.

acteristics of the threonine-corrinoid-β-ME interaction, not discernible by observing CO_2 evolution alone, could be studied by following changes in the aquocobalamin spectrum in the presence either of β-ME or of β-ME plus threonine. Figure 2A shows the progressive change in the aquocobalamin spectrum in the presence of β-ME alone during five days at room temperature. The change in transmittance after 34 minutes is similar to that reported by Peel (8). However, the spectral change continues, with the region above 350 nm becoming almost featureless with time. When threonine was added to a solution containing aquocobalamin and β-ME at pH 8, a slight change in the final appearance of the spectrum was noted. The difference spectrum is shown in Figure 2B. The addition of 50% TCA essentially destroyed the difference spectrum, and, as is apparent from Figure 2A, the corrinoid spectrum was again altered in the region above 350 nm.

Table II. Comparison of Decarboxylation of Various L-[U-^{14}C]Amino Acids[a]

[^{14}C]Amino Acid	[^{14}C]CO_2 cpm[b]	Relative Extent of Decarboxylation
L-Threonine	25,387	100
L-Serine	23,753	94
L-Histidine	7,823	31
L-Glutamic Acid	7,624	30
L-Arginine	6,921	27
L-Leucine	6,127	24
L-Tyrosine	3,982	16
L-Alanine	3,776	15
L-Ornithine	498	2
β-Alanine	248	1

[a] Assay conditions: 0.1 M Tris-HCl, pH 8.0, containing 25 mM β-ME, 0.065 mM aquocobalamin, 0.035 mM [^{14}C]amino acids (0.5 μCi); incubation 2 hr, [^{14}C]CO_2 collection 1 hr. [b] Average from duplicates; total threonine cpm per assay: 8.26 x 10^5 cpm.

From this and other data in the literature (reviewed in 10), we feel that the interaction at pH 8 between β-ME and aquocobalamin results in a chemically altered corrin cobalt to which threonine might become bound as a ligand. Ligand binding of amino acids is known to occur and to cause changes in the corrinoid spectrum (11,12).

Decarboxylation of Other Amino Acids. Ten L-amino acids were assayed. As may be seen from Table II, the reactivity of L-threonine and L-serine was much greater than that of the other amino acids tested. It appears that (a) the β-hydroxyl group promotes decarboxylation, and (b) the carboxyl group must be next to the amino group, since β-alanine is decarboxylated much more

poorly than alanine. We feel that in this case one function of the hydroxyl might be to stabilize an intermediate carbanion (e.g. after decarboxylation, $CH_3CHOH\bar{C}HNH_3$); further, it is possible that binding of threonine as a ligand to corrinoid, as evinced by our spectral studies, may depend on the hydroxyl group.

The present novel system which brings about release of CO_2 from threonine thus appears to possess intriguing specificities related to the biosynthetic threonine utilization for corrinoid biosynthesis. The reaction takes place at a moderate pH, and is specific not only for the "right" amino acid, i.e., threonine, but also for the "right" corrinoid, cobyric acid, which upon incorporation of (R)-1-amino-2-propanol directly yields cobinamide. Further studies of the reaction, to answer such questions as thiol specificity, products other than CO_2, and possible mechanisms, are in progress.

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