BACTERIAL AND ENZYMATIC DEGRADATION OF BIOTIN*

Robert N. Brady, Lan-Fun Li, Donald B. McCormick, and Lemuel D. Wright

Graduate School of Nutrition and
Biochemistry Section of the Division of Biological Sciences
Cornell University, Ithaca, New York

Received May 14, 1965

A bacterium which grows on biotin as a sole source of C, N, and S was isolated from soil by enrichment culture and assigned to the genus Pseudomonas (Li, 1964). Maximum growth occurs within a week at room temperature during aeration of a liquid medium at pH 7 containing salts (Rodwell et al., 1958), trace elements (Hoagland and Synder, 1933), and 0.3% biotin. Disappearance of biotin during growth of this pseudomonad was followed with Neurospora crassa 77a. Biotin-l-sulfoxide was found by paper chromatography and bioautography to be an early product of oxidation; complete degradation of biotin leads to the formation of carbon dioxide, ammonia, sulfate, and H_2S (Brady et al., 1965).

This study reports the differential loss of C^{14} from carboxyl and carbonyl labelled biotin with both whole cells and broken-cell preparations. The rates of appearance of final products in culture filtrates and the general characteristics of biotin degradation in the crude extracts are also documented.

Materials and Methods

Crystalline D-biotin was purchased from Nutritional Biochemicals Corp. The C^{14}-biotin was obtained as carbonyl labelled from Nuclear of Chicago and as carboxyl labelled from Hoffmann-La Roche and Co. of Basel.

Biotinyl CoA was synthesized from C^{14}-carbonyl labelled biotin by conversion to biotinyl chloride with excess $SOCl_2$ (Wolf et al., 1951).

*Supported by Research Grant AM-08721 from the U.S. Public Health Service

The acid chloride was reacted with thiophenol in pyridine to form the thiophenylate. The latter was converted to the thiolester of coenzyme A with about 50% yield (Vagelos, 1963). The crude product was chromatographed on Whatman No. 3 MM paper with isopropyl alcohol-pyridine-water (1:1:1) as developing solvent. Biotinyl CoA (R_f 0.6) was detected with alkaline nitroprusside as a slowly developing reddish colored spot (Toennies and Kolb, 1951).

Growth of the pseudomonad was measured by the increase in turbidity (optical density at 650 mμ) in the aerated biotin medium. Bicarbonate production was measured in culture filtrates by acidification with HCl, flushing with nitrogen, and trapping the evolved CO_2 in 0.2 N $Ba(OH)_2$-$BaCl_2$. The excess $Ba(OH)_2$ was titrated with 0.1 N HCl to the phenolphthalein end point and the precipitated $BaCO_3$ collected by filtration, dried, and weighed. Ammonia production was assayed by nesslerization and spectrophotometric determination following aeration of the culture filtrates after treatment with saturated K_2CO_3 (Daly, 1933). Inorganic sulfate was determined gravimetrically as $BaSO_4$ by treating the culture filtrates with excess $BaCl_2$ (Peters and Van Slyke, 1956).

Bacteria were obtained following growth for a week on the biotin medium. Cells were harvested by centrifugation, washed in isotonic saline, and lyophilized. Suspensions of from 1 to 10 mg. per ml. frozen cells were prepared in cold 0.05 M potassium phosphate buffer, pH 6. For broken-cell preparations, the suspensions were subjected to sonic oscillation for 3 to 5 min. at 0° in a Model LS75 Branson Sonifier. The broken cells retained activity for a week when frozen.

Loss of C^{14} from biotin was determined by incubating 0 to 4.1 X 10^{-6} M C^{14}-biotin or C^{14}-biotinyl CoA with 0.067 M buffers, usually potassium phosphate at pH 6, and 0 to 10 mg. of whole or broken cells in 3 ml. of total volume. Reactions were normally run at 30° for 60 min. with shaking and terminated by the addition of 1 ml. of 20% trichloroacetic

acid. The mixtures were heated in a boiling water bath for a minute, cooled, centrifuged at 25,000 X g for 20 min., and 0.1 ml. aliquots of the supernatant solution added to 10 ml. of Bray's solution (Bray, 1960) for counting of radioactivity in a Packard Tricarb Liquid Scintillation Spectrophotometer.

Results and Discussion

The data in Fig. 1 shows the production of HCO_3^-, NH_4^+, and $SO_4^=$ during growth of the pseudomonad on the 0.3% biotin medium.

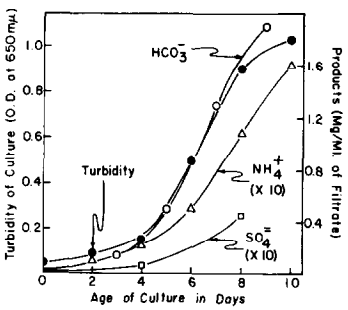

Fig. 1. Increases of HCO_3^-, NH_4^+, and $SO_4^=$ in the culture filtrates from the pseudomonad growing on an aerated 0.3% biotin-salts medium.

Total oxidation of biotin gives principally carbon dioxide, less ammonia, and small amounts of sulfate. A considerable portion of the sulfur in the original molecule is converted to H_2S, mainly lost during aeration of the medium.

In Table I, comparisons are made of the C^{14} lost during incubation of differentially labelled biotin with whole cells and broken cells.

Table I

Comparisons of C^{14} Lost from Biotin Differentially Labelled in Carboxyl and Carbonyl Positions

System*	Biotin added mμmoles	Biotin degraded: Carboxyl labelled mμmoles	%	Carbonyl labelled mμmoles	%
Whole cells	1.23	0.38	31	0.24	20
Broken cells	1.23	0.33	27	0.21	17

*Incubations were for 1 hr. at 30° with 0.75 mg. of original cells and 0.067 M potassium phosphate buffer, pH 6, in a total volume of 3 ml.

Considerable variation in the total activities of broken-cell preparations was noted as depending on the extent of sonic fragmentation; how-

ever, loss of carboxyl carbon as CO_2 from biotin was always more rapid and extensive than loss of the carbonyl carbon with both whole cells and broken cells.

The amounts of C^{14}-biotin degraded as influenced by the amount of broken cells and of biotin is shown by the data in Fig. 2. Fig. 3 illustrates the effects of temperature and of pH on such enzymatic degradation. In all cases, loss of C^{14} from carboxyl labelled biotin was greater than from the carbonyl labelled compound. Apparently, breakdown of the aliphatic chain preceeds cleavage at the ureido carbon. The extent of biotin degradation becomes maximal when less than half of the added substrate has disappeared. Such catabolic reactions *in vitro* become saturated near 10^{-5} M biotin under optimal conditions near 25° and pH 6.5.

Fig. 2

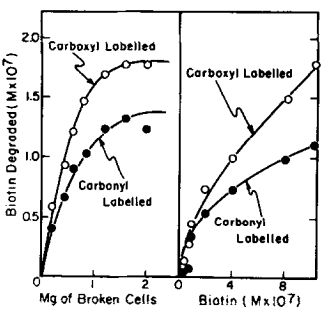

Fig. 3

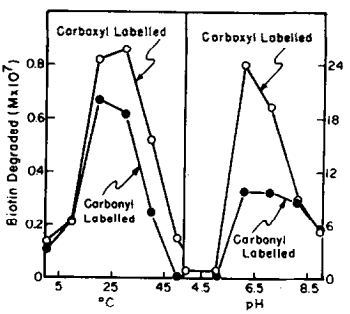

Fig. 2. Effects of amount of broken cells and of biotin on the loss of carboxyl and carbonyl carbons from C^{14}-biotin. With variations in the amount of broken cells, mixtures initially contained 4.1×10^{-7} M biotin. With variations in the amount of biotin, mixtures contained 0.5 mg. of broken cells. All incubations were for 1 hr. at 30° with 0.067 M potassium phosphate buffer, pH 6, in a total volume of 3 ml.

Fig. 3. Effects of temperature and of pH on the loss of carboxyl and carbonyl carbons from C^{14}-biotin. With variations in the temperature, mixtures at pH 6 contained 4.1×10^{-7} M biotin, 0.067 M potassium phosphate buffer, and 0.4 mg. of broken cells in 3 ml. With variations in the pH, mixtures at 30° contained 4.1×10^{-6} M biotin, 0.067 M buffers as sodium acetate at pH 4 and 5, sodium phosphate at pH 6 and 7, and Tris-HCl at pH 8 and 9, and 5 mg. of broken cells in 3 ml. All incubations were for 1 hr.

The rates of degradation and effects of a second addition of C^{14}-biotin with broken-cell preparations is shown in Fig. 4.

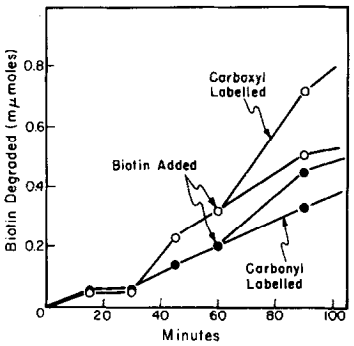

Fig. 4. Rates of degradation and effects of a second addition of C^{14}-biotin with broken cells. Original mixtures at 30° contained 4.1×10^{-7} M biotin, 0.067 M potassium phosphate at pH 6, and 0.75 mg. of broken cells in 3 ml. After 1 hr., an additional 1.5 ml. of 8.2×10^{-7} M biotin was added.

The increased rate of loss of $C^{14}O_2$ from both carboxyl and carbonyl positions of biotin after a second equivalent amount of biotin (1.23 mμ-moles) is added suggests that product inhibition may partly account for the apparent saturation of activity seen when less than half the substrate is degraded.

As indicated by the recent finding of biotinyl adenylate and its probable conversion to biotinyl CoA in bacterial extracts (Christner et al., 1964), the participation of biotinyl CoA in the initial β-oxidative cleavage of the side chain may be suggested by the present observation of its more rapid breakdown in cell-free extracts. During incubation under the usual conditions described herein, carbonyl labelled C^{14}-biotinyl CoA was poorly degraded by whole cells, but showed greater loss of C^{14} with broken cells than did the correspondingly labelled C^{14}-biotin. These findings probably reflect that permeability of whole cells to biotin is greater than to biotinyl CoA, but hydrolysis of the ureido ring may occur after cleavage of the side chain.

Studies are now underway to elucidate the exact nature of intermediates and enzymes involved in the catabolism of biotin.

References

Brady, R. N., Li, L. F., McCormick, D. B., and Wright, L. D., Federation Proc., 24, 625 (1965).

Bray, G. A., Anal. Biochem., 1, 279 (1960).

Christner, J. E., Schlesinger, M. J., and Coon, M. J., J. Biol. Chem., 239, 3997 (1964).

Daly, C. A., J. Lab. Clin. Med., 18, 1279 (1933).
Hoagland, D. B., and Synder, W. C., Proc. Am. Soc. Hort. Sci., 30, 288 (1933).
Li, L. F., The Microbiological Degradation of Biotin, Ph.D. thesis, Cornell University, June, 1964.
Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, Methods, Vol. II, Williams and Wilkins Co., Baltimore, 1956, p. 892.
Rodwell, V. W., Volcani, B. E., Ikawa, M., and Snell, E. E., J. Biol. Chem., 233, 1548 (1958).
Toennies, G., and Kolb, J. J., Anal. Chem., 23, 823 (1951).
Vagelos, P. R., in S. P. Colowick and N. O. Kaplan (Eds.), Methods in Enzymology, Vol. VI, Academic Press, Inc., New York, 1963, p. 544.
Wolf, D. E., Valiant, J., and Folkers, K., J. Am. Chem. Soc., 73, 4142 (1951).