

Biocytin in Bacterial Deamination of Aspartic Acid. (17897)

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Lichstein and co-workers(1-4) reported that the aspartic acid, serine, and threonine deaminase activity of certain bacteria may be inactivated by exposure of the cells for a short time to pH 4 in M phosphate buffer. The deaminase activity of such inactivated cells could be restored with biotin, adenylic acid, or yeast extract. Yeast extract was found to be particularly active in stimulating the system. Lichstein reported (3) that yeast extract is approximately 100 times more active than can be accounted for on the basis of its biotin content. It was concluded that there exists in yeast extract a more highly active, presumably coenzyme, form of biotin that yields biotin on hydrolysis. A preliminary concentration of the so-called coenzyme of aspartic acid deaminase has been described(5). Wright *et al.*(6) have confirmed Lichstein to the extent that they showed that bacterial preparations with a reduced ability to deaminate aspartic acid can be obtained and that, when the deaminase reaction is run at pH 7, after inactivation of the cells at pH 4 in M phosphate buffer, activity can be restored by either biotin or yeast extract. Attempts at reactivating cells with biotin when the deaminase reaction is run at pH 4 in M/2 phosphate buffer, such as described by Lichstein in his paper(3) demonstrating special activity for yeast extract, have been unsuccessful. In contrast to Lichstein and coworkers, when the amounts

of biotin and yeast extract used to stimulate deamination at pH 7 were titrated downward, biotin usually was found to be more active in the system than an equivalent of biotin as it occurs in yeast extract.

Recently Wright *et al.*, have announced(7) the isolation in crystalline form of a component of yeast extract, termed biocytin, that is available as a source of biotin to *Lactobacillus casei* but not to *Lactobacillus arabinosus*. Acid or alkaline hydrolysis yields as one moiety biotin or its microbiological equivalent for all biotin-requiring microorganisms studied. Chromatographic studies have failed to demonstrate the existence in yeast extract of more than two forms of biotin, namely free biotin and biocytin.

A biocytin concentrate has been furnished to Dr. Lichstein who has reported(8) that biocytin is inactive in stimulating the aspartic acid deaminase activity of cells that have been exposed to pH 4 M phosphate buffer. Studies reported in this paper with crystalline biocytin demonstrate that under the experimental conditions recently described(6) biocytin may be shown to have activity. The activity of biocytin has varied with various bacterial preparations used from essentially no activity to activity equal to that shown by biotin itself.

Experimental. Procedures that have been employed in the aspartic acid deaminase experiments have been described in detail(6). The biocytin used was crystalline material (7) and contained no free biotin by microbiological assay with *Lactobacillus arabinosus* (9).

Results and discussion. The results that have been obtained are summarized in Table

1. Lichstein, H. C., and Umbreit, W. W., *J. Biol. Chem.*, 1947, v170, 423.

2. Lichstein, H. C., and Christman, J. F., *J. Biol. Chem.*, 1948, v175, 649.

3. Lichstein, H. C., *J. Biol. Chem.*, 1949, v177, 125.

4. Lichstein, H. C., *J. Biol. Chem.*, 1949, v177, 487.

5. Lichstein, H. C., and Christman, J. F., *J. Bact.*, 1949, v58, 565.

6. Wright, L. D., Cresson, E. L., and Skeggs, H. R., *Proc. Soc. Exp. Biol. and Med.*, 1949, v72, 556.

7. Wright, L. D., Cresson, E. L., Skeggs, H. R., Wood, T. R., Peck, R. L., Wolf, D. E., and Folkers, K., *J. Am. Chem. Soc.*, 1950, v72, 1048.

8. Lichstein, H. C., Christman, J. F., and Boyd, W. L., *J. Bact.*, 1950, v59, 113.

9. Wright, L. D., *Biological Symposia*, 1947, v12, 290.

TABLE I.
 Summary of Aspartic Acid Deaminase Experiments.

Exp. No.	Organism	pH of deaminase reaction	Ammonia N found per tube		
			No supplement, γ	Biotin, γ	Biocytin, γ
1	<i>Proteus vulgaris</i>	7	9.	13.6 (.001 γ)	—
2	" "	7	5.4	11.1 (.001 γ)	—
3	" "	7	5.4	9.0 (.001 γ)	7.00 (.001 γ)
4	" "	7	3.7	6.6 (.001 γ)	4.5 (.001 γ)
5	" "	7	7.5	34.5 (.001 γ)	36.7 (.001 γ)
6	" "	7	5.8	31.0 (.0001 γ)	28.5 (.0001 γ)
				28.5 (.001 γ)	26.3 (.001 γ)
7	" "	7	11.	20.5 (.00001 γ)	18.7 (.00001 γ)
				33.0 (.0001 γ)	31.5 (.0001 γ)
8	" "	7	17.2	29.0 (.001 γ)	25.8 (.001 γ)
9	" "	4	0.	0. (.001 γ)	0. (.001 γ)
10	<i>Escherichia coli</i>	7	10.8	11.8 (.001 γ)	15.2 (.001 γ)

Figures in parentheses indicate micrograms of biotin or biocytin calculated as biotin per tube.

I. As previously reported, it has been impossible to demonstrate stimulation of aspartic acid deaminase with biotin at pH 4. At pH 7 activity is demonstrable with both biotin and biocytin. There is a suggestion that biocytin is less active than biotin in those instances where relatively small amounts of ammonia are produced with biotin (see Exp. 3 and 4). In those instances where relatively large amounts of ammonia are produced with biotin, biocytin has activity equal to that of biotin (see Exp. 6 and 7). Thus it would appear that the ability to utilize biocytin may be a function of the metabolic activity of the bacterial preparation employed.

In no experiment has biocytin shown sig-

nificantly greater activity in stimulating the aspartic acid deaminase system than that obtained with biotin. Since chromatographic data have failed to show the existence of more than 2 forms of biotin in yeast extract, these data would indicate that, if a highly active coenzyme for the aspartic acid deaminase system exists, biotin and/or biocytin is concerned with its formation rather than as one of its components.

Summary. Biocytin, like biotin, has been shown to be active in stimulating the aspartic acid deaminase system of bacterial cells that have been inactivated by exposure to pH 4 M phosphate buffer.

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