Biotin in the Bacterial Deamination of Certain Amino Acids. (17496)

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Lichstein and coworkers(1,2,3,4) have 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. In their experiments the deaminase activity of such inactivated cells could be restored with biotin. Subsequent investigators(5) have been unable with biotin to restore deaminase activity to bacterial cells similary treated and the fundamental observation that biotin somehow functions in deaminase activity has remained unconfirmed by any other group. Although certain discrepancies exist between the results obtained by Lichstein and those found in this laboratory, the activation of treated cells with biotin under certain defined conditions has been obtained, thus furnishing corroborative evidence for the involvement of biotin in the bacterial deamination of certain amino acids.

Procedure. Eighteen-hour cultures of the organisms used were obtained following growth at 37°C on a medium composed of 1% yeast extract (Difco), 1% tryptone (Difco), 0.5% disodium hydrogen phosphate, and 0.1% sodium formate. The medium was dispensed in 100 ml quantities in 250 ml Erlenmeyer flasks. The pH before autoclaving at 120°C for 15 minutes was 7.35. Following growth the organisms were centrifuged down rapidly (Servall angle centrifuge, plastic cups), resuspended in 50 ml of distilled water, and recentrifuged. The washed cells thus obtained were suspended in about 3 ml of M phosphate buffer of pH 4. Inactivation with respect to deaminase activity was accomplished on aliquots of this suspension. These aliquots were dispensed in open test tubes that usually were immersed in a water bath at 37°C. Thirty minutes was the usual inactivation time. Immediately following inactivation of the cells the deaminase reaction was carried out at 37°C by appropriate additions to the same tubes. A protocol for deamination at pH 7 is given in Table I. When the deamination reaction was carried out at pH 4, M phosphate buffer of pH 4 replaced the Na_3PO_4 solution and was used also as a solvent for the aspartic acid employed.

Following the deamination reaction at either pH enzymatic activity was stopped by the addition of 0.5 ml of 20% trichloroacetic acid to each tube. The tubes then were centrifuged until a completely clear supernatant was obtained. Ammonia formed in each tube was determined by adding to 2 ml of supernatant 2 ml of 10% NaOH and 1 ml of Nessler's reagent (gum ghatti stabilized). The color intensities were read in a Klett-Summerson colorimeter against a series of similarly prepared standard tubes of ammonium sulphate. The ammonia nitrogen found in tube 1 was subtracted from the results obtained in each other tube to give the ammonia nitrogen originating from the deamination of aspartic acid by the bacterial preparations used.

Results. The results obtained in a series of experiments are summarized in Table II. These data confirm those of Lichstein to the extent that they show that cells of *Proteus vulgaris* or *Escherichia coli* can be obtained with a reduced ability to deaminate aspartic acid 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 was 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

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TABLE I.
Protocol for Aspartic Acid Deaminase.

	No. 1, ml	No. 2, ml	No. 3, ml	No. 4, ml
Bacterial suspension in pH 4 M phosphate buffer	0.5	0.5	0.5	0.5
Na_3PO_4 (saturated)	0.5	—	—	—
Aspartic acid* in Na_3PO_4 (saturated)	—	0.5	0.5	0.5
H_2O	1.0	1.0	—	—
Biotin†	—	—	1.0	—
Yeast extract‡	—	—	—	1.0

* 266 mg dl-aspartic acid dissolved in 100 ml saturated Na_3PO_4. Calculated to give 0.005 M dl-aspartic acid in the final reaction mixture.
† 0.0001 γ or 0.001 γ d-biotin/ml.
‡ 0.1 mg or 1.0 mg yeast extract/ml.

TABLE II.
Summary of Aspartic Acid Deaminase Experiments.

Exp. No.	Organism*	pH of deaminase reaction	Ammonia nitrogen formed: No supplement, γ	Biotin, γ	Yeast extr., γ†
1	*Proteus vulgaris*	7	11.7	15.8 (0.001 γ)	15.1 (1 mg)
2	" "	7	7.0	14.8 (0.001 γ)	13.7 (1 mg)
3	" "	7	4.3	15.9 (0.001 γ)	17.3 (1 mg)
4	" "	7	2.3	11.3 (0.0001 γ)	2.3 (0.1 mg)
5	" "	7	2.3	16.1 (0.0001 γ)	5.4 (0.1 mg)
6	" "	4	0.0	0.0 (0.001 γ)	0.0 (1 mg)
7	" "	4	0.6	0.6 (0.001 γ)	0.0 (1 mg)
8	" "	4	0.1	0.5 (0.0001 γ)	1.0 (0.1 mg)
9	*Escherichia coli* (Gratia)	4	0.0	0.0 (0.001 γ)	0.0 (1.4 mg)
10	*Escherichia coli* (Gratia)	4	3.0	3.0 (0.001 γ)	0.0 (1.4 mg)

* All bacterial preparations were washed with water and inactivated by exposure for 30 minutes at 37°C to M phosphate buffer of pH 4 except in experiments 7, 9 and 10, where inactivation was at 25°C, and in experiment 10, where in addition to inactivation at the lower temperature the cells also were unwashed.
† 1 mg of yeast extract contains after vigorous acid hydrolysis prior to microbiological assay about 0.001 γ of biotin.

unsuccessful. When the amounts of biotin and yeast extract used to stimulate deamination at pH 7 are titrated downward, contrary to Lichstein who worked at pH 4, biotin was found to be more active in the system than is an equivalent of biotin as it occurs in yeast extract.

Despite the fact that inactivation of bac-

terial deaminase by exposure to molar phosphate buffer at pH 4 is not yet understood, is not always consistent, and doubtlessly is influenced by a variety of factors, these data afford confirmation that biotin somehow is concerned in activation of the system. Further study of the variables involved during inactivation and reactivation of the deaminase system is indicated before final conclusions can be drawn concerning the significance of biotin in these reactions.

Summary. Inactivation of bacterial aspartic acid deaminase activity by exposure of cells to pH 4 M phosphate buffer and its reactivation with biotin has been confirmed.

Received October 6, 1949. P.S.E.B.M., 1949, v72.

Binding of Phenolsulfonephthalein by Tissues and the Effect of Denaturation Thereon.* (17497)

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The present experiments were conducted to study (1) the binding of the organic anionic dye phenolsulfonephthalein (PSP) by homogenates of several tissues of the rat; (2) the effect of denaturation of tissue proteins on this binding; and (3) the competitive effect of native and denatured tissues on the binding of PSP by crystallized bovine plasma albumin. It has been previously established that certain proteins can form complexes with organic anions on both the acid and basic side of the isoelectric point. De Haan(1) and Marshall and Vickers(2) demonstrated that the anion PSP was bound appreciably by serum proteins and Grollman(3) showed that at the pH of normal blood the binding was primarily by serum albumin, the globulins binding very little dye. Klotz and Urquhart(4) showed that anions are also bound to β-lactoglobulin, hemocyanin and α_2 globulin, although to a much smaller extent than occurs with serum albumin. Ohta(5) working with tissue brei preparations presented evidence that both anionic and cationic dyes were adsorbed by tissue proteins. Denaturation of serum albumin by heat(6,7) or urea(7) abolishes the binding capacity for oleate and methyl orange anions. A different effect occurs with egg albumin in that heat denaturation increases the binding of alkylbenzenesulfonate detergents(8) and methyl orange.(7)

Experimental. Albino rats were exsanguinated by cardiac puncture or decapitation and weighed samples of the tissues were homogenized for approximately two minutes in M/15 phosphate buffer, pH 6.5. In the experiments, measured volumes containing 250-500 mg of the original tissue together with 1.0 ml of a solution of PSP in phosphate buffer pH 7 were placed in a cellophane bag 15 cm long and 1.5 cm in diameter. The samples were dialyzed at 5°C against M/15 phosphate buffer pH 6.5 in flasks which were slowly rotated for 40 hours to reach equilibrium; the total volume of the system was 100 ml. The bound PSP was calculated from the concentration of free PSP in the dialysate

* This investigation was supported by grants from Mr. Sewell L. Avery, from the American Cancer Society recommended by the Committee on Growth of the National Research Council, and from the Committee for Research in Problems of Sex of the National Research Council.

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