COMMUNICATIONS TO THE EDITOR

THE FORMATION OF S-ADENOSYLHOMOCYSTEINE IN ENZYMATIC TRANSMETHYLATION REACTIONS[1]

Sir:

It has been assumed for many years that homocysteine might be formed biologically in transmethylation reactions from methionine.[2] Recent studies have shown that enzymatic activation is a prerequisite to the transfer of the methyl group of methionine.[3] The product of this enzymatic activation is S-adenosylmethionine (AMe).[4,5] It follows, therefore, that adenosylhomocysteine, S-(5′-desoxy-adenosine-5′)-homocysteine, rather than homocysteine should be the primary product resulting from transmethylations involving methionine.[6,7] This deduction has now been verified experimentally and the present communication describes the enzymatic preparation of adenosylhomocysteine.

The enzyme GA methylpherase,[8] which catalyzes the reaction

$$GA + AMe \xrightarrow{GSH} ASR + Creatine + H^+$$

was used for the preparation of adenosylhomocysteine. Guanidinoacetic acid (120 μm.) and AMe (90 μm.) labeled with S^{35} were incubated for 150 minutes in phosphate buffer (0.05 M, pH 7.4) and BAL (0.002 M) with 70 units (80 mg.) of a partially purified preparation of GA methylpherase. The incubation was terminated by addition of trichloroacetic acid. Unreacted AMe was removed from the protein filtrate by precipitation with ammonium reineckate. After removal of the excess reineckate, the supernatant containing ASR, was made 0.02/N with respect to HCl and passed through a small column of Norite A (500 mg.); the Norite was then washed with 100 ml. of water. The filtrate and washings which contained no radioactivity were discarded. The Norite column was then eluted with four 50-ml. portions of aqueous pyridine (10%). The first two eluates, which contained over 90% of the total counts, were pooled, freed from pyridine and concentrated under reduced pressure. This material in addition to ASR contained creatinine, guanidinoacetic acid and traces of pyridine. These contaminants were removed by descending chromatography on Whatman no. 1 paper (solvent system EtOH, acetic and water, 75:5:20).

Examination of the chromatogram revealed only one area exhibiting radioactivity, ultraviolet absorbency and ninhydrin positive reaction. This area was cut out, and eluted with water. On the basis of adenine content 29 μm. of purified ASR was obtained. The purified material behaved as a single substance when chromatographed with several different solvents; it had an ultraviolet absorption spectrum characteristic for adenine nucleosides, with a maximum at 260 mμ; for each mole of adenine it contained one mole of pentose; it gave a positive reaction with the ninhydrin test and with the nitroprusside test for methionine.[9] Finally, the purified material was found to be identical with synthetic adenosylhomocysteine, the preparation of which will be described in a separate communication.[10]

The behavior of ASR in various biological systems is under investigation in this laboratory.

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(1) Aided by grants from the Williams–Waterman Fund and the American Cancer Society.

(2) L. W. Butz and V. du Vigneaud, *J. Biol. Chem.*, **99**, 135 (1932).

(3) G. L. Cantoni, in W. D. McElroy and B. Glass, "Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., 1952, Vol. 2, p. 129.

(4) These abbreviations will be used: AMe for S-adenosylmethionine, *i.e.*, active methionine; ASR, S-adenosylhomocysteine; ATP; adenosinetriphosphate; GSH, reduced glutathione, GA, guanidinoacetic acid; BAL, British Anti-Lewisite, *i.e.*, Dimercaptopropanol.

(5) G. L. Cantoni, *J. Biol. Chem.*, **204**, 403 (1953).

(6) J. Baddiley, G. L. Cantoni and G. A. Jamieson, *J. Chem. Soc.*, 2662 (1953).

(7) D. W. Woolley, *Nature*, **171**, 323 (1953).

(8) G. L. Cantoni and P. T. Vignos, Jr., *J. Biol. Chem.*, **209**, 647 (1954).

(9) T. E. Mcarthy and M. X. Sullivan, *ibid.*, **141**, 871 (1941).

(10) J. Baddiley and G. A. Jamieson, in press.

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FLAVIN MONONUCLEOTIDE-DEPENDENT MENADIONE REDUCTASE COUPLED TO CYTOCHROME C REDUCTION IN CELL-FREE EXTRACTS OF *Achromobacter fischeri*[1]

Sir:

A menadione reductase linked to DPN[2] has been demonstrated by Wosilait and Nason[3] in cell-free extracts of *E. coli* and other organisms including *Achromobacter fischeri*. These workers were unable to demonstrate a flavin requirement with their system. A requirement for FAD or FMN in the menadione reductase system of cell-free extracts of *Streptococcus faecalis* has been demonstrated by M. I. Dolin (personal communication). We observed that both cytochrome c and menadione in small amounts will inhibit cell-free bacterial luminescence, the results suggesting that menadione might mediate hydrogen transfer between flavoprotein and cytochrome c. Since $FMNH_2$ is required for bacterial luminescence,[4,5] the nature of cytochrome c and menadione inhibition could be a competition with the luminescent pathway for the hydrogens of the flavoprotein. The function of menadione as proposed would be important because of the vitamin K

(1) Work performed under United States Atomic Energy Commission Contract No. W-7405-eng-26.

(2) DPN = diphosphopyridine nucleotide; FAD = flavin adenine dinucleotide; FMN = flavin mononucleotide; $FMNH_2$ = reduced flavin mononucleotide; BAL = British antilewisite (2,3-dimercaptopropanol).

(3) W. D. Wosilait and A. Nason, *J. Biol. Chem.*, **208**, 785 (1954).

(4) B. L. Strehler, E. N. Harvey, J. J. Chang and M. J. Cormier, *Proc. Natl. Acad. Sci. U.S.*, **40**, 19 (1954).

(5) W. D. McElroy, J. W. Hastings, V. Sonnenfeld and J. Coulombre, *Science*, **118**, 385 (1953).

activity of this compound in man and animals[6] and its possible relation to Slater's factor.[7]

In ammonium sulfate-fractionated extracts of *A. fischeri*,[8] the menadione reductase activity was present chiefly in the 30–40% saturated fraction. With this preparation it was observed that the reaction required FMN and that FAD was only slightly active (Table I). The specificity for FMN is in contrast to the *S. faecalis* menadione reductase system of Dolin.

TABLE I

EFFECT OF FLAVINS ON THE *A. fischeri* MENADIONE REDUCTASE SYSTEM

Reaction components: 1.0 ml. tris-(hydroxymethyl)-aminomethane (0.1 *M*), *p*H 8.2; 200 μg. $DPNH_2$; 25 μg. menadione; 29 μg. FMN; 50 μg. FAD; 0.05 ml. enzyme; final vol., 3.0 ml.; components incubated with enzyme for 5 min. at room temp. before $DPNH_2$ addition. The $-\Delta E_{340}$ was measured.

Reaction components	Enzyme units[a]
Enzyme	12
Enzyme + FAD	32
Enzyme + FMN	240
Enzyme + FMN (no menadione added)	26

[a] One enzyme unit = amount of enzyme which gives a change in log I_0/I of 0.001 per min. calculated from the change between 15- and 45-sec. readings.

Crude cell-free extracts of *A. fischeri* reduced cytochrome c at a relatively rapid rate but only in the presence of menadione. The purified enzyme, however, required the addition of FMN before a significant increase in the rate of cytochrome c reduction was observed (Table II).

TABLE II

EFFECT OF FMN AND MENADIONE ON CYTOCHROME C REDUCTION IN *A. fischeri*

Reaction components: 1.0 ml. tris-(hydroxymethyl)-aminomethane (0.1 *M*), *p*H 8.2; 200 μg. $DPNH_2$; 25 μg. menadione; 90 μg. FMN; 0.2 ml. cytochrome c (2×10^{-4} *M*); 0.06 ml. crude and 0.02 ml. purified enzyme [30–40% $(NH_4)_2SO_4$ fraction] used; final vol., 3.0 ml.; components incubated for 5 min. at room temp. before addition of $DPNH_2$. The $+\Delta E_{550}$ was measured.

Reaction components	Enzyme units
Crude enzyme	4
Crude enzyme + menadione	76
Purified enzyme + FMN	16
Purified enzyme + menadione	8
Purified enzyme + menadione + FMN	172

A cell-free extract of *S. faecalis*[9] from which cytochrome c reductase activity had been removed by ammonium sulfate fractionation and adsorption on calcium phosphate gel and which contained menadione reductase reduced cytochrome c at a rapid rate after the addition of menadione. Explanation of this involves two possibilities: (a) that menadione is the "prosthetic group" of cytochrome c reductase, or (b) that the product of menadione reductase reaction enzymically or spontaneously reduces cytochrome c. The latter hypothesis is favored by the observation that reduced menadione rapidly reduces cytochrome c non-enzymically. Boiled crude extracts of *S. faecalis* did not reactivate the purified extract.

A. fischeri menadione reductase is inhibited by BAL, while both BAL and $FMNH_2$ reverse menadione and cytochrome c inhibition of bacterial luminescence. It seems likely that the menadione reductase and luminescent systems are competing with each other.

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(6) S. Ansbacher and E. Fernholz, THIS JOURNAL, **61**, 1924 (1939).
(7) E. C. Slater, *Biochem. J.*, **46**, 484 (1950).
(8) B. L. Strehler and M. J. Cormier, *Arch. Biochem. and Biophys.*, **47**, 16 (1953).
(9) M. I. Dolin, manuscript in preparation.

PYRIDOXAL PHOSPHATE AND METAL IONS AS COFACTORS FOR HISTIDINE DECARBOXYLASE

Sir:

In contrast to other amino acid decarboxylases, histidine decarboxylase has not been resolved into apoenzyme and coenzyme.[1,2] The failure to detect pyridoxal phosphate (PLP) in purified cell-free preparations of histidine decarboxylase, the comparative insensitivity of this enzyme to inhibition by ferrous ions or carbonyl-trapping reagents, and the fact that histidine decarboxylase (in contrast to ornithine decarboxylase) was not reduced in amount in cells of a *Lactobacillus* grown with reduced amounts of vitamin B_6, have led to the view that this enzyme may not require PLP for activity.[3,4,5] However, long incubation of resting cells of *Escherichia coli* with pyridoxine does increase their histidine decarboxylase activity.[6] This uncertainty concerning the role of PLP in the activity of histidine decarboxylase stimulated the following investigation.

Lactobacillus 30a of Rodwell,[5,7] proved to require vitamin B_6 for growth in a defined medium containing D-alanine[8]; however, at minimal levels of vitamin B_6, the histidine decarboxylase activity per mg. of cells obtained was less than 4% of that of cells grown with optimal levels of the vitamin (Table I). It should be noted that the concentration of vitamin B_6 required for production of an active ornithine decarboxylase is very much higher.

Cells from a complete medium were sonically disintegrated and the fraction soluble in 45% ammonium sulfate but insoluble in 55% ammonium sulfate separated. On the basis of protein content, this fraction was several hundred times more active than intact cells in decarboxylating histidine. Dialysis of this fraction at room temperature for 24 hours against 0.2 *M* acetate buffer, *p*H 3.8, resulted in almost complete loss of enzymatic activity. Addition of a boiled cell suspension restored 60 to 100% of the initial activity; PLP additions gave erratic results (A and B, Fig. 1), higher amounts frequently failing to show any reactivating

(1) O. Schales, "The Enzymes," Vol. II, Part A, J. B. Sumner and K. Myrback, Academic Press, New York, N. Y., 1951, p. 222.
(2) E. E. Snell, *Physiol. Revs.*, **33**, 509 (1953).
(3) E. S. Taylor and E. F. Gale, *Biochem. J.*, **39**, 52 (1945).
(4) H. M. R. Epps, *ibid.*, **39**, 42 (1945).
(5) A. W. Rodwell, *J. Gen. Microbiol.*, **8**, 233 (1953).
(6) E. Werle and W. Koch, *Biochem. Z.*, **319**, 305 (1949).
(7) A. W. Rodwell, *J. Gen. Microbiol.*, **8**, 224 (1953).
(8) The medium is similar to that used by Craig and Snell (*J. Bact.*, **61**, 283 (1951)). A more detailed account of the nutrition of this organism will appear later.