An enzymically catalyzed incorporation of amines into proteins*

In experiments reported some years ago the enzyme-catalyzed exchange of the amide group of glutamine and asparagine with ^{15}N ammonia was demonstrated but an exchange of the amide groups of protein bound dicarboxylic amino acids could not be explored owing to the unfavorable experimental conditions[1]. Recently the problem of the metabolism of protein-amide groups was approached again with the aid of ^{14}C-labeled amines, such as ethanolamine or cadaverine. Soluble protein fractions obtained from sucrose homogenates (0.25 *M*) of the livers of guinea pigs, rats, mice, and rabbits by centrifugation at 100,000 × *g* were incubated in the presence of Ca^{++} with the ^{14}C-labeled amines. Appreciable radioactivity was recovered in the proteins precipitated and purified by the method of SIEKEVITZ[2] (Table I). The incorporating system was activated by Ca^{++} but not by Mn^{++} and Mg^{++} or other metal ions—a fact which makes it unlikely that we are dealing with the enzyme system responsible for glutamo or asparto-transferase activities.

TABLE I

INCORPORATION OF ^{14}C-AMINES AND LYSINE INTO SOLUBLE PROTEIN FRACTIONS OF GUINEA PIG LIVER

Counts/min at infinite thickness

(1) ^{14}C-Amines added	Ethanolamine		Cadaverine		Lysine	
(2) Protein fraction	SP	PP	SP	PP	SP	PP
(3) No Ca^{++}	22	530	25	460	23	420
(4) With Ca^{++}	480	5000	690	7270	470	5000
(5) (4) + spermine (4 μmoles)	300	2420	360	3100	330	2500
(6) (4) + hydroxylamine (4 μmoles)	40	120	30	100	50	80
(7) (4) + putrescine (4 μmoles)	270	3060	300	3950	290	3260
(8) (4) + $(NH_4)^+$ (8 μmoles)	300	3900	340	5000	330	4700

The incubation mixture (1 ml) contained 4 μmoles ^{14}C-amine or ^{14}C-lysine; 20 μmoles $CaCl_2$; 20 μmoles tris(hydroxymethyl)aminomethane, pH 8.0 and 10–12 mg soluble protein (SP) or about 1 mg purified protein (PP). Incubation 1 h at 37°. Incubation was terminated by addition of trichloroacetic acid. Added ^{14}C-lysine and ^{14}C-cadaverine counted 290,000 and 340,000 counts/min at infinite thickness, respectively. ^{14}C-Ethanolamine counted approximately 240,000 counts/min. All protein samples were counted at infinite thickness (11 mg). For counting of experiments with PP, 10–11 mg inert protein was added at the termination of incubation.

BORSOOK *et al.*[3] and SCHWEET[4] have described an enzymically catalyzed incorporation of ^{14}C-lysine into a soluble liver protein fraction. This incorporating system is likewise activated by Ca^{++} and may be identical with the enzyme system active in the incorporation of amines.

Under the conditions of our experiments the soluble liver protein fraction incorporated ^{14}C-lysine as well as ^{14}C-ethanolamine and ^{14}C-cadaverine. The experiments were carried out in tris buffer since phosphate buffer proved to be a strong inhibitor of the incorporation of amines. The pH optimum for the incorporation of the amino acid was found to be 6.6 while that for incorporation of the amines was 8.0 to 8.5. The soluble protein fractions of liver, kidney, and brain of guinea pigs show enzymic activity in incorporating cadaverine in decreasing order. The active protein fraction from guinea pig liver was purified about 10–12 times by ammonium sulfate fractionation and adsorption on calcium phosphate gel and isoelectric precipitation. The purified protein fraction incorporated ^{14}C-cadaverine to an extent of 1 to 2% of the total protein. While nothing definite can be said at present as to the linkage of ^{14}C-cadaverine to the protein, a major portion of the ^{14}C-cadaverine was recovered as mono-dinitrophenyl-cadaverine after hydrolysis of the protein treated with dinitrofluorobenzene.

Inhibition of cadaverine incorporation by other amines (Table I) suggests competition by a variety of amines for the active sites of the protein. Mescaline (4 μmoles) was not inhibitory, a finding of some interest, since it has been reported that it is incorporated into liver proteins[5].

The occurrence of enzyme systems incorporating biologically occurring amines (and diamino acids) raises the interesting question as to whether or not the enzyme specificity is restricted to aliphatic amines. The findings also open the way to a test whether or not this enzymic reaction,

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if occurring *in vivo*, could produce modified body proteins which might interfere with enzymic activities or cell permeability, or might possess antigenic properties.

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1 H. WAELSCH, P. OWADES, E. BOREK, N. GROSSOWICZ AND M. SCHOU, *Arch. Biochem.*, 27 (1950) 237.
2 P. SIEKEVITZ, *J. Biol. Chem.*, 195 (1952) 549.
3 H. BORSOOK, E. L. DEASY, A. J. HAAGEN-SMIT, G. KEIGHLEY AND P. H. LOWY, *J. Biol. Chem.*, 184 (1950) 529.
4 R. SCHWEET, *Federation Proc.*, 15 (1956) 350.
5 W. BLOCK, *Z. physiol. Chem.*, 296 (108) 1954.

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A natural guanine-containing analogue of vitamin B_{12}

A preparation containing two vitamin B_{12} analogues has been obtained from the fermentation broths of a *Nocardia* strain isolated in our laboratory[1].

The extraction from the microorganisms was performed by autoclaving a cell suspension supplemented with cyanide. The substances under investigation have been separated from vitamin B_{12} and factor B, likewise present in fermentation broths, and purified by a procedure involving: adsorption on charcoal, elution in *iso*propanol–water, removal of vitamin B_{12} as dicyanidocomplex in benzyl alcohol, adsorption on ion-exchangers (Amberlite IRC-50, Amberlite IRA-401, Dowex 1) and electrophoresis in a column of Whatman cellulose powder[2]. The eluate from the cellulose column was concentrated *in vacuo* and the red substances were precipitated by acetone addition.

The cobalt content of the product, dried over $CaCl_2$ *in vacuo* at room temperature, was found to be 3.2%; two atoms of phosphorus and one molecule of ribose are present per atom of cobalt.

No benziminazole derivatives were found after hydrolysis by 6*N* HCl. Hydrolysis by 2*N* HCl at 100° for 2–4 h sets free a purine whose ultra-violet spectrum and chromatographic behavior are similar to those of guanine. Differential spectrophotometry of the hydrolysate in acid and alkaline solutions and FOLIN-CIOCALTEU's reaction show that the preparation contains one molecule of guanine per molecule of ribose.

The absorption spectrum of aqueous solutions of the product has maxima at 273, 320, 356, 500, 530 mμ over a wide range of pH values (from 1 to 10). By addition of cyanide to neutral and alkaline solutions the maxima are shifted to 276, 307.5, 367.5, 540 and 580 mμ.

Paper chromatography in butanol–acetic acid–water and paper electrophoresis in 0.5*N* acetic acid followed by bioautography[3] revealed the presence of two growth factors for *E. coli* 113/3. Most of the activity was due to an electronegative component showing an R_F value relative to vitamin B_{12} of about 0.12. The other factor, which is present in smaller amount, has an R_F of about 0.27 and behaves like vitamin B_{12} in electrophoresis. Deamination by HNO_2 apparently does not affect the latter component while the former is changed into a more electronegative growth factor.

A purine, not distinguishable from xanthine in its chromatographic behavior and ultra-violet absorption spectrum, has been found after hydrolysis of the deaminated mixture by 2*N* HCl.

Therefore we may conclude that the preparation consists mainly of a guanine-containing analogue of vitamin B_{12}. The chromatographic and electrophoretic properties of this substance are closely similar to those described for factor C[3].

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1 A. DI MARCO, C. G. ALBERTI, G. BORETTI, M. GHIONE, A. MIGLIACCI AND C. SPALLA, in H. C. HEINRICH, *Vitamin B_{12} und Intrinsic Faktor*, Enke Verlag, 1957, p. 55.
2 H. DELLWEG, E. BECHER AND K. BERNHAUER, *Biochem. Z.*, 328 (1956) 88.
3 J. E. FORD, E. S. HOLDSWORTH AND S. K. KON, *Biochem. J.*, 59 (1955) 86.

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