Proceedings of the

NATIONAL ACADEMY OF SCIENCES

Volume 44 · Number 12 · December 15, 1958

*PROTEIN BIOSYNTHESIS BY A CELL-FREE BACTERIAL SYSTEM. II. FURTHER STUDIES ON THE AMINO ACID INCORPORATION ENZYME**

BY MIRKO BELJANSKI† AND SEVERO OCHOA

DEPARTMENT OF BIOCHEMISTRY, NEW YORK UNIVERSITY COLLEGE OF MEDICINE, NEW YORK, NEW YORK

Communicated October 27, 1958

INTRODUCTION

The incorporation of amino acids into the protein of particulate preparations of *Alcaligenes faecalis* is stimulated by an enzyme present in the supernatant extract. This enzyme, which was isolated in highly purified form and is distinct from the known amino acid–activating enzymes catalyzing the amino acid–dependent exchange of PP^{32} with ATP,[1] appears to be concerned with some phase of protein biosynthesis.[2] The enzyme was found in extracts of *A. faecalis*, *Azotobacter vinelandii*, and *Escherichia coli*. Pending elucidation of its nature and mode of action, the new enzyme is referred to as the "amino acid incorporation enzyme."

Preliminary experiments, to be reported in this paper, indicate that the amino acid incorporation enzyme is involved in protein biosynthesis in mammalian as well as in bacterial cells. Thus (*a*) there are indications that the incorporation enzyme is present in liver extracts, and (*b*) highly purified preparations of the *A. faecalis* enzyme completely replace the liver supernatant or pH 5 enzymes of Hoagland *et al.*[3] in stimulating the incorporation of amino acids into protein of rat liver microsomes. We further wish to report that highly purified preparations of the *A. faecalis* incorporation enzyme catalyze a Mg^{++}-dependent exchange of radioactive ADP with ATP, a reaction which appears to be related to their amino acid incorporation activity. This finding may be of significance in view of the fact that glutathione synthetase, an enzyme involved in the synthesis of a typical peptide, catalyzes a similar exchange of ADP with ATP.[4]

MATERIALS AND METHODS

The preparation of *A. faecalis* particles, determination of the incorporation of C^{14}-labeled amino acids into protein, and purification of the amino acid incorporation enzyme have been previously described.[2] Microsomes, supernatant fluid, and pH 5 enzymes were prepared from the livers of young rats (80–100 gm.) as described by Keller and Zamecnik.[5] For measurement of the exchange of C^{14}-labeled ADP with ATP, samples were prepared and incubated as described in the legend to Table 3. The reaction was stopped by addition of 0.05 ml. of 50 per cent trichloroacetic

acid, and aliquots of the protein-free supernatant fluid were chromatographed in the isobutyric acid–ammonia system.[6] The ADP and ATP spots were located with an ultraviolet lamp, cut out, and eluted with 1.0 N HCl. The eluates were dried and their radioactivity measured with an end-window counter. The per cent exchange was calculated according to De Moss and Novelli.[7] 8-C^{14}-ADP was obtained from the Schwarz Laboratories, Mount Vernon, New York. GDP and crystalline ATP were purchased from the Sigma Chemical Company, St. Louis, Missouri. C^{14}-labeled amino acids were obtained from the New England Nuclear Corporation, Boston, Massachusetts. Phosphoenolpyruvate and crystalline pyruvic kinase were purchased from Böhringer and Sons, Mannheim, Germany.

RESULTS

Amino Acid Incorporation Enzyme in Rat Liver Supernatant.—As shown in the previous paper,[2] amino acid incorporation enzyme preparations, free of conventional amino acid–activating enzymes, stimulate the incorporation of C^{14}-labeled amino acids into the protein of washed *A. faecalis* particles. Table 1 shows that rat liver supernatant, the pH 5 enzymes derived from it, and the supernatant extract from *A. faecalis* cells produce the same degree of stimulation of incorporation of amino acids into protein of washed *A. faecalis* particles. These results strongly suggest that the liver fractions contain amino acid incorporation enzyme. However, as previously noted for the *A. faecalis* extract, part of the activity of the liver fractions is not destroyed by heat (Table 1) and is, presumably, non-enzymatic in nature.

TABLE 1

EFFECT OF RAT LIVER SUPERNATANT OR pH 5 ENZYMES ON INCORPORATION OF C^{14}-LABELED AMINO ACIDS INTO PROTEIN OF WASHED *A. fæcalis* PARTICLES*

PARTICLES	ADDITIONS	LABELED AMINO ACID INCORPORATED INTO PARTICLES (MILLIMICROMOLES PER MG. PROTEIN N OF PARTICLES PER HOUR)		
		Glycine	D,L-Valine	D,L-Phenylalanine
Unwashed	None	26	..	..
Washed	None	13	5	21
	Heated liver supernatant (100 mg. protein)	17	..	..
	Liver supernatant (50 μg. protein)	19	..	..
	Liver supernatant (100 μg. protein)	21	10	32
	Liver supernatant (200 μg. protein)	22	..	..
	Alcaligenes supernatant (100 μg. protein)	23	..	..
	Heated pH 5 enzymes (100 μg. protein)	17	..	..
	pH 5 enzymes (50 μg. protein)	20	..	..
	pH 5 enzymes (100 μg. protein)	22	9	30

* The samples contained: Tris buffer, pH 7.0, 50 μmoles; $MgCl_2$, 5.0 μmoles; K_2HPO_4, 5.0 μmoles; C^{14}-labeled amino acid (specific radioactivity, 80,000–124,000 cpm per μmole), 0.25 μmole; and *A. fæcalis* particles with 300 μg. of protein. Rat liver supernatant, pH 5 enzymes, or *A. fæcalis* supernatant added as indicated. Final volume, 1.0 ml. Incubation in air, 1 hour at 32°.

Effect of Incorporation Enzyme on Amino Acid Incorporation by Rat Liver Microsomes.—Table 2 shows, in agreement with the results of previous workers, that liver pH 5 enzymes strongly stimulate the incorporation of C^{14}-leucine into the protein of rat liver microsomes. This effect has been ascribed to the amino acid–activating enzymes present in this preparation. However, as further shown in Table 2, small amounts (10 μg. of protein) of purified *A. faecalis* incorporation enzyme, free of amino acid–activating enzymes, were as effective as the pH 5 enzymes (1.5 mg. of protein) in stimulating the incorporation of leucine into microsomal protein. Contrary to the *A. faecalis* particles, in which the ATP required for amino acid

incorporation is generated by oxidative phosphorylation,[2] the microsome system functions anaerobically, ATP being provided as such and regenerated from ADP by transfer of phosphate from phosphoenolpyruvate in the presence of pyruvic kinase. Therefore, the results of Table 2 reinforce previous evidence[2] that the action of the amino acid incorporation enzyme is not due to an effect on oxidative phosphorylation.

TABLE 2

EFFECT OF AMINO ACID INCORPORATION ENZYME ON INCORPORATION OF C^{14}-LEUCINE INTO PROTEIN OF RAT LIVER MICROSOMES*

Microsomes		C^{14}-Leucine Incorporated (cpm per mg. Microsomal Protein)
Unwashed	None	20
	Heated pH 5 enzymes	20
	Heated incorporation enzyme	21
	pH 5 enzymes	95
	Incorporation enzyme	98
Washed†	None	12
	Heated pH 5 enzymes	13
	Heated incorporation enzyme	11
	pH 5 enzymes	58
	Incorporation enzyme	59

* The samples contained; D,L-C^{14}-leucine, 0.2 μmole (144,000 cpm); ATP, 1.0 μmole; GDP, 0.25 μmole; phosphoenolpyruvate, 10 μmoles; pyruvic kinase, 50 μg.; and microsomal suspension (in a solution [Keller and Zamecnik, *J. Biol., Chem.*, 221 45, 1956] containing 0.35 *M* sucrose, 0.035 *M* $KHCO_3$, 0.025 *M* KCl, and 0.004 *M* $MgCl_2$) with 4.0 mg. of protein. Other additions: pH 5 enzymes with 1.5 mg. of protein; *A. fæcalis* amino acid incorporation enzyme, specific activity (glycine) 24[2], with 10 μg. of protein. Final volume, 1.0 ml. Incubation in 95 per cent N_2–5 per cent CO_2, 20 minutes at 37°.

† Washed once with suspending solution.

ADP-ATP Exchange Reaction with Incorporation Enzyme.—Highly purified preparations of the amino acid incorporation enzyme have been found to catalyze a rapid exchange of C^{14}-ADP with ATP which is strictly dependent on the presence of Mg^{++} (Table 3). There is no change in the concentration of either nucleotide. The rate of this reaction is not influenced by the addition of a mixture of amino acids (total concentration 2×10^{-5} to 2×10^{-3} *M*) and there is no inhibition by 2,4-dinitrophenol (up to 2×10^{-4} *M*) or chloramphenicol (up to 0.1 mg. ml.). The enzyme preparations were free of polynucleotide phosphorylase, inorganic pyrophosphatase, and adenylic kinase (myokinase). No exchange of P^{32} or PP^{32} with ATP was obtained whether in the absence or presence of amino acids. The virtual absence of adenylic kinase (which catalyzes the reaction 2 ADP = ATP + AMP) was established through (*a*) the absence of formation of labeled AMP and ATP on incubation of the incorporation enzyme with C^{14}-ADP, in the presence of Mg^{++}, and (*b*) the lack of oxidation of DPNH on incubation of the enzyme with ATP, AMP, Mg^{++}, phosphoenolpyruvate, pyruvic kinase, and lactic dehydrogenase. Had adenylic kinase been present, phosphoenolpyruvate would have transferred P to ADP (formed from ATP + AMP), and the resulting pyruvate would have been reduced to lactate by DPNH. Exclusion of adenylic kinase is important because the action of this enzyme would lead to ADP-ATP exchange. Although the absence of other enzymes, such as nucleoside monophosphokinases other than adenylic kinase and nucleoside diphosphokinases, has not yet been excluded, there is a fact that strongly suggests a relationship between the stimulation of amino acid incorporation and the ADP-ATP exchange. As previously reported,[2] highly purified preparations of the incorporation enzyme are very unstable and lose most of their activity in about 2 weeks when stored at −18°. We find that the amino acid

incorporation and the ADP-ATP exchange activity of these preparations both disappear at about the same rate. If the exchange is indeed related to the amino acid incorporation activity, the enzyme might be concerned with some kind of activation reaction, since a Mg^{++}-dependent ADP-ATP exchange is also brought about, in the absence of any other reactants, by the phosphorylating enzyme[8] and, as already mentioned, by glutathione synthetase.[4] The former enzyme catalyzes the reaction of succinate with ATP and CoA to succinyl CoA, ADP, and P, while the latter catalyzes the reaction of γ-glutamylcysteine, glycine, and ATP to glutathione, ADP, and P. Glutathione synthetase, kindly provided by Dr. Konrad Bloch, Harvard University, failed to replace the amino acid incorporation enzyme for the incorporation of glycine, valine, or phenylalanine, by washed *A. faecalis* particles.

TABLE 3

EXCHANGE OF C^{14}-ADP WITH ATP*

EXPERIMENT No.	SYSTEM	INCUBATION TIME (MINUTES)	RADIOACTIVITY (cpm) ADP	RADIOACTIVITY (cpm) ATP	EXCHANGE Per Cent	EXCHANGE μMoles/mg. Protein/Hour
1	Complete	0	3,600	60	0	0
	Complete	5	2,900	720	39	594
	Complete	10	2,389	1,206	64	610
2	Complete	0	4,330	72	0	0
	Complete	5	3,520	830	37	552
	Complete	10	3,000	1,331	59	536
	No $MgCl_2$	10	4,350	62	0	0
	No enzyme	10	4,340	70	0	0
	No ATP	10	4,346	65	0	0

* The complete system contained: Tris buffer, pH 7.0, 50 μmoles; $MgCl_2$, 1.0 μmole; C^{14}-ADP, 1.0 μmole (4,500–9,000 cpm); crystalline ATP, 1.0 μmole; and freshly prepared *A. faecalis* amino acid incorporation enzyme, specific activity (glycine) 24 (experiment 1) or 21 (experiment 2), with 5 μg. of protein. Final volume, 0.5 ml. Incubation in air at 34°.

SUMMARY

1. Indications have been obtained for the presence of the amino acid incorporation enzyme in the supernatant fraction from rat liver and in the precipitate obtained by acidification of this fraction to pH 5.2 (pH 5 enzymes). Highly purified amino acid incorporation enzyme from *A. faecalis* completely replaces the pH 5 enzymes in stimulating the incorporation of C^{14}-leucine into protein of rat liver microsomes. These observations show that the amino acid incorporation enzyme is involved in protein biosynthesis in both mammalian and bacterial cells.

2. Highly purified preparations of the *A. faecalis* incorporation enzyme catalyze a rapid, Mg^{++}-dependent exchange of radioactive ADP with ATP an activity which appears to be related to their amino acid incorporation activity. This finding may be of significance, since glutathione synthetase, which catalyzes the synthesis of a typical peptide, brings about a similar exchange.

* Aided by grants from the National Institute of Arthritis and Metabolic Diseases (Grant A-1845) of the National Institutes of Health, United States Public Health Service, and the Rockefeller Foundation.

† Present address: Service de Biochimie Cellulaire, Institut Pasteur, 28 rue du Docteur Roux, Paris XV, France.

[1] Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine-5′-monophosphate; P, orthophosphate; PP, pyrophosphate; DPNH, reduced diphosphopyridine nucleotide; Tris, tris(hydroxymethyl)aminomethane; cpm, counts per minute.

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*ON THE SEPARATION OF THE TRYPTOPHAN SYNTHETASE OF ESCHERICHIA COLI INTO TWO PROTEIN COMPONENTS**

BY IRVING P. CRAWFORD† AND CHARLES YANOFSKY

DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD UNIVERSITY, STANFORD, CALIFORNIA

Communicated by E. L. Tatum, October 6, 1958

INTRODUCTION

Tryptophan synthetase (TSase), the enzyme catalyzing reaction (1),

$$\text{Indole} + \text{L-serine} \rightarrow \text{L-tryptophan}, \quad (1)$$

has been demonstrated in a variety of micro-organisms.[1] Recent studies with partially purified TSase preparations from *Neurospora crassa* have shown that this enzyme also acts on indoleglycerol phosphate (InGP), a precursor of tryptophan, according to reactions (2) and (3).[2]

$$\text{Indoleglycerol phosphate} \rightleftharpoons \text{indole} + \text{triose phosphate}, \quad (2)$$

$$\text{Indoleglycerol phosphate} + \text{L-serine} \rightarrow \text{L-tryptophan} + \text{triose phosphate}. \quad (3)$$

These studies further indicated that reaction (3) rather than reactions (1) plus (2) probably represents the principal physiological mechanism of tryptophan synthesis in *N. crassa*, since evidence was obtained excluding free indole as an intermediate in this reaction.

Investigations with *Escherichia coli* have shown that two proteins, designated components A and B, are required for the catalysis of reaction (2) and that one of these, component B, is also involved in reaction (1).[3] In view of these findings and the recent observations with preparations from *N. crassa*, a study of reactions (1), (2), and (3) in extracts of *E. coli* was undertaken. It was soon found that the same two separable protein components were required for the catalysis of all three reactions. This paper is concerned with the demonstration of the separability of these two proteins and with certain unusual features of their interaction.

MATERIALS AND METHODS

Organisms.—Two tryptophan auxotrophs of *E. coli* K-12 were used as enzyme sources, strains T-3 and T-8. Mutant T-3 is blocked in the synthesis of anthranilic acid and forms large amounts of TSase when grown on low levels of anthranilic acid.[4] It was considered equivalent to wild-type for the purposes of this study. Mutant T-8 responds to indole or tryptophan but not to anthranilic acid and