Incorporation of leucine into microsomal albumin by microsomes and pH-5 enzymes from normal rat liver

The uptake of [^{14}C]amino acids into microsomal protein by cell-free systems has been a subject of interest since the study of KELLER AND ZAMECNIK[1]. PETERS[2] demonstrated, using chick-liver slices, that the microsome is the main site of serum-albumin synthesis. CAMPBELL *et al.*[3] further noted [^{14}C]amino acid uptake into microsomal albumin in a system consisting of microsomes and cell sap of the regenerating rat liver. The present experiments were undertaken to elucidate the participation of amino-acid-activating enzymes, soluble RNA and the microsome system in albumin synthesis by normal liver cells.

Rat-liver pH-5 enzymes* and microsomes, prepared by the method of KELLER AND ZAMECNIK[1], were incubated with uniformly labeled [^{14}C]-L-leucine at 30° for 30 min with some modifications according to the method of LITTLEFIELD *et al.*[7]. After incubation, Peter's desoxycholate extraction[2] of the microsome fraction was carried out, and microsomal albumin was then precipitated from the dialyzed deoxycholate-soluble fraction by the addition of a suitable amount of rabbit antiserum** against the rat serum albumin***. The identification of the precipitate was made by the Ouchterlony's gel diffusion[9] and immuno-electrophoretic methods. The protein fractions were then subjected to purification and their specific activities were determined as reported in our previous papers [5,10,11]. The specific activity of microsomal albumin was corrected by the factor for the antigen-antibody ratio according to the method of HEIDELBERGER[8,10,11].

The results are summarized in Table I. It was of interest that GTP was indispensable for the incorporation of [^{14}C]leucine into microsomal albumin, together with ATP and an ATP-generating system. In agreement with the results of experiments by PETERS[2] with liver slices, the specific activity of microsomal albumin was considerably higher than that of the total microsome desoxycholate-soluble protein fraction, indicative of active albumin synthesis by this system. The requirement of pH-5 enzymes for the incorporation was demonstrated by the use of washed microsomes (Table I, B). The pre-incubation of the reaction mixture with a small amount of RNase resulted in a remarkable decrease in the incorporation (Table I, C). The fact that the pre-incubation of pH 5 enzymes with RNase followed by the precipitation at pH 4.7 resulted in marked inhibition of [^{14}C]leucine uptake into microsomal albumin, suggested the participation of soluble RNA in albumin synthesis (Table I, D). The further addition of the pH 5-supernatant fraction[6] increased the incorporation into both protein fractions, and its lability to heat suggested that it is enzymic in nature. The result is in agreement with recent investigations of ZAMECNIK *et al.*[6] and GROSSI *et al.*[12]. The 2,4-dinitrofluorobenzene treatment of the pooled microsomal albumin and ribonucleoprotein by the method of SANGER[13] indicated that [^{14}C]-leucine was not bound to the free amino groups of the proteins.

Abbreviations: RNA, ribonucleic acid; ATP, adenosine triphosphate; GTP, guanosine triphosphate; Tris, tris(hydroxymethyl)aminomethane.

* Isoelectric precipitation of pH 5 enzyme was made once at pH 4.7.

** Antiserum was absorbed with rat serum globulin and the titer was determined by HEIDELBERGER's method[8] prior to use.

*** Rat serum albumin was purified by $(NH_4)_2SO_4$ fractionation, followed by starch zone electrophoresis.

TABLE I

INCORPORATION OF [^{14}C]LEUCINE INTO ALBUMIN AND RIBONUCLEOPROTEIN OF RAT LIVER MICROSOMES

	Specific activity (counts/min/mg)	
	Albumin	*Ribonucleoprotein*
(A) Complete system	2500 (1390)*	3390
minus ATP	19	150
minus phosphocreatine, creatine kinase	220	394
minus GTP	40	340
(B) Complete system	1220 (505)*	1615
minus pH-5 enzymes	292	189
(C) Complete system	2700 (1040)*	3260
plus RNase	290	133
(D) Complete system	2020 (575)*	2220
with RNase-treated pH-5 enzymes	850	191

* The specific activities of total microsome deoxycholate-soluble proteins are reported in parentheses.

(A) The complete system: 0.5 mmole sucrose, 10 μmoles $MgCl_2$, 100 μmoles KCl, 20 μmoles $KHCO_3$, 50 μmoles Tris buffer (pH 7.9), 2 μmoles ATP, 0.5 μmole GTP, 40 μmoles phosphocreatine, 0.17 μmole of [^{14}C]leucine (0.5 μC), 0.1 mg creatine kinase, microsomes (12 mg protein), pH-5 enzymes (8 mg protein) in total volume of 2 ml. Incubation at 30° for 30 min.

(B) The same conditions as in (A), except that once-washed microsomes were used and 0.08 μmole [^{14}C]leucine was added.

(C) The incubation mixture without [^{14}C]leucine was pre-incubated without or with 0.5 μg RNase/2 ml for 10 min at 30°.

(D) pH-5 enzymes pre-incubated without or with RNase (5 μg/ml) for 30 min at 10° were used.

The results would seem to raise the possibility that albumin synthesis in liver cells could be formulated through amino-acid-activating enzymes, soluble RNA and the microsome system in analogy to the hemoglobin synthesis by reticulocytes reported by SCHWEET *et al.*[14].

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[1] E. B. KELLER AND P. C. ZAMECNIK, *J. Biol. Chem.*, 221 (1956) 45.
[2] T. PETERS, JR., *J. Biol. Chem.*, 229 (1957) 659, *Federation Proc.*, 17 (1958) 452.
[3] P. N. CAMPBELL, O. GREENGARD AND B. A. KERNOT, *Biochem. J.*, 68 (1958) 18P; 73 (1959) 24P.
[4] M. B. HOAGLAND, M. L. STEPHENSON, J. F. SCOTT, L. I. HECHT AND P. C. ZAMECNIK, *J. Biol. Chem.*, 231 (1958) 241.
[5] K. OGATA AND H. NOHARA, *Biochim. Biophys. Acta*, 31 (1959) 149.
[6] P. C. ZAMECNIK, M. L. STEPHENSON AND L. I. HECHT, *Proc. Natl. Acad. Sci., U.S.*, 44 (1958) 73.
[7] J. W. LITTLEFIELD AND E. B. KELLEY, *J. Biol. Chem.*, 224 (1957) 13.
[8] M. HEIDELBERGER AND F. E. KENDALL, *J. Exptl. Med.*, 50 (1929) 809; 58 (1933) 137; 62 (1935) 697.
[9] M. W. WILSON, B. H. PRINGLE, *J. Immunol.*, 73 (1954) 232.
[10] S. OMORI, *Niigata Med. J. (Japan)*, 74 (1960) 94, 105.
[11] K. OGATA, M. OGATA, Y. MOCHIGUKI, T. NISHIYAMA, *J. Biochem. (Japan)*, 43 (1956) 653.
[12] L. G. GROSSI, K. MOLDAVE, *Biochim. Biophys. Acta*, 35 (1959) 275.
[13] F. SANGER, *Biochem. J.*, 39 (1945) 507; 45 (1949) 563.
[14] R. SCHWEET, H. LAMFROM AND E. ALLEN, *Proc. Natl. Acad. Sci., U.S.*, 44 (1958) 1029.

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