Incorporation *in vitro* of [^{14}C]amino acids into proteins of rat-liver microsomal particles

When [^{14}C]amino acids are injected intravenously into a rat, the subcellular components of liver which show the greatest initial incorporation of the amino acid into protein are the microsomal particles[1] and it is generally accepted that the RNA of these particles constitutes a template on which amino acids are assembled into polypeptide chains. The microsomal particles are most probably identical with the ribonucleoprotein particles of the endoplasmic reticulum[2] seen in electronmicrographs of liver and other tissues and they can be separated from the lipoprotein of the reticulum by treating the microsome pellet with sodium deoxycholate[1,3] which solubilizes the lipoprotein[4]. Particles prepared by the deoxycholate method were reported[5] to be incapable of incorporating amino acids into protein in a cell-free system[6], although similar particles, which can be obtained from Ehrlich ascites tumours by use of 0.5 *M* NaCl, actively incorporate amino acids into proteins *in vitro*[5].

Ribonucleoprotein fractions of rat-liver microsomes which will incorporate amino acids into proteins *in vitro* have been prepared[7] with Lubrol W and with sodium perfluoro-octanoate but the RNA/protein ratios given for these fractions (0.22 and 0.4 respectively), which are much lower than those of ribonucleoprotein particles (0.7–1.0)[1,3,5,8], show that fractions obtained in this way are not microsomal particles[7]. This communication reports experiments in which microsomal particles, prepared by the deoxycholate method, were shown to incorporate [^{14}C]amino acids into protein *in vitro*.

Rat liver was homogenized in 0.44 *M* sucrose and a microsome pellet obtained by differential centrifugation. The microsomal particles were separated from it in the usual way with sodium deoxycholate at a final concentration of 0.5 %[1,3]. The RNA/protein ratios for several different preparations of particles were all between 0.75 and 0.95. The pictures obtained on sedimentation in the Spinco (model E) ultracentrifuge were similar to those obtained by others[8].

These particles actively incorporate radioactive amino acids into protein under suitable conditions (Table I). The specific activity of the protein of the particles after a 30-min incubation period is greater than the specific activity of the protein of whole microsomes gained from the same weight of liver. More than twice as much amino acid was, however, incorporated into protein in the system containing microsomes than in the system with microsomal particles. When the soluble fraction of the cell, or the pH 5 enzymes[9], were omitted from the incubation mixture the amount of amino acid incorporated was reduced to 10–12 % of that of the normal system. In Expt. 1, where three to four times as much microsomal particle protein was incubated as in Expt. 2, the incorporation in the absence of added soluble fraction was almost 24 % of that obtained when soluble fraction was added. Little incorporation occurred in the absence of the ATP-generating system and the presence of ribonuclease in the incubation mixture was, as expected, inhibitory.

The protein of the particles is labelled rapidly and reaches a maximum specific activity after about 15 min incubation. Reseparation of the microsomal particles from the soluble fraction after various periods of incubation showed that radioactively labelled protein has been transferred from the particles to the soluble fraction.

Abbreviation: RNA, ribonucleic acid; ATP, adenosine triphosphate.

TABLE I

IN VITRO INCORPORATION OF DL-[1-^{14}C]LEUCINE INTO THE PROTEIN OF MICROSOMAL PARTICLES

The complete system consisted of (a) microsomes or microsomal particles suspended in 0.03 *M* 2-amino-2-hydroxymethylpropane-1:3-diol hydrochloride (Tris) buffer at pH 7.5 containing 0.08 *M* KCl, 0.05 *M* NaCl, 0.005 *M* $MgCl_2$ and 0.0025 *M* glutathione, (b) the soluble fraction of the cell (*i.e.* the supernatant of a 105,000 × *g* centrifugation of a rat-liver homogenate in 0.44 *M* sucrose), *or* the pH 5 enzymes prepared from this fraction[9], (c) 0.001 *M* ATP, 0.02 *M* creatine phosphate, 0.0006 *M* guanosine triphosphate and 0.03 mg creatine phosphokinase, and (d) 0.4 μC DL-[1-^{14}C]leucine. The total vol. was 1 ml. Incubation at 37° for 30 min. The reaction was stopped by addition of cold 0.5 *N* $HClO_4$ containing DL-leucine and the precipitated proteins were repeatedly washed with 0.5 *N* $HClO_4$. RNA and lipid were extracted, the proteins were dried and plated and the radioactivity measured in a Geiger-Müller gas flow counter. The results are expressed as counts/min/mg protein. In Expt. 1 three or four times as much particle protein was incubated as in Expt. 2.

	counts/min/mg microsome or microsomal protein
Expt. 1	
microsomes with complete system	425
microsomal particles (a) with complete system	625
(b) soluble fraction omitted	150
Expt. 2	
microsomes with complete system	278
microsomal particles (a) with complete system	433
(b) soluble fraction omitted	51
(c) pH 5 enzymes in place of soluble fraction	478
(d) ATP-generating system omitted	19
(e) ribonuclease added (0.3 mg)	16

Treatment of microsomes with concentrations of deoxycholate varying from 0.1–1.0 % final concentration yielded particles with lower RNA/protein ratios and lower abilities to incorporate amino acids into proteins than the particles obtained with 0.5 % deoxycholate.

Similar results to the ones described here have been obtained with L-[^{14}C]leucine and DL-[1-^{14}C]valine.

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