DIRECT EVIDENCE FOR ALBUMIN BIOSYNTHESIS BY MEMBRANE BOUND POLYSOMES IN RAT LIVER

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Recently, much evidence has accumulated indicating that there are two kinds of polysomes in the liver cells, that is, membrane-bound polysomes and free polysomes. Isolation procedures of these two kinds of polysomes were reported by several authors (Webb et al, 1964; Loeb et al, 1965; Cammarano et al, 1965; Sugano et al, 1967; Blobel and Potter, 1967). Importance of the presence of RNase-inhibitor during the preparation of two kinds of polysomes, especially of bound polysomes, was also shown (Blobel and Potter, 1966; Sugano et al, 1967).

As to the functional difference of these two kinds of polysomes, Birbeck and Mercer (1961) suggested, from electron microscopic studies, that free ribosomes synthesize proteins for intracellular purposes and that bound ribosomes synthesize proteins to be secreted. And from their previous work (Sargent and Campbell, 1965) in the biosynthesis of serum albumin by liver microsomes, Campbell et al, (1967) have recently proposed that serum albumin is synthesized by bound polysomes. Direct evidence, however, is still lacking if serum albumin is synthesized only on bound polysomes, since their microsomal fraction might also contain free polysomes and the activity of free polysomes in the synthesis of serum albumin has not yet been investigated.

In order to obtain direct evidence for the difference in the activity in the synthesis of serum albumin between two kinds of polysomes, the following experiments were undertaken by the present authors.

Materials and Methods

^{14}C-leucine (311 mc/mM) and ^{3}H-leucine (29.1 c/mM) were purchased from Radiochemical Center, Amersham, England. Rats of the Wistar strain weighing about 150-180 g were sacrificed after overnight fasting. The liver was homogenized in 2.5 volumes of medium A (0.25M sucrose, 0.005M $MgCl_2$, 0.025M KCl, 0.05M Tris-HCl pH 7.6) and two kinds of polysomes were prepared according to the method described by Sugano et al (1967) except that; (1) The microsomal membrane fraction, which was obtained as a turbid zone after the 1st discontinuous gradient centrifugation at 244,500 x g for 2 hr, was used without the subsequent centrifugation at 105,000 x g. Instead, after the addition of desoxycholate (DOC, final concentration, 1.3%), the fraction was directly placed on 4 ml of 2M sucrose solution in Medium A' (0.25M sucrose, 0.005M $MgCl_2$, 0.05M KCl, 0.01M $KHCO_3$, 0.05M Tris-HCl pH 7.9) and the 2nd discontinuous gradient centrifugation at 244,500 x g for 2 hr was carried out for the preparation of bound polysomes. (2) Free polysomes, obtained by the 1st discontinuous gradient centrifugation, was further purified by 1.3% DOC treatment, followed by centrifugation at 244,500 x g for 2 hr to remove completely the contaminated membrane fraction.

RNase-inhibitor which was partially purified from the post-microsomal supernatant of rat liver by DEAE-cellulose column chromatography (Shortman, 1961) was added to all the solutions used in the procedures. Judging from the ratio of absorbancy at 260/280mμ or 260/235mμ, these two kinds of polysomes were almost completely free from the membrane. The typical polysomal patterns were always demonstrated in these preparations by the analyses with an analytical ultracentrifuge.

Each of two kinds of polysomes and the supernatant obtained after the

1st discontinuous gradient centrifugation were incubated at 37°C for a given period with ^{14}C-leucine or ^{3}H-leucine. The supernatant fraction was employed for the determination of radioactivity of the albumin fraction. The albumin fraction and the control non-specific protein fraction were then prepared according to the immunological method described by Hirokawa and Ogata (1962).

Results

The time course of the incorporation of ^{14}C-leucine into the total proteins showed that the rate of protein synthesis by two kinds of polysomes was almost the same (Fig. 1). It was noted that free polysomes prepared without DOC-treatment had somewhat higher activity than bound polysomes, in agreement with the results of other investigators (Bloemendal et al, 1967;

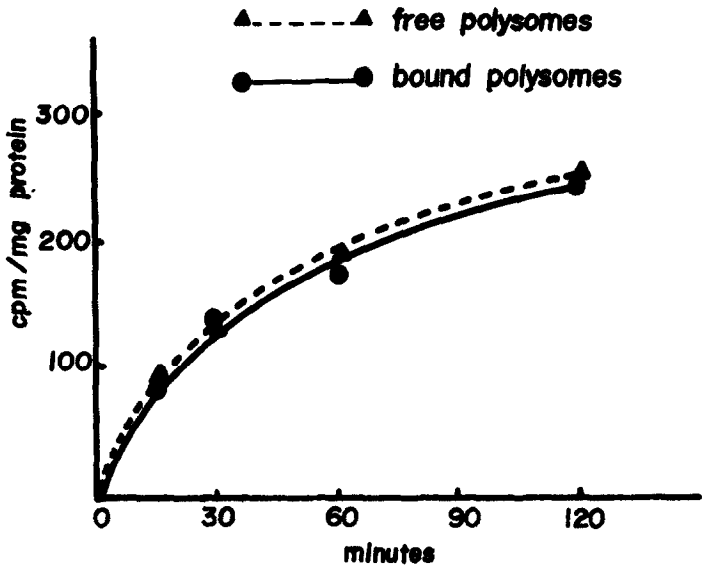

Fig. 1. The rate of incorporation by free and bound polysomes:

Three tenth mg of protein of free or bound polysomes and 1mg of proteins of the supernatant after the first discontinuous gradient centrifugation were incubated with 100mμc of ^{14}C-leucine at 30°C in the complete reaction mixture of the total volume of 0.4 ml, containing ATP 1mM, GTP 0.25mM, glutathione 10mM, phosphocreatine 10mM and creatine kinase 50μg/ml in medium A'. The reaction was stopped with TCA and the radioactivity of the total proteins was determined with a gas flow counter.

Table 1. Incorporation of leucine into albumin fraction by free and bound polysomes:

Eight tenth mg of proteins of free or bound polysomes and 7mg of proteins of the supernatant after the first discontinuous gradient centrifugation were incubated with 12.5μc of ^{14}C-leucine or 100μc of ^{3}H-leucine in the complete reaction mixture of 1 ml. In the 1st experiment, free polysomes were labeled with ^{3}H- and bound polysomes with ^{14}C-leucine and in the 2nd, the reverse labeling was employed. After the incubation at 30°C for 2 hr the ^{3}H- and ^{14}C-labeled supernatant fraction were combined in each experiment and used for the preparation of albumin fraction. After removal of non-specific proteins three times with ovalbumin and its antiserum, the supernatant was divided into two parts. From the one, the albumin fraction was precipitated with carrier rat serum albumin and its antiserum, and from the other, the control non-specific proteins with ovalbumin and its antiserum. Each antigen-antiserum complex was washed with cold 0.9% NaCl, hot and cold TCA, ethanol-ether and finally dissolved in 0.4 ml hyamine. After addition of toluene-scintillator, ^{3}H- and ^{14}C-radioactivity were determined with a liquid scintillation counter, Beckman LS 150.

cpm/0.3 ml reaction mixture

	total proteins (A)	non-specific fr. (B)	albumin fr. (C)	(C-B)
No. 1 experiment				
^{3}H-free (a)	48600	313	900	587
^{14}C-bound (b)	6880	51	481	430
(b) x Factor*	48600	359	3390	3031
$\frac{\text{(b) x Factor}}{\text{(a)}}$	1.00	0.87	3.77	5.17
No. 2 experiment				
^{3}H-bound (a)	41600	564	3191	2627
^{14}C-free (b)	7100	159	230	71
(b) x Factor*	41600	947	1235	398
$\frac{\text{(a)}}{\text{(b) x Factor}}$	1.00	0.60	2.37	6.60

Factor*; Calculated to make ^{14}C radioactivity equal to ^{3}H radioactivity in total protein fraction.

Sugano et al, 1967). About 30% of the total labeled proteins were released into the 105,000 x g supernatant after 2 hr of incubation. DEAE-cellulose column chromatography of the supernatant showed that all the supernatant

proteins were labeled. Furthermore, the radioactivity of the albumin fraction of the nascent proteins of liver ribosomes, obtained by EDTA-treatment followed by the density gradient centrifugation (Ogata et al, 1967), was lower than that from the supernatant. From these reasons the incorporation of radioactive leucine into the albumin fraction was compared between the supernatants after the incubation of two kinds of polysomes.

The results are summarized in Table 1. In the 1st experiment ^{3}H- and ^{14}C-leucine were respectively incubated with free and bound polysomes and in the 2nd experiment the situation was reversed. For the direct comparison of the two kinds of radioactivities, ^{14}C counts in the table were multiplied by factors which were ratios of ^{3}H counts to ^{14}C counts in the total proteins. Since the incorporation of ^{14}C-leucine into the total proteins by two kinds of polysomes was almost the same (Fig. 1), such a correction may be allowable to compare the activity in the synthesis of serum albumin between the polysomes. The incorporation into the albumin fraction by bound polysomes was 5.2 and 6.6 times higher than that by free polysomes in two experiments respectively.

Discussion and Conclusion

The results mentioned above indicate that serum albumin is synthesized rather exclusively on bound polysomes of the liver. It must be added that the results of our recent experiment *in vivo* (Takagi et al, unpublished data) showed more clearly that nascent albumin exist only on bound polysomes. The indication is of importance, since it points out the possibility that the distribution of messenger RNA is different between two kinds of polysomes, at least in the case of serum albumin. Furthermore, the results appear to refuse the hypothesis that free and bound polysomes are interchangeable each other in the cytoplasm and tend to support the idea that they are discrete components of the liver cell, having different functions.

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