GROWTH HORMONE STIMULATION OF PROTEIN SYNTHETIC ACTIVITY OF MEMBRANE-BOUND RIBOSOMES

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1. Introduction

Both membrane-bound and free ribosomes occur in rat liver and qualitative, and possibly quantitative, differences exist between them. Bound ribosomes synthesize proportionately more serum protein [1, 2] than free ribosomes, but less ferritin [3, 4]. These findings and the report [5] that free, but not bound, ribosomes react with antibodies to undefined, soluble liver antigens lend credence to the current view that a ribosome will synthesize proteins for export from the cell if it is membrane-bound and that it will synthesize proteins for the cell's own use if it is free. This functional specialisation is probably not absolute [6].

We thought it of interest to see whether the known effect of growth hormone in stimulating the protein synthetic activity of liver ribosomes [7, 8] involved free or bound ribosomes or both. This paper shows that the hormone affected membrane-bound but not free ribosomes.

2. Materials and methods

Hypophysectomised male rats were obtained from Charles River Laboratories, USA. Growth hormone (NIH-GH-B14) was a generous gift of the National Pituitary Agency, Washington, USA and was reported to have a potency of 1.04 I.U./mg. It was dissolved in 1 M tris (pH approx. 10), diluted with 0.9% saline and 100 μg of it was injected intraperitoneally to each rat twelve hours before death. Control rats received tris-saline. Rats were without food for about 17 hr before death.

Free (F), light membrane-bound (LMB) and heavy membrane-bound (HMB) ribosomes were isolated from the livers essentially by the procedure of Bloemendal et al. [9]. Livers were rinsed, minced and homogenized in 3 vol. Medium A (50 mM tris pH 7.6, 25 mM KCl, 10 mM magnesium acetate and 6 mM mercaptoethanol) containing 0.35 M sucrose. The supernatant fluid from a 10 min, 10,000 *g* centrifugation was layered over 3.5 ml 1.5 M sucrose which had been layered over 4 ml 2 M sucrose, both in medium A, and centrifuged at 200,000 *g* for 4 hr. The 0.35 M sucrose layer was removed and discarded, the opaque interphase between 0.35 and 1.5 M sucrose layers contained the LMB and the yellow bands in the 1.5 M sucrose contained the HMB. The pellet contained F which was rinsed and suspended in medium A. LMB and HMB were diluted with medium A to allow sedimentation at 200,000 *g* within 1.5 hr and the pellets were rinsed and suspended in medium A. In some experiments LMB and HMB were treated with 1% (v/v) Triton X-100 in medium A and spun through 1.0 M sucrose containing medium A. RNA and protein of the fractions were determined as previously described [10] and fractions were diluted so that equal amounts of ribosomes were used for cell-free incorporation assay. This was carried out in final vol. 0.2 ml containing 68 mM tris pH 7.6, 27.5 mM KCl, 17.5 mM Na^+, 11 mM magnesium acetate, 0.05 mM of 19 amino acids, 5 mM ATP, 1 mM GTP (both as sodium salts), 10 mM creatine phosphate, 60 μg/ml creatine phosphokinase, 9 mM mercaptoethanol, 1 μCi/ml ^{14}C-L-leucine (312 mCi/mmole) or ^{14}C-L-phenylalanine (475 mCi/mmole), 10 μg ribosomal

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RNA and 250–500 mg cell-sap protein. The preparation of the protein for radioactivity assay has been described [10].

3. Results

The light membrane-bound (LMB) constituted 20%, the heavy-membrane bound (HMB) 30% and the free ribosomes (F) 50% of the recovered material. RNA: protein ratios were 0.05–0.122, 0.2–0.388 and 0.563–0.87 respectively.

The absence of KCl during preparation, which is a feature of the methods used by some authors [11, 12] resulted in a marked fall in the protein synthetic activity of LMB and F but not of HMB ribosomes (table 1). Omission of KCl also resulted in a smaller yield of F ribosomes so we have always included it in the preparative sucrose gradients.

Table 1 shows that F ribosomes were more active than HMB ribosomes which, in turn, were more active than LMB ribosomes. This result remained true when ^{14}C-aminoacyl-tRNA was used as a source of radioactive precursor in place of ^{14}C-labelled amino acids, thus eliminating the possibility that the difference in activity resulted from differences in membrane bound ATPases or in contaminating concentrations of unlabelled amino acids. Amino acid incorporation in the absence of added cell-sap, which is a rough indication of contamination by cell-sap factors, showed that the LMB ribosomes had more sap contamination than HMB ribosomes which were more contaminated than F ribosomes. Removal of the membranes with Triton X-100 markedly reduced the ability of LMB and HMB ribosomes to incorporate amino acids unless cell-sap was added.

No differences were detected between the protein synthetic activity of LMB, HMB and F ribosomes from normal, hypophysectomized or growth hormone-treated rats. The experiments were therefore repeated after removing the ribosomes from the membranous material with Triton X-100. The results are shown in table 2. Growth hormone had significantly stimulated the protein synthetic activity of the ribosomes originally bound to membranes in LMB and HMB fractions but had no affected the free ribosomes.

It was noted that treatment of the LMB, but not the HMB, ribosome fraction with detergent increased

Table 1
Comparison of the incorporating activity of heavy membrane bound (HMB), light membrane bound (LMB) and free (F) ribosomes of liver from normal rats.

	Incorporation into liver ribosome fractions		
	HMB	LMB	F
Cell-sap and ^{14}C-ieucine	207,785	102,660	277,593
KCl omitted from preparation	215,700	64,000	165,400
Transferases and ^{14}C-aminoacyl-tRNA	46,282	–	72,242

HMB, LMB and F ribosomes were prepared as described in the Methods section or in the absence of KCl from the preparative gradients. The incorporating activity was assayed as described using either ^{14}C-leucine as precursor or replacing it and the cell-sap with ^{14}C-aminoacyl-tRNA and a crude transferase I and II preparation. Results are expressed as cpm/mg ribosomal RNA.

Table 2
Effect of growth hormone treatment on incorporating activity of ribosome fractions.

	HMB		LMB		F	
	Control	GH	Control	GH	Control	GH
1) ^{14}C-Leucine (4)	121,309	155,944	161,901	217,356	197,721	195,690
2) ^{14}C-Phenylalanine (2)	89,410	105,705	117,894	154,421	144,679	149,601

Hypophysectomized rats were given 100 μg bovine growth hormone or saline by injection. Free, light and heavy membranous fractions were prepared as described in Methods and the membranous fractions treated with 1% Triton. Mean activity is expressed as cpm/mg ribosomal RNA. Number of experiments in parenthesis.

the activity of the ribosomes. Probably some material is attached to the membranes of the LMB fraction which is inhibitory to protein synthesis.

4. Discussion

Free ribosomes were, in our hands, more active than bound ones at cell free protein synthesis. This result confirms that of some workers [1, 2, 5, 9, 13, 14] but differs from other reports [11, 12, 15, 16]. Differences in methods of preparation, in particular the lack of KCl (table 1) and of contaminating materials or redistribution of the ribosomes during preparation [17] may help to explain the discrepancies.

We were surprised not to find a difference in the activity of any of the three ribosomal fractions as a result of growth hormone treatment since differences had been apparent when liver microsomes and ribosomes were assayed [7, 18]. These earlier studies had used different isolation media and techniques and these differences may explain the difference in results. Clear differences in activity were seen, however, when the membranes were removed from both the heavy and light membranous fractions before assay. No difference was apparent in the free ribosomes as a result of growth hormone treatment.

It has been reported [12, 19, 20] that free and heavy bound ribosomes from hypophysectomized rats are less active *in vitro* than normal ones and that treatment with growth hormone together with triiodothyronine for three days enhanced the activity of both fractions. The effect of growth hormone alone was not studied in these experiments. We did not compare normal and hypophysectomized rats because the differences in body weight and food intake might have influenced the results. The ability of the free ribosomes to respond to hormone treatment may reflect a difference in the response to growth hormone accompanied by triiodothyronine compared with growth hormone alone; or a difference in response to three day treatment with hormone compared with 12 hr; or a difference in the free and bound ribosomes used by these workers and ourselves. In his experiments, Tata [19] found free ribosomes were the least active fraction and in our experiments they were the most active fraction. A recent report [20] of results obtained in vivo confirmed our in vitro findings that growth hormone stimulated bound but not free ribosomes.

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References

[1] M.Takagi and K.Ogata, Biochem. Biophys. Res. Commun. 33 (1968) 55.
[2] C.M.Redman, Biochem. Biophys. Res. Commun. 31 (1968) 845.
[3] C.M.Redman, J. Biol. Chem. 244 (1969) 4308.
[4] S.J.Hicks, J.M.Drysdale and H.N.Munro, Science 164 (1969) 584.
[5] M.C.Ganoza and C.A.Williams, Proc. Natl. Acad. Sci. U.S. 63 (1969) 1370.
[6] G.Ragnotti, G.R.Lawford and P.N.Campbell, Biochem. J. 112 (1969) 139.
[7] A.Korner, Biochem. J. 81 (1961) 292.
[8] A.Korner, in: CIBA Foundation Symposium. Control Processes in Multicellular Organisms, eds. G.E.W.Wolstenholme and Julie Knight (Churchill, London, 1970) p. 86.
[9] H.Bloemendal, W.S.Bont, M.de Vries and E.L.Benedetti, Biochem. J. 103 (1967) 177.
[10] A.J.Munro, R.J.Jackson and A.Korner, Biochem. J. 92 (1964) 289.
[11] P.N.Campbell, C.Cooper and M.Hicks, Biochem. J. 92 (1964) 225.
[12] J.R.Tata and H.G.Williams-Ashman, European J. Biochem. 2 (1967) 366.
[13] S.Kwan, T.E.Webb and H.P.Morris, Biochem. J. 109 (1968) 619.
[14] P.N.Campbell and G.R.Lawford, Proc. 4th Meeting FEBS, Oslo, 1968, Vol. 6, p. 57.
[15] E.C.Henshaw, T.B.Bojarski and H.H.Hiatt, J. Mol. Biol. 7 (1963) 122.
[16] T.H.Hallinan and H.N.Munro, Biochim. Biophys. Acta 80 (1964) 166.
[17] D.Lowe, E.Reid and T.Hallinan, FEBS Letters 6 (1970) 114.
[18] A.Korner, Biochem. J. 73 (1959) 61.
[19] J.R.Tata, Biochem. J. 104 (1967) 1.
[20] J.R.Tata, Biochem. J. 116 (1970) 617.