# PROTEIN PHOSPHOKINASES OF BOUND AND FREE THYROID POLYRIBOSOMES

M. PAVLOVIC-HOURNAC, D. DELBAUFFE, A. VIRION and J. NUNEZ

*Unité de Recherche sur la Glande Thyroide et la Régulation Hormonale, I.N.S.E.R.M., Hopital de Bicêtre, 78, Avenue du Général Leclerc, 94270, Bicêtre, France*

Received 29 March 1973

## 1. Introduction

Several recent reports have clearly demonstrated that ribosomal proteins are phosphorylated both *in vivo* [1–3] and *in vitro* [1, 2, 4–7]. It has also been shown that polyribosomes from different tissues contain protein phosphokinase activities as well as endogenous protein-substrate(s) [2, 5, 7–11]. However, the relationship between these observations and the process of protein synthesis is still not clear.

On the other hand, it is well established that cells from higher organisms contain two classes of polyribosomes, free in the cytoplasm and bound to the membranes of the rough endoplasmic reticulum [12]. Increasing evidence accumulates that bound polyribosomes are involved in the formation of exportable proteins, whereas free ribosomes are specialized in the synthesis of intracellular proteins [13–15].

Vassart and Dumont [16] have recently shown that thyroid bound polyribosomes synthesize thyroglobulin, an exportable protein, whereas free ribosomes are involved mainly in the formation of cellular proteins. For this reason this work was performed with free and bound thyroid polyribosomes.

The results reported here show that both free and bound thyroid polyribosomes contain protein phosphokinase activities. However, when compared to endogenous and exogenous substrates these activities behave differently.

## 2. Material and methods

### *2.1. Preparation of free and bound polysomes*

Horse thyroid glands were homogenized first by an omni-Mixer Sorvall (3 × 15 sec, 90 V) and then by an Ultra-Turrax (2 × 15 sec, 850 rpm) in the following medium: Tris-HCl buffer (0.05 M, pH 7.6) containing: 0.025 M KCl; 0.005 M $MgCl_2$; 0.006 M β-MSH and 0.35 M sucrose. The homogenate was centrifuged 10 min at 3 000 *g* and the supernatant was used for purification of free and bound polysomes by the technique of Bont et al. [17] with slight modifications. The supernatant was centrifuged on a discontinous sucrose gradient (2 M and 1.5 M) for 21 hr at 30 000 rpm in a Spinco 50-1 rotor. The pellet from the centrifugation represents free polysomes. Polysomes still bound to the membranes were collected from the interface between 2 M and 1.5 M sucrose and then homogenized in the presence of liver RNAase inhibitor [18] and sodium deoxycholate (DOC, final conc. 1%). This homogenate was centrifuged on a discontinous sucrose gradient (2 M – 1.5 M) during 3.5 hr at 41 000 rpm in a Spinco 50-1 rotor. The pellet of this second centrifugation represents bound polysomes. Pellets of both free and bound polysomes were washed, resuspended and the aggregates eliminated by a centrifugation at 3 000 *g* for 5 min. The ratio RNA/protein (table 1) and the profiles obtained after sucrose gradient ultracentrifugation (fig. 1) were used to characterize free and bound polysomes.

### 2.2. Protein phosphokinase activity

Protein phosphokinase activity was measured at 30° in aliquots of the above suspensions of free and bound polysomes in a medium containing: β-glycerophosphate 50 mM; EGTA 0.3 mM; theophylline 2 mM; and magnesium acetate 10 mM in a potassium phosphate buffer 1 mM, pH 7; [$\gamma$-$^{32}$P]ATP was added at a concentration of $1 \times 10^{-4}$ M (specific activity 50–100 cpm/pmole). Histones (Sigma, Type II A) and 3′5′ *c*AMP (Sigma) when added, were, respectively, at concentrations of 4 mg/ml and $5 \times 10^{-6}$ M. Incorporation of $^{32}$P into polysomal proteins and histones was evaluated according to the method of Mans and Novelli [19] described for the acellular study of protein synthesis.

### 2.3. cAMP binding activity

Aliquots of free and bound polysomes were incubated (10 min) in the presence of tritiated *c*AMP ($6 \times 10^{-8}$ M) and the capacity of ribosomes to bind this nucleotide was evaluated according to the technique of Walton and Garren [20].

### 2.4. Analytical methods

Proteins were analyzed according to Lowry et al. [21] and RNA according to Schmidt and Tannhauser [22]. The samples were counted in water in an Intertechnique counter. Results are expressed in picomoles of $^{32}$P incorporated per mg ribosomal proteins or RNA.

## 3. Results

### 3.1. Ultracentrifugation profiles of free and bound thyroid polyribosomes

Fig. 1 represents the ultracentrifugation profiles of bound and free thyroid polyribosomes. These profiles, very similar to those obtained by Vassart and Dumont [16], show that bound polyribosomes contain much heavier material than free ones. Both preparations are completely degraded to single ribosomes (80 S) and their dimer (110 S) after RNAase treatment (fig. 1). The 260/280 nm absorption ratio and RNA/protein ratio (table 1) are almost identical. From these data,

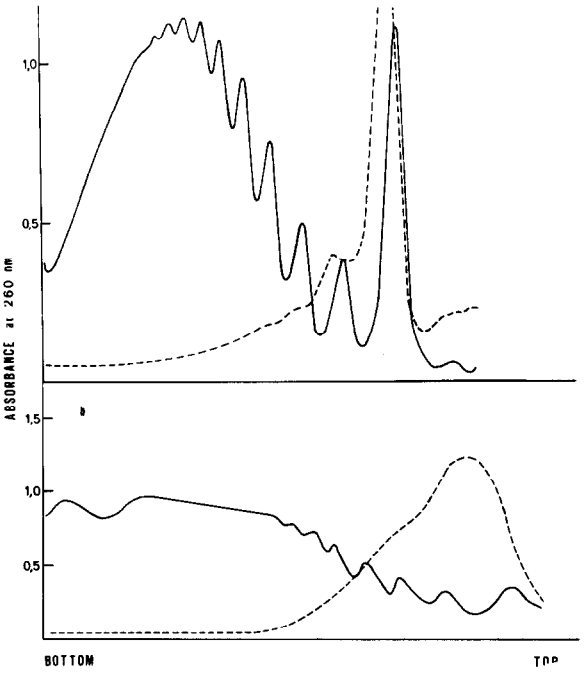


Fig. 1. Sucrose gradient ultracentrifucation profiles of free (a) and bound (b) thyroid polysomes. Polyribosomes were analyse before (——) and after (- - - -) pancreatic RNAase treatment (2 μg/ml final conc., 15 min, 20°) by sucrose gradient (5 ml; 20–50%) ultracentrifugation (50 000 rpm, 50 min, Spinco rotor SW 50); a 0.2 ml sucrose 2 M cushion was present at the bottom of the tube. 260 nm absorbance was recorded automatically.

both polyribosomes preparations seem to be suitably purified and not contaminated by membranes.

### 3.2. Protein phosphokinase activities and phosphorylation of ribosomal endogenous substrates (autophosphorylation)

Protein phosphokinase activities measured in the absence of any added exogenous substrate was found in both classes of polyribosomes. Phosphorylation of endogenous substrate is not *c*AMP dependent, despite the presence of a *c*AMP binding activity in both types of polyribosomes (table 1).

Furthermore, the results depicted in fig. 2 show that protein kinase specific activities were much higher in free than in bound polyribosomes. However, some protein kinase activity or/and some endogenous protein substrate could be removed by DOC during

Table 1

| Polysomes | 260/280 nm absorbance ratio | RNA / protein | cAMP binding activity (cpm)* | |
|---|---|---|---|---|
| | | | 40 μg protein | 40 μg protein |
| Free | 1.67 ± 0.03 | 0.74 ± 0.06 | 300 | 560 |
| Bound | 1.64 ± 0.03 | 0.78 ± 0.06 | 150 | 310 |

* The cAMP binding activity was measured as described in Material and methods.

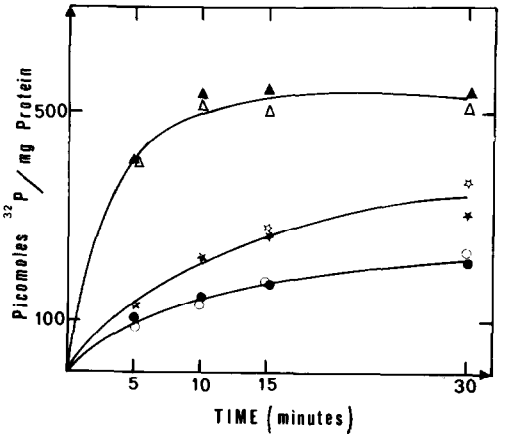


Fig. 2. Autophosphorylation of free and bound thyroid polysomes. Polysomes were incubated in the presence of [$\gamma$-$^{32}$P]-ATP as described in Material and methods. Aliquots (in duplicate) were taken at indicated time intervals and the incorporation of $^{32}$P into proteins was determined. Δ) free polysomes; ⋆) free polysomes with DOC (1% final conc.); ○) bound polysomes. Open signs: phosphorylation in the absence of cAMP and filled signs: phosphorylation in the presence of $5 \times 10^{-6}$ cAMP. 4 Separate experiments were analysed.

the preparation of bound polyribosomes thus explaining the difference in the specific activity when compared to that of free polyribosomes. Actually, DOC treatment of free ribosomes removes some enzymatic activity but the specific activity remains higher than that of bound polyribosomes (fig. 1).

### *3.3. Histone kinase activity of free and bound polyribosomes*

Phosphorylation, when measured in the presence of histones and of bound polyribosomes, was always higher than autophosphorylation (fig. 3a). With free polyribosomes the results were reversed, autophosphorylation being always much higher (fig. 3b). It seems in fact that histones (from 0.3 to 5 mg/ml) inhibit autophosphorylation catalysed by free polyribosomes; when the histone concentration is increased a higher inhibition is observed.

DOC treatment of free polyribosomes reduced the histone kinase activity; however after treatment by the detergent, the histone kinase activity of free polyribosomes was again lower than autophosphorylation.

As reported above histone kinase activity is clearly cAMP dependent with both categories of polyribosomes whereas autophosphorylation is not. When added to bound polyribosomes cAMP stimulates the phosphorylation of histones. With free polyribosomes the cyclic nucleotide seems rather to counteract the inhibitory effect of histones.

## 4. Discussion

Free and bound purified polyribosomes contain both protein phosphokinase and cAMP binding activities. The phosphorylation of endogenous protein-substrates (which belong perhaps to the ribosomal structure) is not cAMP dependent. The two types of polyribosomal preparations do not differ in these respects. Kabat [2] obtained similar results for reticulocyte polyribosomes.

However a clear difference appears, when one compares the protein kinase activity of the two types of ribosomes in the absence (autophosphorylation) and in the presence of histones. Whereas bound polyribosomes phosphorylate poorly endogenous substrate(s) as compared to phosphorylation in the presence of histones, free polyribosomes catalyse much more actively the former reaction than the latter. The two classes of polyribosomes seem therefore to be different in these respects. However, histone-kinase activity is cAMP dependent for both types of polyribosomes. On the other hand, there exists some parallelism between the development of rough endoplasmic reticulum i.e. bound polyribosomes, and thyroglobulin synthesis [23–25]. Moreover, TSH regulates thyroid metabolism including protein synthesis [26–29]; cAMP mimics these effects of TSH [30–32]; its action is likely mediated by a protein kinase [33]. The

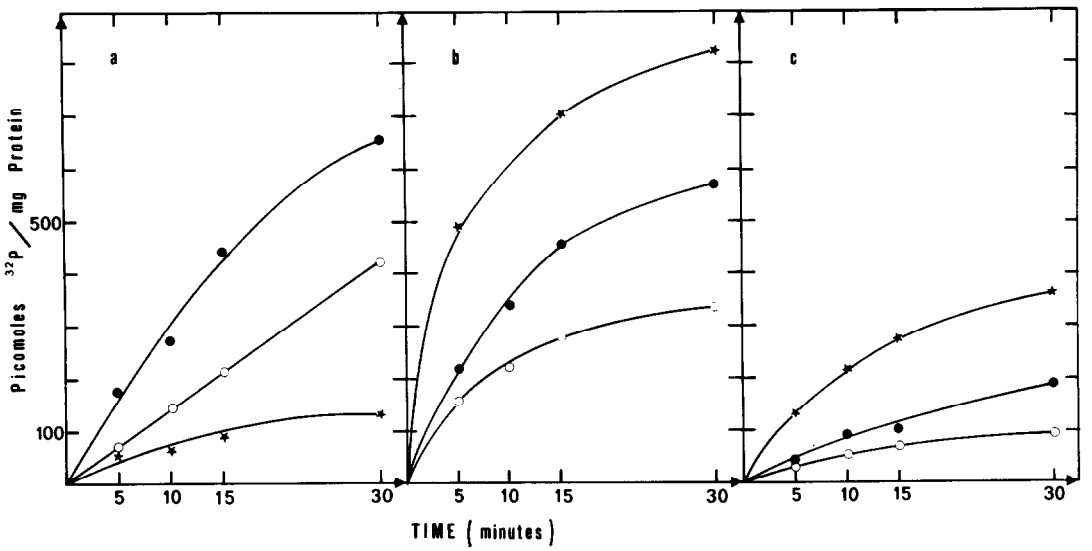


Fig. 3. Phosphorylation in the presence of exogenous substrates. Polysomes were incubated as in fig. 2. Histones (Sigma type II A) were added at concentration of 4 mg/ml. a) Bound; b) free; c) free polysomes + DOC. (★—★—★) autophosphorylation; phosphorylation in the presence of histones with (●—●—●) and without *c*AMP (○—○—○). Other details as in fig. 2.

difference in behaviour towards endogenous and exogenous substrates of the protein kinase activities associated respectively with bound and free polyribosomes could therefore have some significance in the mechanism of action of TSH (and *c*AMP) on the total and/or specific protein synthesis in the thyroid gland.

## Acknowledgement

The authors wish to thank Mrs. R. Ohayon for her excellent technical assistance.

## References

[1] J.E. Loeb and C. Blat, FEBS Letters 10 (1970) 105.
[2] D. Kabat, Biochemistry 9 (1970) 4160.
[3] C. Correze, P. Pinell and J. Nunez, FEBS Letters 23 (1972) 87.
[4] Ch. Li and H. Amos, Biochem. Biophys. Res. Commun. 45 (1971) 1398.
[5] D. Kabat, Biochemistry 10 (1971) 197.
[6] G.M. Walton, G.N. Gill, I.B. Abrass and L.D. Garren, Proc. Natl. Acad. Sci. U.S. 48 (1971) 880.
[7] C. Eil and I.G. Wool, Biochem. Biophys. Res. Commun. 43 (1971) 1001.
[8] J.A. Fontana, D. Picciano and W. Lovenberg, Biochem. Biophys. Res. Commun. 49 (1972) 1225.
[9] B. Jergil, European J. Biochem. 28 (1972) 546.
[10] J.A. Trough and R.R. Traut, Biochemistry 11 (1972) 2503.
[11] L. Bitte and D. Kabat, J. Biol. Chem. 247 (1972) 5345.
[12] G.E. Palade and P. Siekevitz, J. Biophys. Biochem. Cytol. 2 (1956) 171.
[13] S.J. Hicks, J.W. Drysdale and H.N. Munro, Science 164 (1969) 584.
[14] C.M. Redman, J. Biol. Chem. 244 (1969) 4308.
[15] P. Gaye and R. Denamur, Biochem. Biophys. Res. Commun 41 (1970) 266.
[16] G. Vassart and J.E. Dumont, European J. Biochem. 32 (1973) 322.
[17] W.S. Bont, J. Geels, A. Huizinga, K. Mekkelholt and P. Emmelot, Biochim. Biophys. Acta 262 (1972) 514.
[18] R.R. Mac Gregor and H.R. Mahler, Biochemistry 8 (1969) 3036.
[19] R.J. Mans and D.G. Novelli, Arch. Biochem. Biophys. 94 (1961) 48.
[20] G.M. Walton and L.D. Garren, Biochemistry 9 (1970) 4223.
[21] O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193 (1957) 265.
[22] G. Schmidt and S.J. Thannhauser, in: Techniques de laboratoire, Tome I, Fascicule 2, ed. J. Loiseleur (Masson et Cie, Paris 1963) p. 1229.
[23] V. Pantic, M. Pavlovic-Hournac and L. Rappaport, J. Ultrastr. Res. 31 (1970) 37.

[24] P. Olin, R. Ekholm and S. Almquist, Endocrinology 27 (1970) 1000.
[25] E. Regard and J. Mauchamp, J. Ultrastr. Res. 77 (1971) 664.
[26] E. Raghupathy, W. Tong and J.L. Chaikoff, Endocrinology 72 (1963) 620.
[27] R.R. Cavalieri and G.L. Searle, Proc. Soc. Exp. Biol. Med. 126 (1967) 459.
[28] W. Tong, Endocrinology 80 (1967) 1101.
[29] M. Pavlovic-Hournac, L. Rappaport and J. Nunez, Endocrinology 89 (1971) 1477.
[30] B. Wilson, E. Raghupathy, T. Tonoue and W. Tong, Endocrinology 83 (1968) 877.
[31] V. Macchia and S. Varrone, FEBS Letters 13 (1971) 342.
[32] R.E. Lococq and J.E. Dumont, Biochim. Biophys. Acta 289 (1972) 434.
[33] L. Rappaport, J.F. Leterrier and J. Nunez, Biochimie 53 (1971) 721.