BIOCHEMICAL EVIDENCE FOR A LOW MOLECULAR WEIGHT PROTEIN (PROFILIN-LIKE PROTEIN) IN HOG THYROID GLAND AND ITS INVOLVEMENT IN ACTIN POLYMERISATION

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1. Introduction

Actin was characterized ubiquitously in muscle fibers and in non-muscle cells from the most primitive to the most advanced organisms. However, in non-muscle cells, this protein is found together in microfilamentous and monomeric states (F- and G-actin, respectively). It has been suggested that cell constituents in non-muscle cells interact with G-actin to maintain it in non-polymerized form. In this context, it has been shown that a low molecular weight protein named profilin prevents the actin polymerisation [1].

In thyroid cells actin microfilaments appear to be involved in the endocytotic process [2]. The organization of these microfilaments depends on the physiological state of the gland. The bundles of actin filaments in the resting gland are transformed into a network in response to thyrotropin stimulation; concomitantly, an increase of the ratio G/F actin is observed [3].

Here, we have initiated a search for effectors which may have an influence on the state of actin polymerisation in hog thyroid cells.

2. Materials and methods

Rabbit skeletal muscle actin was purified according to [4]. Its concentration was calculated from A_{290} using $E = 0.62$ ml . mg^{-1} . cm^{-1} [5]. The method in [6] was used for all other protein concentration determinations. The method in [7] was applied for polyacrylamide slab-gel electrophoresis. Amino acid analyses were performed on a Beckman model 120C autoanalyzer using a single column. Tryptophan was determined by colorimetry [8] and spectrophotometry [9].

Actin polymerisation was followed at 25°C by measuring the increase in A_{232} on a Cary model 219 spectrophotometer. In order to have a better resolution, 0.437 cm lightpath cells were used. The spectral bandwidth was 3 nm.

3. Results

3.1. *Preparation of thyroid profilin*

Profilin was isolated from fresh hog thyroid glands. All steps were carried out at 4°C.

Routinely, 120 g thyroid slices were washed in 3 vol. 0.15 M NaCl, 0.1 mM PMSF, stirred for 30 min and filtered on gauze. The tissue slices were suspended in 2 vol. 0.9 M KCl, 15 mM pyrophosphate, 5 mM $MgCl_2$, 3 mM DTE, 0.1 mM sodium azide, 0.1 mM PMSF, 30 mM imidazole buffer (pH 7), stirred for 30 min and then homogenized for 2 min in a waring blendor. The homogenization was achieved using 2 strokes of a glass–teflon homogenizer. After 30 min stirring, the homogenate was centrifuged for 20 min at 15 000 × *g*. The supernatant was diluted with 3 vol. 2 mM $MgCl_2$ under stirring and adjusted to pH 6.4. After 15 min, a precipitate which is in major part actomyosin was discarded by centrifugation for 45 min at 17 000 × *g*. Solid ammonium sulfate was then added to the supernatant to 30% satu-

Abbreviations: PMSF, phenylmethylsulfonyl fluoride; DTE, 1,4-dithioerythritol; M_r, relative molecular mass

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ration. After centrifugation at 17 000 × *g* for 45 min, the salt concentration of the resulting supernatant was increased to 85% saturation. The suspension was kept overnight. The precipitate was collected by centrifugation and suspended in a minimal volume of 25 mM Tris–HCl, 5 mM $MgCl_2$, 0.1 mM DTE, 0.1 mM PMSF, 2 M urea buffer (pH 7.5). Insoluble material was discarded. The sample (~250 ml) was applied to a (5.5 × 110 cm) column of Sephadex G-100 equilibrated and eluted with the buffer mentioned above. Fractions containing low molecular weight proteins were pooled, concentrated by partial lyophilisation and filtrated on a (3.2 × 75 cm) column of Sephadex G-50 fine eluted with the same buffer. The profilin peak was then salted out either by dialyses (spectrapor membrane tubing, cutoff app. M_r 3500) or by Sephadex G-15 filtration using 10 mM imidazole, 0.1 M KCl, 0.1 mM DTE, 0.1 mM PMSF buffer (pH 7.5) then passed through a DEAE-cellulose column (3 × 18 cm). Equilibrated with the same buffer. The eluted fractions were pooled and concentrated on UM 0.05 diaflo membrane. A schematic outline of the purification procedure is given in table 1. The material obtained is homogeneous on SDS–polyacrylamide gel (fig.1).

3.2. *Characterisation of thyroid-like profilin*

The homogeneous product obtained was M_r 13 000 ± 500 as determined by SDS–polyacrylamide gel electrophoresis. This M_r was also found by filtration on Sephadex G-75 under non-denaturing conditions (fig.2) indicating that this protein is a monomer. Furthermore, it behaves as a basic molecule since it does not bind to DEAE-cellulose at pH 7.5.

The results of the amino acid composition are presented in table 2. Profilin contains in particular 2 tryptophans, 3 tyrosines and 1 cysteinyl residue.

Table 1
Summary of the purification of profilin from hog thyroid

Purification step	Protein (mg)
Extract	14 000
Supernatant after $MgCl_2$ treatment	13 000
18–85% Saturation $(NH_4)_2SO_4$	12 500
Sephadex G-100 chromatography	200
Sephadex G-50 fine chromatography	8
DEAE-cellulose chromatography	3.5

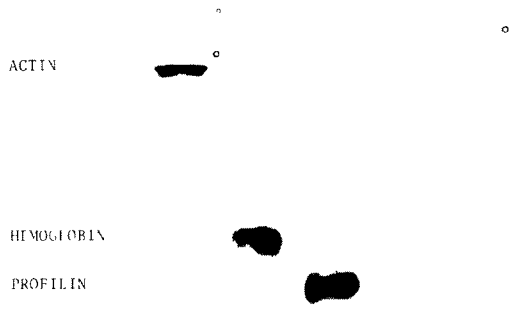

Fig.1. Electrophoresis analysis of thyroid profilin (20 μg) compared to standards: actin (10 μg); hemoglobin (20 μg); SDS–15% polyacrylamide slab gel.

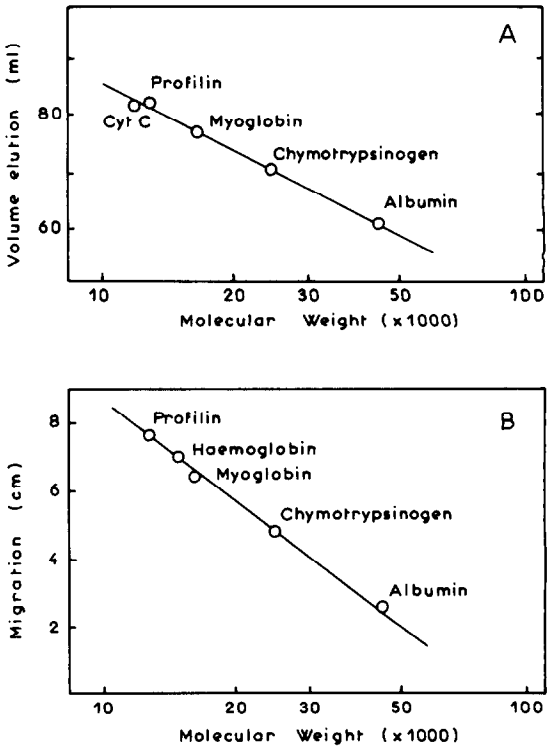

Fig.2. Molecular weight determination of thyroid profilin by: (A) Sephadex G-75 filtration, 0.1 M Tris–HCl, 0.05 mM DTE buffer (pH 7.5), (1 × 100 cm) column. (Standards: cytochrome *c*; myoglobin; chymotrypsinogen; albumin.) (B) SDS–15% polyacrylamide gel electrophoresis. (Standards: hemoglobin; myoglobin; chymotrypsinogen, albumin.)

Table 2
Amino acid composition of hog thyroid profilin, comparison with values reported for profilins from other sources

Amino acid	Hog thyroid profilin		Calf spleen[a] mol%	Human platelets[a] mol%	*Acanthamoeba*[a] mol%
	Residues	mol%			
Asp	11	9.1	10.6	10.7	10.2
Thr	7	5.8	8.5	8.3	8.3
Ser	8	6.6	6.3	8.3	6.5
Glu	15	12.4	6.3	6.9	8.3
Pro	9	7.4	2.8	3.4	5.5
Gly	11	9.1	12.0	12.1	13.0
Ala	10	8.3	7.7	7.6	14.8
Cys	1	0.8	2.1		
Val	6	5.0	7.7	8.3	7.4
Met	2	1.6	4.2	3.1	0.9
Ile	5	4.1	4.9	4.1	4.6
Leu	10	8.3	7.7	10.7	6.5
Tyr	3	2.5	2.8	2.8	2.8
Phe	4	3.3	3.5	2.1	2.8
Try	2	1.6	1.4		
Lys	10	8.3	6.3	6.9	3.7
His	2	1.6	1.4	1.4	0.9
Arg	5	4.1	3.5	3.4	2.8

[a] The data for calf spleen, human platelets and *Acanthamoeba* are from [17], [14] and [13], respectively

3.3. *Interaction of profilin with actin*

To test the biological properties of the 13 K M_r protein, its effect towards the inital rate of monomeric muscle actin polymerisation has been measured spectrophotometrically. As shown in fig.3, profilin reacts with G-actin and inhibits its polymerisation. The level of this inhibition depends on the ratio profilin/G-actin.

In addition, we detected profilinactin during thyroid actin preparation essays according to [10]. After Sephadex filtration G-actin was eluted in two fractions as judged by DNase 1 inhibitory activity [11]. The SDS electrophoresis of the 60 K M_r fraction results in numerous protein bands. Two of them comigrate with actin and profilin, respectively. This fraction was filtered through a Sephadex G-50 fine column and eluted with 2 M urea buffer (see profilin preparation). A low molecular weight fraction was then obtained. It migrates on SDS slab gel as thyroid profilin and under hemoglobin. Consequently, the 60 K M_r fraction could correspond to the complex of actin plus profilin.

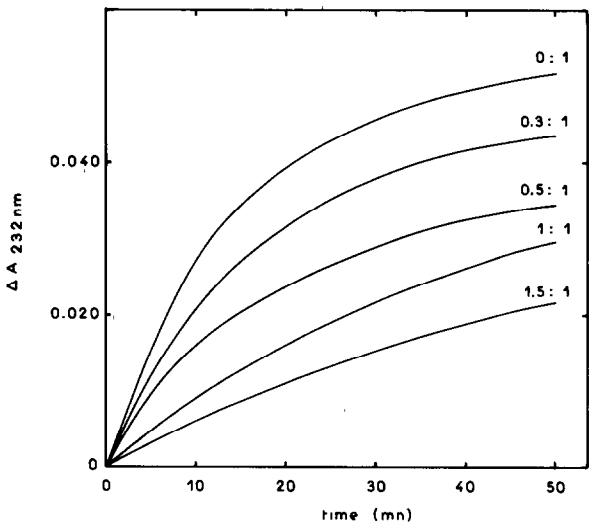

Fig.3. Inhibition of the rate of the polymerisation of the rabbit muscle actin by thyroid profilin. G-actin: 0.28 mg in 0.6 mM imidazole, 4 mM KH_2PO_4, 0.02 mM $CaCl_2$, 0.1 mM DTE, 0.067 mM ATP buffer (pH 7.5) at 25°C. Polymerisation was started by 2 mM $MgCl_2$ and 100 mM KCl and followed by the increase in A_{232}. Profilin: G-actin molar ratio is varied from 0:1 to 1.5:1.

4. Discussion

A small protein exhibiting similar chemical and physiological properties with profilin has been isolated from hog thyroid gland.

The first step of this purification method is based on the preparation of myosin in [12]. It consists in an extraction of thyroid tissue at high ionic strength, then in a precipitation of actomyosin at lower ionic strength (≃0.23) pH 6.4. The supernatant obtained after centrifugation of actomyosin, contains actin under G form as estimated by DNase I inhibitory effect. If we consider the amount of G-actin in this fraction (26 mg/100 g gland) the yield of the preparation of profilin is ~45%, assuming that all no polymerised actin is complexed with profilin. In second step, the profilactin complex is dissociated in 2 M urea buffer and the profilin is directly isolated. Direct purification of *Acanthamoeba* profilin with a similar yield has been reported [13]. Lower recoveries of profilin were obtained when profilactin complex was first isolated [1,14,16]. Like other profilins, thyroid profilin is a monomer protein as judged by filtration on Sephadex G-75 and SDS-polyacrylamide gel electrophoresis. It would be also a basic molecule since it is recovered on DEAE-cellulose at pH 7.5 from the flow-through fraction. Furthermore, its amino acid composition which presents many similarities with those published for calf spleen [17] human platelets [14] and *Acanthamoeba* [13], however differs significantly at the level of glutamic acid and prolin. If one compares the ratio of acidic to basic amino acids for all these profilins it does not differ for thyroid (1.5), spleen (1.5) platelets (1.5) but is higher for *Acanthamoeba* profilin (2.5). Thyroid profilin is found to be ~13 K M_r and therefore are smaller than other mammalian profilins yet known. In contrast, it is closer to *Acanthamoeba* profilin which is 12 K M_r [13]. A possible proteolysis [18] during extraction and purification is not excluded although PMSF was added in all steps of the preparation. Moreover, the isolated protein possesses one of the characteristic properties of profilin as refered to its interaction with G-actin in the thyroid extracts and in the reconstituted system; in fact the purified profilin decreases the rate of G-actin polymerisation under conditions where actin alone would polymerize. The profilactin complex could have an est. K_d ~2 μM assuming a profilin:actin molar ratio in profilactin of 1:1 [1].

Thyroid profilin is the first such protein to be isolated from an endocrine gland. The instability of profilactin underlined in [18] suggests that unknown factors would probably stabilize or destabilize the complex. Our purpose is to identify these factors and to study what role profilin would play in the microfilament rearrangement observed in thyroid cells under TSH stimulation.

References

[1] Carlsson, L., Nystrom, L. E., Sundkirst, I., Markey, F. and Lindberg, U. (1977) J. Mol. Biol. 115, 465–483.
[2] Gabrion, J., Travers, F., Benyamin, Y., Sentein, P. and Thoai, N. V. (1980) Cell Biol. Int. Rep. 4, 59–68.
[3] Martin, F., Gabrion, J. and Pradel, L. A. (1980) Cong. Printemps Soc. Chim. Biol. 28–29 Avril, Montpellier, France, abst.
[4] Spudich, J. A. and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871.
[5] Rich, S. A. and Estes, J. E. (1976) J. Mol. Biol. 104, 777–792.
[6] Bradford, M. M. (1976) Anal. Biochem. 72, 248–254.
[7] Laemmli, U. K. (1970) Nature 227, 680–685.
[8] Opienska-Blauth, J., Charezinski, M. and Berbec, H. (1963) Anal. Biochem. 6, 69–76.
[9] Beaven, G. H. and Holiday, E. R. (1952) Adv. Prot. Chem. 7, 319–386.
[10] Pardee, J. D. and Bamburg, J. R. (1979) Biochemistry 18, 2245–2251.
[11] Blikstad, I., Markey, F., Carlsson, L., Persson, T. and Lindberg, U. (1978) Cell 15, 935–943.
[12] Pollard, T. D., Thomas, S. M. and Niederman, R. (1974) Anal. Biochemistry 60, 258–266.
[13] Reichstein, E. and Korn, E. D. (1979) J. Biol. Chem. 254, 6174–6179.
[14] Markey, F., Lindberg, U. and Eriksson, L. (1978) FEBS Lett. 88, 75–79.
[15] Harris, H. E. and Weeds, A. G. (1978) FEBS Lett. 90, 84–88.
[16] Blikstad, I., Sundkirst, I. and Eriksson, S. (1980) Eur. J. Biochem. 105, 425–433.
[17] Nyström, L. E., Lindberg, U., Kendrick-Jones, J. and Jakes, R. (1979) FEBS Lett. 101, 161–165.
[18] Malm, B., Nyström, E. and Lindberg, U. (1980) FEBS Lett. 113, 241–244.