# DISTRIBUTION OF MYOSIN, ACTIN AND ACTIN-BINDING PROTEIN IN MEMBRANE AND SOLUBLE FRACTION OF HUMAN BLOOD PLATELETS

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## 1. Introduction

Contractile proteins play an important role in a number of platelet functions such as aggregation, clot contraction and serotonin release [1]. The understanding of the molecular mechanism of these basic platelet activities requires the elucidation of the structure, biological activity and localization of the contractile proteins. Both actin [2] and myosin [3] were found in the plasma membrane as well as in the soluble fraction of platelets. Actin-binding protein, another contractile protein with apparent subunit molecular weight of 260 000 was described recently in platelets [4], however its localization was not reported. Considering the role of contractile proteins in platelet function, it seemed of interest to quantitatively determine the distribution of contractile proteins between the membrane and cytosol fractions.

## 2. Experimental procedure

*Platelets*

Blood sample was collected from healthy donors, anticoagulated with 0.32% sodium citrate and platelet rich plasma (PRP) was prepared immediately from the sample by centrifugation at 100 × *g* for 15 min at room temperature. Platelets were collected from the PRP by centrifugation at 10 000 × *g* for 10 min. The sedimented platelets were resuspended in 145 mM NaCl, 4 mM EDTA, 0.3 mM glucose and 10 mM Tris-acetate, pH 7.4 and washed twice in this buffer by centrifugation (10 000 × *g* for 10 min).

*Fractionation of platelets*

Three different methods were used for the fractionation of platelets. The first was osmotic lysis where packed platelets ($\sim 8 \times 10^8$) were resuspended in either 5 mM Na-phosphate, pH 8.0, or in 10 mM Tris-acetate, pH 7.4. After 5-min incubation at 0°C the suspension was centrifuged at 27 000 × *g* for 15 min. The supernatant containing the cytosol fraction was stored and the sedimented membranes were washed twice with the buffer used for the osmotic lysis and finally resuspended in 10 mM Tris-acetate, pH 7.4.

In the second method freeze-thawing was used to rupture the platelets which were then extracted with

0.9 M KCl, 5 mM $MgCl_2$, 2 mM DTT and 30 mM Tris-acetate, pH 7.4 (extraction solution) in ice for 30 min. The suspension was centrifuged (10 000 × *g*, 4°C, 30 min) the supernatant collected and the pellet washed twice and finally resuspended in 10 mM Tris-acetate, pH 7.4.

In the third method the platelet membranes were solubilized by the nonionic detergent Triton X-100. Platelets were subjected to 1% Triton X-100 in 0.35 M sucrose, 1 mM EDTA, 1 mM DTT and 10 mM Tris-acetate, pH 7.4, for 30 min at 4°C. The suspension was centrifuged (10 000 × *g*, 30 min), the extract was collected and the sedimented residue washed twice with the same solution and resuspended in 10 mM Tris-acetate, pH 7.4.

*Electrophoresis*

SDS-polyacrylamide gel electrophoresis was performed according to Porzio and Pearson [5] on 10% gels. The gels were stained for proteins with Coomassie brilliant blue or for glycoproteins with a periodic acid Schiff (PAS) reagent [6] and densitometer scanning was performed by a Kipp and Sonen densitometer. Quantitative densitometry of the gels stained for proteins was carried out in the Quick Scan Densitometer of Helena Laboratories. The relative amounts of myosin (heavy chains only), actin and actin-binding protein was estimated by integrating the area under the peaks of the densitometer scans of at least two Coomassie blue stained gels with different amounts of the same sample. The positions of myosin heavy chain, actin and actin-binding protein were verified by using authentic markers.

*Protein determination*

Protein was determined by the method of Lowry et al [7].

*Assay of myosin ATPase*

K-EDTA activated ATPase activity [8], which is characteristic for the myosin ATPase [1], was assessed in platelet fractions in a reaction mixture containing 600 mM KCl, 5 mM EDTA, 2 mM ATP and 20 mM Tris–HCl, pH 8.0. After incubation at 37°C for 30 min the reaction was stopped with 5% trichloroacetic acid (final concentration) and the liberated inorganic phosphate (Pi) was determined [9]. The enzymic activity was expressed as μmol Pi liberated per mg myosin in the protein sample per min. The amount of myosin in the fraction was estimated from the electrophoregrams.

## 3. Results and discussion

The densitometric analysis of the electrophoregrams of the SDS-polyacrylamide gels of whole platelets revealed that myosin, actin and actin-binding protein comprised 6.61 ± 1.77, 16.2 ± 3.27 and 3.34 ± 1.07 percent of the total platelet protein respectively. Thus myosin, actin and actin-binding protein account for 26.2 percent of the total platelet protein. A much higher value of 60 percent was reported recently by Lucas et al. [4], which included in their calculation also the polypeptide of about 240 000 apparent molecular weight. The discrepancy between our results and those reported above may result from the different gel systems used, and possibly also from the staining and densitometric procedures employed in the two studies.

The distribution of the contractile proteins in the soluble and insoluble fractions which were prepared by several procedures from human blood platelets is presented in table 1, which includes also the K-EDTA activated ATPase of myosin.

Actin, myosin and actin-binding protein were detected in both the soluble and membrane fractions of the platelets. A considerable part of these proteins is tightly bound to the membrane and could not be separated from it even by 1% Triton X-100 treatment which solubilized 86% of the platelet proteins (table 1).

Actin-binding protein could not be detected in the KCl extract of the platelets nor in its residue. A possible explanation for the elimination of the actin-binding protein from the preparations may be the occurrence of a protease activity which was released as a result of freeze-thawing and became activated by high concentration of KCl. Some indication for the role of KCl in this process was provided by the observation that actin-binding protein was found in both the extract and the residue when freeze-thawing of the platelets was followed by extraction with a solution devoid of KCl. Interestingly, a polypeptid with electrophoretic mobility similar to human actin-binding protein was observed in the KCl extract of

Table 1
Distribution of contractile proteins in soluble and insoluble fractions prepared by several procedures from human blood platelets

| Protein | | Fraction | | | | | |
|---|---|---|---|---|---|---|---|
| | | Osmotic lysis | | Freeze and thawing +KCl extraction | | Triton X-100 treatment | |
| | | soluble | residue | soluble | residue | soluble | residue |
| Total protein | Distribution (%) | 49.2 | 50.8 | 45.0 | 55.0 | 85.9 | 14.1 |
| Myosin | Percent of protein in fraction | 4.85 ±0.87 | 8.68 ±0.67 | 8.53 ±2.54 | 6.06 ±1.57 | 5.29 ±0.91 | 6.34 ±1.05 |
| | Percent of myosin in platelet | 35.3 | 64.7 | 53.3 | 46.7 | 83.5 | 16.5 |
| | ATPase specific activity | 0.30 ±0.15 | ,0.19 ±0.07 | 0.26 ±0.12 | 0.13 ±0.03 | 0.25 ±0.09 | 0.083 ±0.043 |
| Actin | Percent of protein in fraction | 18.31 ±3.82 | 14.64 ±2.82 | 16.31 ±1.57 | 15.26 ±1.59 | 12.6 ±0.91 | 17.28 ±2.38 |
| | Percent of actin in platelet | 56.3 | 43.7 | 46.7 | 53.3 | 81.6 | 18.4 |
| Actin-binding protein | Percent of protein in fraction | 4.66 ±1.10 | 2.00 ±0.98 | 0 | 0 | 2.76 ±0.92 | 9.80 ±2.30 |
| | Percent of actin-binding protein in platelet | 66 | 34 | 0 | 0 | 63.1 | 36.9 |

The data are the mean of 27 experiments

the rat platelets (Muhlrad and Eldor unpublished results). The amount of actin-binding protein which associated with the membrane fraction was rather constant (34–37%, table 1) whether the soluble protein was removed by osmotic shock, which removed 50%, or by Triton X-100 treatment, which solubilized 86% of the total platelet protein. This finding may suggest that the membrane bound actin-binding protein differs from the soluble one. Differences between soluble and membrane bound myosins may also be suggested, since the ATPase activity of the former was always higher than the latter independently of the method used for the lysis of the platelets. The possibility that more than one form of myosin and actin-binding protein exists in platelets may be suggested by the results of Abramowitz et al. [10] who showed the presence of two forms of actin in platelets.

Since some evidence was presented for the occurrence of loosely attached glycoproteins on the outer surface of platelets [11,12] polyacrylamide gels of the membrane and the soluble fraction were stained also for glycoprotein by the PAS reagent. Glycoproteins were found only in the membrane fraction

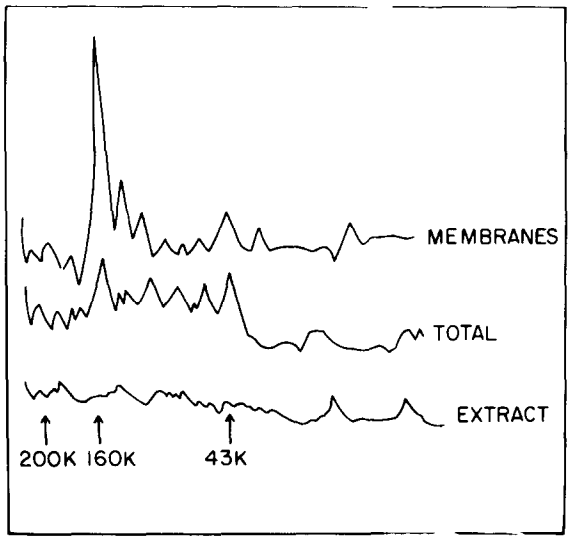


Fig.1. Densitometric tracing of SDS-polyacrylamide gel electrophoregrams of glycoproteins of total platelets, membranes obtained by osmotic shock and KCl extract. The gels were stained for carbohydrates.

and their amount was negligible in the soluble fraction (fig.1). Thus, the present studies are in accordance with a number of investigations and do not support the suggestion of the occurrence of glycocolin [11] or a similar loosely bound membrane glycoprotein [12].

Finally, we may conclude that fairly large amount of contractile proteins are present both in the soluble and membrane compartments of human blood platelets. This large quantity of contractile proteins further stresses their importance in a number of platelet functions which depended on the mobility of these blood elements.

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