GTPγS-induced solubilization of actin and myosin from rabbit peritoneal neutrophil membrane

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Addition of guanosine 5'-(3-*O*-thio)triphosphate (GTPγS) to the membrane fraction isolated from rabbit peritoneal neutrophils results in the solubilization of several proteins from the membrane. The major proteins are of 180 kDa (myosin) and 43 kDa (actin). The effect is observed with a half-maximum GTPγS concentration of 70 μM. The potencies of various nucleotides are compared: GTPγS > GTP > ATP > GDP, GMP, cGMP, cAMP. The effect does not require calcium and is not inhibited by using membranes perpared from cells that have been pretreated with pertussis toxin.

GTPγS *Actin* *Myosin* *Neutrophil membrane*

1. INTRODUCTION

The nonhydrolyzable GTP analogue, guanosine 5'-(3-*O*-thiotriphosphate) (GTPγS), has been used to induce the degranulation of permeabilizing neutrophils [1] and platelets [2] and to demonstrate the role of GTP-binding protein in activating phosphatidylinositol-specific phospholipase C in various systems including neutrophil membranes [3], blowfly salivary gland [4] and liver membranes [5]. In neutrophils, the latter effect is mediated by a pertussis toxin (PT)-sensitive GTP-binding protein (Gp) [6]. Various concentrations of GTPγS, from 10 μM to 1 mM, have been used in these studies.

A novel type of insertion of proteins into biological membranes through phosphatidylinositol linkage has been reported [7]. Since the linkage is sensitive to phospholipase C digestion [7], we reasoned that activation of phospholipase C by Gp may induce dissociation of membrane proteins from the membranes. Here, we have found that addition of GTPγS to the membranes induces the dissociation of actin and myosin from the membranes. However, the effect is not mediated by a PT-sensitive GTP-binding protein in the membrane.

2. MATERIALS AND METHODS

Pertussis toxin was a generous gift from Dr John Munoz; f-Met-Leu-Phe, leupeptin, GTP, GDP, GMP, cGMP, ATP, and cAMP were obtained from Sigma: GTPγS from Boehringer-Mannheim; protein standards for SDS gel electrophoresis from Pharmacia. Buffer A contains 10 mM Hepes, 1 mM EGTA, pH 7.0.

Rabbit peritoneal neutrophils were collected 4–16 h after the intraperitoneal injection of 200–400 ml of 0.1% glycogen in sterile saline. Subcellular fractions were prepared by a slight modification of the methods in [8]. In brief, neutrophils ($2–10 \times 10^9$), were washed in Hanks' buffer (137 mM NaCl, 5 mM KCl, 0.7 mM KH_2PO_4, 10 mM Hepes, 17 mM $NaHCO_3$, pH 7.2). The cells were treated with diisopropyl fluorophosphate [9], centrifuged and suspended in ice-cold washing buffer (11.6% sucrose containing 10 mM Hepes, 1 mM EGTA, pH 7.2) and washed 4 times with the washing buffer. They were

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resuspended in the same buffer and homogenized for 3 min with a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 700 × *g* for 10 min to remove unbroken cells and nuclei and the postnuclear supernatant was centrifuged at 13000 × *g* for 10 min to remove granules. The post-granular supernatant was then centrifuged at 100000 × *g* for 1 h to obtain the cytosol (supernatant) and the membrane (pellet) fractions. The membrane fraction was washed once with buffer A, resuspended in buffer A at a protein concentration of 3–5 mg/ml and used immediately.

In some experiments, cells (10^7 cell/ml, 50 ml) were treated with or without PT (500 ng/ml) at 37°C for 1 h [10] before being subjected to subcellular fractionation. Aliquots of control and PT-treated cells were tested for granule enzyme secretion induced by the chemotactic factor f-Met-Leu-Phe as described [10]. The PT treatment inhibited granule enzyme secretion induced by f-Met-Leu-Phe completely, similarly to what has been reported [10]. The inhibition was paralleled by ADP-ribosylation of a membrane protein of 41 kDa (Gp) [11].

Various concentrations of GTPγS (40 μM to 1 mM) were added to neutrophil membranes (3 mg/ml, 60 μl) in buffer A. After incubation at 37°C for 10 min, the samples were cooled in ice and then centrifuged at 100000 × *g* in a Beckman airfuge for 15 min at 4°C. This procedure has previously been used to isolate membrane pellets in measuring radiolabeled f-Met-Leu-Phe binding to purified membranes [12]. The supernatant (50 μl) was withdrawn and added with 50 μl of 'SDS stopping solution' (9% SDS, 6% mercaptoethanol, 15% glycerol and a trace amount of bromophenol blue dye in 0.186 M Tris-HCl, pH 6.7). Samples were analyzed by SDS gel electrophoresis (7.5% polyacrylamide). Protein bands were stained with Coomassie blue and quantitated by densitometric scanning as described [13]. Protein concentration was determined according to Lowry et al. [14]. Results shown are representative of 2–4 independent experiments.

3. RESULTS AND DISCUSSION

Fig.1 shows that addition of GTPγS to the membrane fraction resulted in an increased amount of several proteins in the supernatant of the centri-

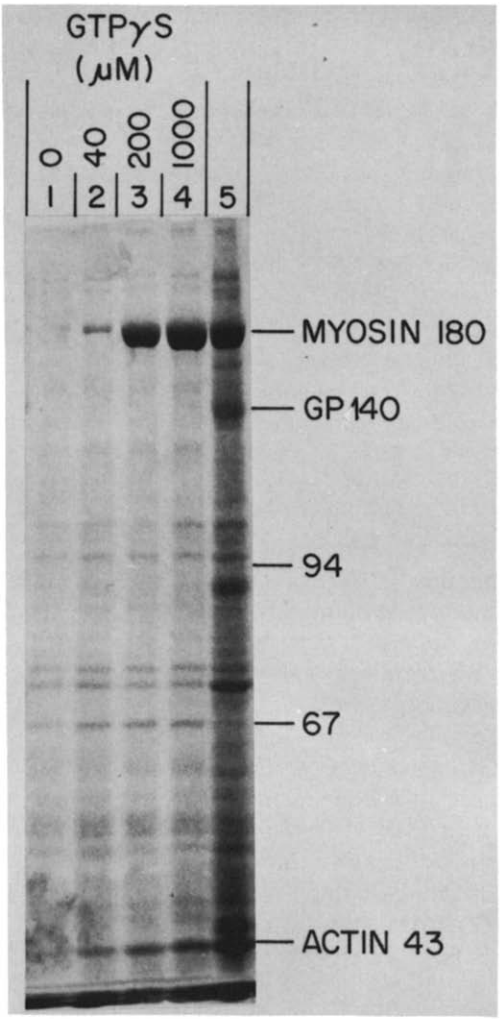

Fig.1. A Coomassie blue-stained gel showing the effect of GTPγS on the dissociation of actin and myosin from rabbit neutrophil membranes. Membrane fractions were treated with various concentrations of GTPγS (lanes: 1, 0 μM; 2, 40 μM; 3, 200 μM; 4, 1 mM) at 37°C for 10 min. Samples were then centrifuged and the supernatant fractions applied to SDS gel electrophoresis as described in the text. Protein bands were stained with Coomassie blue. Lane 5 shows the total membrane protein profile of half of the amount of material used in the experiment. The locations of various protein bands are shown: myosin (180 kDa), a membrane-associated glycoprotein (gp140, 140 kDa) [22], actin (43 kDa) and molecular mass standards (phosphorylase (94 kDa) and bovine serum albumin (67 kDa)).

fuged membrane fraction. Based on their molecular masses and concentrations [13] the major proteins were identified as actin (43 kDa) and myosin (180 kDa). Several minor proteins were also observed. A minor protein of molecular mass similar to acumentin (67 kDa) was detected [14]. A dose-response curve of the effect of GTPγS on myosin is shown (fig.2). The half-maximum concentration of GTPγS for this effect was about 70 μM. The molar ratios of myosin to actin in both the membrane and solubilized fraction were between 0.55 to 0.78. About 37–50% of the membrane-associated actin and myosin was solubilized by GTPγS (1 mM). The effect was not sensitive to the addition of calcium (100 μM), and was not inhibited in membranes prepared from cells pretreated with PT (not shown). Thus, the effect is not likely due to the action of a phospholipase C in the membrane which is regulated by calcium and by a PT-sensitive GTP-binding protein in the neutrophil [3,13].

The effects of various nucleotides on the dissociation of actin and myosin from the membranes are shown in fig.3. The relative potencies are: GTPγS > GTP > ATP; GDP, GMP, cGMP, and cAMP have little effect. Thus, the effect of GTPγS we have observed is different from that of ATP in dissociating the actin-myosin complex in skeletal muscle which is more specific for ATP than GTP [16]. In another experiment (not shown) cytochalasin B (5 μg/ml) had no effect on the

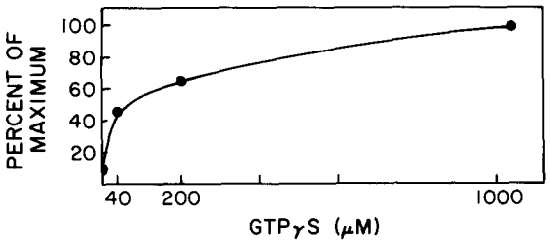

Fig.2. A dose-response curve showing the effect of various concentrations of GTPγS on the dissociation of myosin from the membranes. The amounts of myosin solubilized by various concentrations of GTPγS treatment (shown in fig.1) was estimated by densitometric scanning as described in the text. The amount of myosin solubilized by 1 mM GTPγS was used as the maximum (100%) and was equal to about 50% of the total. A similar curve was obtained when the actin band in fig.1 was used for densitometric scanning.

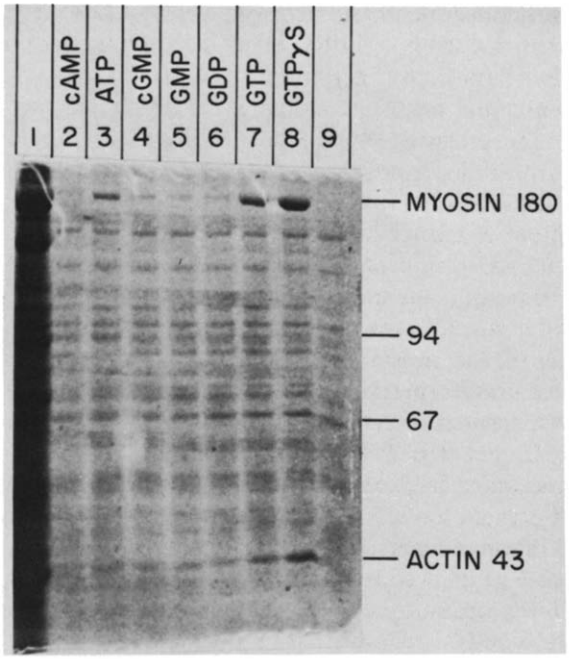

Fig.3. A Coomassie blue-stained gel showing the effect of various nucleotides on the dissociation of actin and myosin from the membranes. Membranes were treated with various nucleotides (100 μM each: lanes: 2, cAMP; 3, ATP; 4, cGMP; 5, GMP; 6, GDP; 7, GTP; 8, GTPγS; 9, control) at 37°C for 10 min. Samples were then centrifuged and the supernatant fraction applied to SDS gel electrophoresis as described in the text. Lane 1 shows the profile of the total membrane protein used in the experiment.

dissociation of actin and myosin from the membrane, suggesting that most of the actin is not from a pool of polymers which undergo polymerization and depolymerization reaction [16]. The exact structure of the actin and myosin in the membrane remains to be studied. The importance of actin and myosin in mediating the function of neutrophils and other nonmuscle cells has recently been reviewed [17,18]. Further studies are required to see whether the GTPγS effect described in this paper is related to changes in the structure of actin, myosin and membrane morphology observed during chemotaxis and phagocytosis [19,20].

The GTP-binding protein which is involved in the activation of membrane phospholipase C can be either PT-sensitive (such as in neutrophils) [6] or PT-insensitive (such as in thyrotropin-releasing

hormone-responsive GH_3 cells) [21]. The exact nature of the proteins which may be involved in the dissociation of actin and myosin from the neutrophil membrane remains to be studied.

The effect of GTPγS on the degranulation of permeabilized neutrophils has been reported [1]. High concentration of GTPγS (up to 1 mM) is required to induce fully the degranulation. Two roles for guanine nucleotide in the stimulus-secretion sequence of neutrophils have been suggested [1]. One is the phospholipase C pathway which is calcium-dependent and PT-sensitive [1,3,6]. The other is calcium-independent and PT-insensitive [1]. Whether the effect of GTPγS on the dissocation of actin and myosin from the membranes may play a role in the calcium-independent and PT-insensitive degranulation of permeabilized cells induced by GTPγS warrants further study.

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REFERENCES

[1] Barrowmann, M.M., Cockcroft, S. and Gomperts, B.D. (1986) Nature 319, 304–307.
[2] Haslam, R.J. and Davidson, M.L. (1984) J. Receptor Res. 4, 605–629.
[3] Cockcroft, S. and Gomperts, B.D. (1985) Nature 314, 534–536.
[4] Litosch, I., Calista, C., Wallis and Fain, J.N. (1986) J. Biol. Chem. 261, 638–643.
[5] Uhing, R.J., Prpic, V., Jiang, H. and Exton, J.H. (1986) J. Biol. Chem. 261, 2140–2146.
[6] Smith, C.D., Lane, B.C., Kusaka, I., Verghese, M.W. and Snyderman, R. (1985) J. Biol. Chem. 260, 5875–5878.
[7] Low, M.G. and Kincade, P.N. (1985) Nature 318, 62–64.
[8] Huang, C.-K., Hill, J.M., Bormann, B.-J., Mackin, N.M. and Becker, E.L. (1983) Biochim. Biophys. Acta 760, 126–135.
[9] Armein, P.C. and Stossel, T.P. (1980) Blood 56, 442–448.
[10] Becker, E.L., Kermode, J.C., Naccache, P.H., Yassin, R., Marsh, M.L., Munoz, J.J. and Sha'afi, R.I. (1985) J. Cell Biol. 100, 1641–1646.
[11] Volpi, M., Naccache, P.H., Molski, T.F.P., Shefcyk, J., Huang, C.-K., Marsh, M.L., Becker, E.L. and Sha'afi, R.I. (1985) Proc. Natl. Acad. Sci. USA 82, 2708–2712.
[12] Mackin, W.M., Huang, C.-K., Bormann, B.-J. and Becker, E.L. (1983) Anal. Biochem. 131, 430–437.
[13] White, J.R., Naccache, P.H. and Sha'afi, R.I. (1983) J. Biol. Chem. 258, 14041–14047.
[14] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[15] Southwick, F.S. and Stossel, T.P. (1981) J. Biol. Chem. 256, 3030–3036.
[16] Korn, E.D. (1982) Physiol. Rev. 62, 672–737.
[17] Geiger, B. (1983) Biochim. Biophys. Acta 737, 305–320.
[18] Stossel, T.P., Chaponnier, C., Ezzell, R.M., Hartwig, J.H., Janmey, P.A., Kwiatkowski, D.J., Lind, S.E., Smith, D.B., Southwick, F.S., Yin, H.L. and Zaner, K.S. (1985) Annu. Rev. Cell Biol. 1, 353–402.
[19] Davies, B.H., Walter, R.J., Pearson, C.B., Becker, E.L. and Oliver, J.M. (1982) Am. J. Pathol. 108, 206–216.
[20] Valerius, N.H., Stendahl, O.I., Hartwig, J.H. and Stossel, T.P. (1981) Cell 24, 195–202.
[21] Martin, T.F.J., Lucas, D.O., Bajjalieh, S.M. and Kowalchyk, J.A. (1985) J. Biol. Chem. 260, 2918–2927.
[22] Williams, D.J. and Becker, E.L. (1984) J. Leukocyte Biol. 35, 71–90.