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Purification and subunit composition of a GTP-binding protein from maize root plasma membranes

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When frozen plasma membranes isolated from maize seedling roots are thawed, a significant portion of GTP-binding activity goes into solution The GTP-binding protein was purified by ion exchange chromatography on Mono-Q and gel filtration on Superose 6 Its molecular weight was estimated at 61 kDa by gel filtration The same molecular weight was obtained upon solubilization of the GTP-binding protein with cholic acid followed by gel filtration in the presence of this detergent SDS-PAGE demonstrated that the isolated GTP-binding protein consists of two types of subunit of molecular weights 27 kDa and 34 kDa

Maize, GTP-binding protein, Isolation, Subunit

1 INTRODUCTION

Higher plants have recently been found to possess GTP-binding activity [1–3] At the same time it has been shown that the plasmalemma of higher plants contains polypeptides that interact with antibodies against a peptide having an amino acid sequence typical for animal GTP-binding proteins [1,2] These data point to the probable existence of GTP-binding proteins in higher plants, but their subunit composition, kinetic properties, and function are still unknown.

To isolate and purify higher-plant GTP-binding proteins, we first tried to make use of the techniques designed for their animal counterparts The usual protocol includes solubilization with sodium cholate and multistage chromatography with detergents [4] While optimizing the conditions for solubilizing the GTP-binding proteins from maize plasmalemma we found out, however, that when membranes stored at −70°C in sucrose buffer are thawed, a significant portion of GTPase activity is solubilized without any detergent. The absence of detergents from chromatographic solutions allowed substantial simplification of the purification procedure for the GTP-binding protein, which proved pure enough for elucidation of its subunit structure

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2 EXPERIMENTAL

2 1 *Reagents*

[γ-^{32}P]GTP (10 Ci/mmol) was from Amersham, [^{35}S]GTPγS (1200 Ci/mmol) from New England Nuclear

2 2 *Plasma membranes*

Plasma membranes were isolated from the roots of maize cv Nadnepryanskaya 4-day seedlings according to [3] Isolated membranes were suspended in 5 mM Tris-HCl, pH 7 3, 0 33 M sucrose and stored at −70°C

2 3 *G-protein solubilization*

Plasma membranes (5–8 mg protein) were thawed, diluted with 20 vols of 20 mM Tris-HCl, pH 8 1, 1 mM EDTA, 1 mM DTT, 0 25 M sucrose (TED buffer), and centrifuged for 30 min at 45 000 rpm in an SW-50 rotor at 4°C The supernatant was subjected to further purification

2 4 *Chromatography*

Chromatography was performed with a Pharmacia FPLC System The solubilized protein was applied on a Mono-Q HR 10/10 column, washed with 2 vols of TED buffer and eluted with a linear 0–1 M NaCl gradient in the same buffer Fractions (2 ml) having GTPase or GTP- binding activity were pooled and rechromatographed in a flatter gradient (0–100 mM NaCl) Active 1-ml fractions were pooled to 4 ml, concentrated on Bio-Rad Ultracent 10 membrane filters, and applied on a Superose 6 HR 10/30 column equilibrated with TED buffer containing 0 1 M NaCl. The sample was eluted with the starting buffer at 0 4 ml/min, fractions of 0 4 ml having GTP-binding activity were pooled, mixed with ethylene glycol (final concentration 20% v/v), and stored at −20°C For molecular weight determination the Superose 6 column was calibrated using Pharmacia gel filtration standards

2 5 *Electrophoresis*

SDS electrophoresis was carried out according to Laemmli [5] in 12% polyacrylamide gels, which were stained with silver [6]

2 6 *GTPase assay*

The protein (0 5 μg) was incubated for 30 min at 30°C in 100 μl of 20 mM Tris HCl, pH 7 5, 1 mM EDTA, 1 mM DTT, 5 mM $MgCl_2$,

0 5 μM [γ-^{32}P]GTP, and 0 1 mM App (NH)p The amount of inorganic phosphate liberated was determined as in [7]

2 7 *Binding assay*

Binding between G-proteins (20–50 μl) and [^{35}S]GTPγS was carried out in 100–200 μl of 25 mM HEPES, pH 7 2, 1 mM DTT, 25 mM $MgCl_2$ containing 2 5 nM [γ-^{35}S]GTP and 10 μM App(NH)p Free label was removed on Sephadex G-50 columns [3]

3. RESULTS

Tabel I shows the data on solubilization of GTP-binding proteins. As can be seen, treatment of the plasmalemma with 1% sodium cholate solubilizes practically all GTP-binding activity. On the other hand, upon thawing of frozen plasmalemmal preparations 10–20% of the GTP-binding activity is recovered in the solution We first tried to purify the GTP-binding protein from the cholate-solubilized fraction. Fig. 1 shows the profile of protein elution from a Superose 6 column equilibrated with cholate-containing buffer. The GTP-binding activity is eluted as a single peak with a retention time corresponding to a 58 kDa protein Further attempts to purify the protein by ion exchange or hydrophobic chromatography failed mainly because of enzyme inactivation.

Greater success was achieved in purifying the GTP-binding protein from the fraction obtained by simple thawing. Fig 2 shows protein separation by ion exchange chromatography on a Mono-Q column. The fraction containing both GTPase and GTP-binding activities is eluted as a single peak at 0 04 M NaCl. This fraction was rechromatographed, concentrated, and applied onto Superose 6 column, pre-calibrated in molecular weight As shown in Fig. 3, the GTP-binding activity elutes as a single peak with retention time corresponding to a mol wt of 61 kDa.

Fig. 4 presents the gel-electrophoretic data testifying that the isolated GTP-binding protein comprises at least

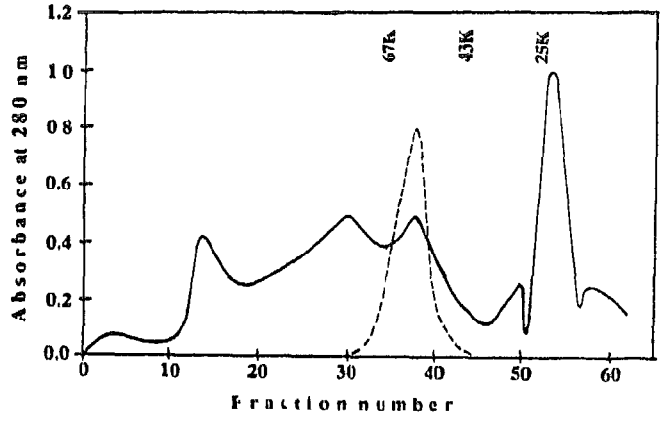

Fig 1 Gel filtration of a specimen obtained by solubilizing the plasmalemma with sodium cholate. Two milligrams of protein were incubated in 300 μl of TED buffer with 1% cholate, centrifuged for 30 min at 45 000 rpm in a SW-50 rotor, and applied to a column equilibrated with the same buffer. --- (dashed line), denotes GTP binding

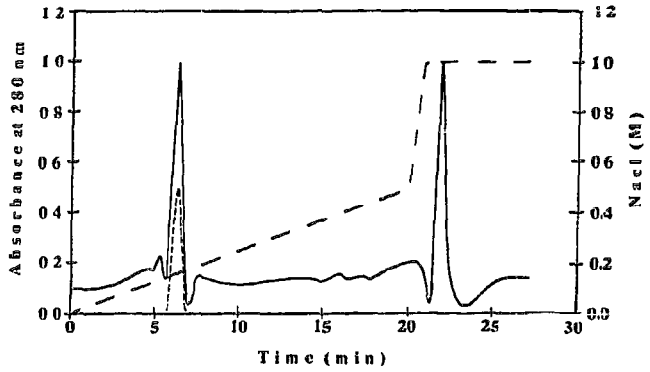

Fig 2 Mono-Q column chromatography Dashed line denotes GTPγS binding

two subunits of 34 kDa and 27 kDa Moreover, the 34 kDa band is clearly split, which can be due either to proteolysis of the α subunit during isolation or to its heterogeneity in maize root plasmalemma

4 DISCUSSION

In animal cells the GTP-binding proteins can be divided in two classes by their subunit composition One class comprises proteins that are most directly involved in the transmembrane signalling These GTP-binding proteins consist of 3 dissimilar subunits, α,β and γ, with molecular weights of 59–39 kDa, 35–24 K, and 8–3 K, respectively [8] The other class includes proteins of a single 20 kDa subunit [7] whose cell functions are not yet clear

Gel electrophoresis of the GTP-binding protein that we have isolated from maize root plasmalemma reveals two types of subunit. α(34 kDa) and β(27 kDa), the sum of their mol wts. equals the mol wt of the protein determined by gel filtration (61 kDa) It cannot be excluded that the isolated protein also contains a γ subunit, because electrophoretic conditions used here do not permit reliable identification of a polypeptide with a presumed mol wt of less than 8 kDa. At the same time, the coincidence of mol wts obtained upon cholate

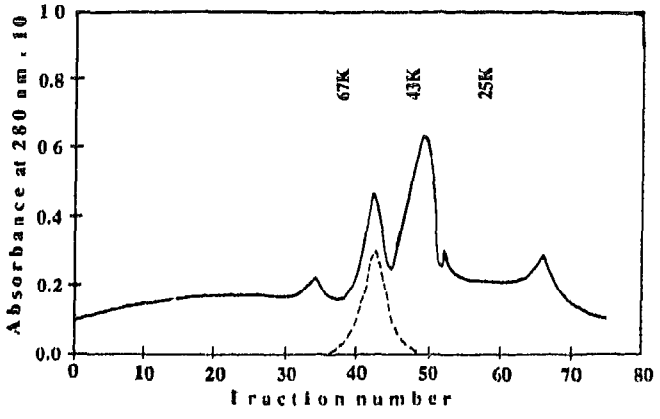

Fig 3 Superose 6 column chromatography Dashed line denotes GTPγS binding

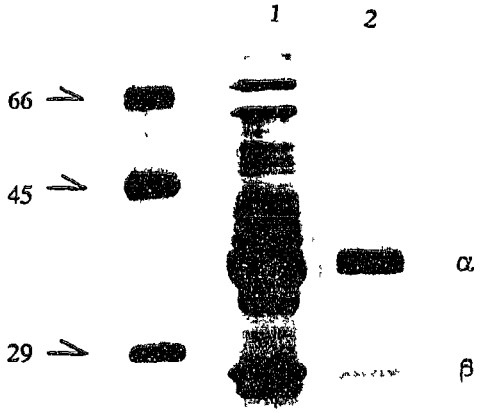

Fig 4 SDS-PAGE electrophoresis (1) after Mono-Q chromatography and (2) after Superose 6 chromatography

treatment and thawing indicates that upon thawing the GTP-binding protein is apparently solubilized without loss of subunits

The α subunit mol. wt agrees nicely with that of the polypeptide detected in higher plants with antibodies to a peptide carrying a conserved amino acid sequence typical for the α subunits of animal GTP-binding proteins [2]. These data together with the plasmalemmal localization and the subunit structure (at least two subunits) of the GTP-binding protein that we have isolated from maize roots argue in favor of the suggestion that it participates in the transmembrane signalling.

Table I

Solubilization of GTP-binding proteins from maize root plasmalemma

	Solubilization	
	Cholic acid	Thawing
Protein (%)	35	5
[^{35}S]GTPγS-binding (%)	50–90	8–17
GTPase activity (%)	40–60	10–17

The data are expressed as percentage of protein, GTPγS binding, and GTPase activity in the post-membrane supernatant of solubilized sample to the total values prior to solubilization

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