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Variability in electrophoretic mobility of G_i-like proteins: effect of SDS

JoLynda Jones, Gregory S. Kopf* and Richard M. Schultz

Department of Biology and **Division of Reproductive Biology, University of Pennsylvania, Philadelphia, PA 19104, USA*

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Two forms of G_i-like protein are resolved in both somatic cells and mouse gametes when Sigma SDS (95% grade) is used during polyacrylamide gel electrophoresis, whereas only a single species is resolved when Bio-Rad SDS (electrophoresis grade) is used. These two G_i-like proteins are likely to reflect two distinct species, since (i) the two species resolved in the presence of Sigma SDS migrate with the same electrophoretic mobility upon re-electrophoresis in the presence of Sigma SDS and (ii) exchanging Sigma SDS for Bio-Rad SDS resolves a single species, whereas exchanging Bio-Rad SDS for Sigma SDS resolves two species.

G-protein; ADP-ribosylation; Pertussis toxin; Electrophoresis; SDS

1. INTRODUCTION

G_i-proteins are heterotrimeric GTP-binding proteins involved in the production of second messengers and regulation of ion channels [1,2]. Three subclasses of $G_{i\alpha}$ subunits, $G_{i\alpha 1}$, $G_{i\alpha 2}$, and $G_{i\alpha 3}$, have been identified and are separate gene products that may prove to be functionally distinct [3,4]. We are examining the temporal pattern of expression of G_i-like proteins in mouse embryos, as detected by pertussis toxin-catalyzed ADP-ribosylation of α_i-like subunits followed by one-dimensional polyacrylamide gel electrophoresis in the presence of SDS. During the course of these studies, we detected two radiolabeled bands in preparations obtained from mouse eggs and sperm, and normal rat kidney (NRK) cells. This result contrasts with a single band reported by others for mouse sperm (M_r 41000) [5] and NRK preparations (M_r 41000) (Woolkalis, personal communication). We report here that this difference is most likely due to differences in the purity of SDS obtained from different commercial sources.

Correspondence address: R. Schultz, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104-6018, USA

2. MATERIALS AND METHODS

2.1. *Preparation of mouse gamete extracts and somatic cell membranes*

2.1.1. Mouse eggs

Eggs were collected from superovulated mice (CF-1, 6 weeks old; Harlan) and freed of cumulus cells by hyaluronidase treatment as described [6]. The ovulated eggs were washed in bicarbonate-free minimal essential medium (Earle's salts) containing pyruvate (100 μg/ml), gentamicin (10 μg/ml), polyvinylpyrrolidone (3 mg/ml), 25 mM Hepes, pH 7.2 (MEM/PVP-bicarbonate) [7] and then homogenized in MEM/PVP-bicarbonate containing 1% Lubrol PX, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μM phenylmethylsulfonyl fluoride, and 1 mM *p*-aminobenzamidine.

2.1.2. Mouse sperm

Caudal epididymal sperm was collected and Lubrol PX extracts were prepared as described in [5].

2.1.3. NRK and chick embryo fibroblasts (CEF)

Membrane preparations of NRK cells and CEF were prepared as in [8,9].

2.2. *Pertussis toxin catalyzed ADP-ribosylation*

Pertussis toxin (0.12 mg/ml) was activated in the presence of 0.15% SDS purchased from either Sigma (St. Louis, MO) or

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Bio-Rad Laboratories (Richmond, CA) [5,9]. The Sigma 95% grade SDS (catalogue no.L5750) contains about 70% lauryl sulfate and the balance consists of higher carbon chain homologs. The Bio-Rad SDS (catalogue no.161-0301) was electrophoresis grade, which is 99.9% pure. The ADP-ribosylation assay was conducted as reported [5,9] using either ovulated egg homogenate (9 μg protein), sperm Lubrol PX extract (5 μg protein), or NRK or CEF crude membranes (2, 1, 0.5 or 0.05 μg protein), except that 3 mM dimyristoylphosphatidylcholine was present. Radiolabeled NAD was synthesized essentially by the method of Cassel and Pfeuffer [10], except that an ATP-regenerating system (pyruvate kinase and phospho*enol*pyruvate) was included. The final concentration of [^{32}P]NAD in the reaction mixture was 0.27 μM (5.28 $\times$ 10^6 cpm/pmol). The reaction was initiated by addition of 5 μl of the activated pertussis toxin to the 25 μl assay volume, the samples incubated at 30°C for 60 min, and the reaction terminated by addition of 4 $\times$ sample buffer [11]. When pertussis toxin was omitted from the reaction mixture, the ADP-ribosylated species of M_r 38000–43000 were not observed (not shown).

2.3. *Gel electrophoresis, autoradiography, and electroelution of ADP-ribosylated proteins*

The radiolabeled samples were subjected to polyacrylamide gel electrophoresis (10% gels) according to Laemmli [11]; the SDS used in the gel and running buffer was either Bio-Rad or Sigma SDS. Molecular masses were calculated using stained standards from Sigma.

ADP-ribosylated NRK membrane preparations (2 μg) were subjected to gel electrophoresis using Sigma SDS as described above and autoradiography was performed on wet, unfixed gels. The two resolved radiolabeled proteins of M_r 38000 and 39000 were then cut out and the proteins electroeluted at 3 W for 1.5 h using running buffer containing Sigma SDS [11]. When the Bio-Rad SDS was used, the single band of M_r 41000 was similarly processed and Bio-Rad SDS used in the electroelution buffer.

2.4. *Exchange of SDS from G_i-like subunits*

The samples obtained from electroelution, which were in a volume of 0.2 ml, were applied to a 0.2 ml column of Extracti-Gel D (Pierce, Rockford, IL), which selectively binds SDS but not proteins. In addition to removing SDS present in the buffer, the Extracti-Gel D also removes SDS bound to proteins. The column was washed with water and fractions containing the radiolabeled proteins were pooled, re-applied to a second Extracti-Gel D column, and eluted under identical conditions. The radiolabeled eluates were pooled and dried under reduced pressure in a Speed-Vac. The samples were then resuspended in Laemmli sample buffer [11] containing either Bio-Rad or Sigma SDS and subjected to electrophoresis in polyacrylamide gels containing either Bio-Rad or Sigma SDS as described above.

3. RESULTS

Pertussis toxin-catalyzed ADP-ribosylation of membrane preparations from NRK cells and mouse sperm revealed a single pertussis toxin substrate of M_r 41000 and 42000, respectively, when the radiolabeling and gel electrophoresis were performed with Bio-Rad SDS (fig.1A). CEF revealed the presence of two pertussis toxin substrates of M_r 41000 and 43000 when Bio-Rad SDS was used (fig.1A). Mouse eggs revealed a single species of slightly lower M_r (M_r 41000) when compared to sperm. In contrast, when these experiments were performed using Sigma SDS, resolution of two pertussis toxin substrates was obtained with NRK cells (M_r 38000, 39000) (fig.1B), which was also observed with several other lots of Sigma 95% grade SDS (not shown). In addition, mouse egg and sperm preparations revealed two pertussis toxin substrates when Sigma SDS was used, and both substrates in mouse eggs (M_r 38000, 39000) were again lower in M_r than those substrates in sperm (M_r 39000, 39500) (fig.1B). The CEF again revealed a doublet (M_r 39000, 41000). It should be noted that in each case a slightly lower M_r was obtained in the presence of Sigma SDS. In addition, a greater degree of separation for the CEF doublet was observed with the Sigma SDS (fig.1B).

The basis for this difference in electrophoretic mobility was likely to be due to differences in the SDS preparations. Both the upper and lower bands, which were resolved in the presence of Sigma SDS, migrated as a single species of the same initial M_r following electroelution and re-electrophoresis of each band in the presence of Sigma SDS (fig.2A). In contrast, the single species resolved in the presence of Bio-Rad SDS was split into a doublet following electroelution and re-electrophoresis in the presence of Sigma SDS

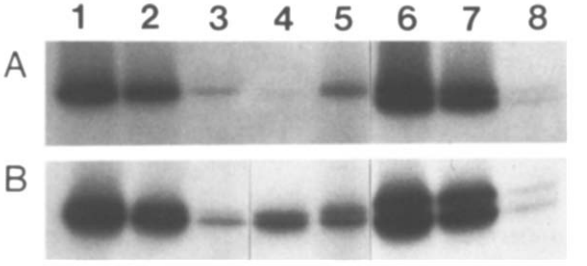

Fig.1. Regions of autoradiograms of pertussis toxin catalyzed ADP-ribosylation of somatic cells and mouse gametes. The experiment was performed as described in section 2. (A) Bio-Rad SDS. (B) Sigma SDS. Lanes: (1–3) 1, 0.5 and 0.05 μg protein of NRK membranes, respectively; (4) mouse eggs; (5) mouse sperm; (6–8) 1.0, 0.5 and 0.05 μg protein of CEF membranes, respectively.

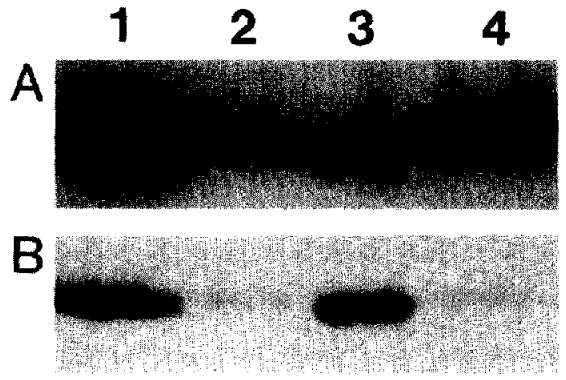

Fig.2. Regions of autoradiograms of pertussis toxin catalyzed ADP-ribosylation of NRK membranes following exchange of SDS. The experiment was performed as described in section 2; 2 μg of protein were used. (A) Electrophoresis in the presence of Sigma SDS. For lanes 1–3, upper and lower bands were resolved in the presence of Sigma SDS, SDS removed by Extracti-Gel D chromatography, and subjected to electrophoresis in the presence of Sigma SDS. Lanes: (1) resolved upper and lower bands pooled; (2) resolved upper band; (3) resolved lower band; (4) single band resolved in presence of Bio-Rad SDS was isolated, SDS removed by Extracti-Gel D chromatography, and subjected to electrophoresis in the presence of Sigma SDS; note the doublet, which is similar to that observed in lane 1. (B) Electrophoresis in the presence of Bio-Rad SDS. For lanes 1–3, upper and lower bands were resolved in the presence of Sigma SDS, SDS removed by Extracti-Gel D chromatography, and subjected to electrophoresis in the presence of Bio-Rad SDS. Lanes: (1) resolved upper and lower bands pooled; (2) resolved upper band; (3) resolved lower band; (4) single band resolved in presence of Bio-Rad SDS was isolated, SDS removed by Extracti-Gel D chromatography, and subjected to electrophoresis in the presence of Bio-Rad SDS.

(fig.2A). In addition, both the upper and lower bands resolved in the presence of Sigma SDS migrated as a single species following separation of the lower and upper bands and re-electrophoresis of each band in the presence of Bio-Rad SDS (fig.2B). The single NRK species obtained in the presence of Bio-Rad SDS migrated as single species following electroelution and electrophoresis in the presence of Bio-Rad SDS (fig.2B).

4. DISCUSSION

We resolve two species of G_i-like proteins from both somatic and germ cells when Sigma 95% grade SDS is used, but only observe a single species, as have others ([5], Woolkalis, personal communication) when Bio-Rad electrophoresis grade SDS is used. These two G_i-like proteins are likely to reflect two distinct species, since (i) the two species resolved in the presence of Sigma SDS migrate with the same electrophoretic mobility upon re-electrophoresis in the presence of Sigma SDS and (ii) exchanging Sigma SDS for Bio-Rad SDS resolves a single species, whereas exchanging Bio-Rad SDS for Sigma SDS resolves two species.

The basis for this difference is most likely the source of SDS. Different sources of SDS contain variable chain length homologs of SDS and this has been shown to correlate with alterations in both the resolution and M_r estimation of viral polypeptides [12]. Sigma 95% grade SDS contains significant amounts of C_{14-16} species, whereas the Bio-Rad SDS contains much less of these species. These differences in composition may allow differential interactions of the multiple chain lengths present in the Sigma SDS with the different forms of G_i-like proteins and this could result in the observed differences in electrophoretic mobility. In contrast, the more uniform composition of the Bio-Rad SDS may mask these differences and result in the resolution of only a single species. Consistent with this hypothesis is the observation that when other commercially available sources of high purity SDS (>99%) are used for electrophoresis, a single G_i-like species is resolved for mouse sperm (Kopf, G.S., unpublished).

Differential effects of SDS on the resolution of two other GTP-binding proteins – sea urchin sperm guanylate cyclase ([13] and Ward, G.E., personal communication) and sheep brain tubulin [14] – have been reported; resolution of the different forms is only observed with the less pure Sigma SDS. This is attributed, at least in the case for the tubulins, to the presence of C_{14} and C_{16} chain lengths in the Sigma SDS, and these homologs are at much reduced concentrations in the more purified SDS preparations [14]. Presumably, the differences in composition of the SDS, i.e. the greater abundance of higher chain length homologs present in the Sigma SDS, result in differences in the interaction of the detergent with different forms of these proteins and leads to the observed changes in electrophoretic mobility.

Multiple forms of G_i-like proteins are identified by pertussis toxin catalyzed ADP-ribosylation [1,2] and these proteins are coded for by an ever-growing multigene family [3,4]. The ability to resolve further multiple forms of G_i-like species

with different preparations of SDS is consistent with this multigene family, although we cannot exclude the possibility that the differences are due to post-translational modifications (see [13]). In either case, our results suggest that caution should be exercised in considering the type of SDS used when determining the types of G-proteins detected by ADP-ribosylation and polyacrylamide gel electrophoresis.

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