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ENHANCEMENT OF *CLOSTRIDIUM BOTULINUM* C3-CATALYSED ADP-RIBOSYLATION OF RECOMBINANT rhoA BY SODIUM DODECYL SULFATE

INGO JUST,*† CHRISTIANE MOHR,*‡ BARBARA HABERMANN,‡ GERTRUD KOCH* and KLAUS AKTORIES*‡

*Institut für Pharmakologie und Toxikologie der Universität des Saarlandes, D-6650 Homburg; and ‡Rudolf-Buchheim-Institut für Pharmakologie, D-6300 Gießen, Federal Republic of Germany

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Abstract—The influence of sodium dodecyl sulfate (SDS) on ADP-ribosylation by *Clostridium botulinum* C3 exoenzyme (C3) was studied. SDS increased the ADP-ribosylation of recombinant rhoA and human platelet cytosolic proteins maximally at 0.01% whereas higher concentrations of the detergent (>0.01%) inhibited the ADP-ribosylation. In contrast, ADP-ribosylation of human platelet membranes and of recombinant rhoB was inhibited by the detergent. The K_m for NAD of the ADP-ribosylation of rhoA was decreased by SDS from about 10 to 0.6 μM. Whereas in the absence of SDS, the C3-induced ADP-ribosylation of recombinant rhoA is not affected by the amphiphilic wasp venom mastoparan, in the presence of SDS (0.01%) mastoparan (100 μM) inhibited the ADP-ribosylation. C3-associated NAD-glycohydrolase activity was maximally and half-maximally inhibited by 0.1 and 0.013% SDS, respectively. Inhibition of NAD-glycohydrolase activity was reversed by diluting out SDS indicating that C3 was not irreversibly denatured by SDS treatment. SDS (0.01%) completely inhibited the [^{3}H]GTP binding of rhoA whereas the release of previously bound nucleotide was not affected. The data indicate that changes in the lipophilicity of rhoA protein largely affect its ability to serve as a substrate for C3-like ADP-ribosyltransferases.

GTP-binding proteins are substrates for various ADP-ribosylating toxins. Diphtheria toxin and *Pseudomonas* exotoxin A ADP-ribosylate elongation factor 2 thereby inhibiting protein synthesis (for reviews see Refs 1–3). Cholera toxin, *Escherichia coli* heat-labile toxins and pertussis toxin modify heterotrimeric GTP-binding proteins (G-proteins) which are involved in transmembrane signal transduction (for reviews see Refs 4–6). A family of bacterial exoenzymes ADP-ribosylate low relative molecular mass (M_r 21,000) GTP-binding proteins. Members of this family are *Clostridium botulinum* C3 exoenzyme (C3§) [7–9] and the recently described *C. limosum* ADP-ribosyltransferase [10], the epidermal cell differentiation inhibitor produced by *Staphylococcus aureus* [11] and an exoenzyme from *Bacillus cereus* [12]. Substrates of these exoenzymes are the rho proteins (rhoA,B,C) [13–16]. The precise function of these small GTP-binding proteins is unknown. However, some evidence exists that the rho proteins are involved in the regulation of the microfilament network [17–20].

ADP-ribosylation of small GTP-binding proteins catalysed by C3 has been shown to be regulated by various cytoplasmic factors [21] and/or by detergents [22]. So far the site and the mechanism of action of these factors and compounds are not clear. Studies on the C3-induced ADP-ribosylation with cell lysates or with partially purified small GTP-binding proteins are difficult to interpret because these preparations usually contain various C3 protein substrates (rhoA,B,C). Therefore, we were prompted to use *E. coli*-expressed recombinant rho proteins to investigate the interaction of sodium dodecyl sulfate (SDS) with the C3-catalysed ADP-ribosylation reaction. Here we report that SDS largely enhances C3-catalysed ADP-ribosylation but inhibits GTP binding to recombinant rhoA.

MATERIALS AND METHODS

Materials. Mastoparan was obtained from Sigma (Deisenhofen, F.R.G). Nucleotides were obtained from Boehringer (Mannheim, F.R.G.). [^{32}P]NAD and [^{3}H]GTP were purchased from NEN (Bad Homburg, F.R.G.). All other chemicals were analytical grade and purchased from commercial sources. ADP-ribosyltransferase C3 was purified as described [7, 23].

Preparation of rho proteins. The rhoA and B proteins were expressed in *E.coli* as described earlier [17, 24] and were partially purified as described for ras proteins. Briefly, *E.coli* lysate supernatant was dialysed overnight in buffer A [30 mM Tris–HCl (pH 7.6), 1 mM dithiothreitol, 10 mM $MgCl_2$, 1 mM NaN_3] and applied to a Q-Sepharose column (equilibrated in buffer A). The protein was eluted with a gradient of sodium chloride (0–400 mM NaCl in buffer A). The rho-containing fractions were pooled, concentrated to about 1 mL and applied to a G 75-Sephadex column equilibrated in buffer B,

† Corresponding author: Dr Ingo Just, Institut für Pharmakologie und Toxikologie der Universität des Saarlandes, 6650 Homburg-Saar, F.R.G. FAX (49) 6841 166402.

§ Abbreviations: SDS, sodium dodecyl sulfate; C3, *Clostridium botulinum* C3 exoenzyme; SDS–PAGE, SDS–polyacrylamide gel electrophoresis; GDI, guanine nucleotide dissociation inhibitor; ARF, ADP-ribosylation factor.

containing 100 mM NaCl, 10 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride. The column was eluted with buffer B and rho-containing fractions were pooled, concentrated and stored in aliquots at −80°. Some of the experiments were repeated with recombinant rhoA protein purified to more than 95% homogeneity from a glutathione *S*-transferase fusion protein by thrombin treatment of glutathione-agarose-bound fusion protein [20]. The rho protein from porcine brain cytosol was purified as described [14]. The amount of GTP-binding proteins present in the individual preparations used was determined by the [^{3}H]GTP-binding activity.

Preparation of human platelet membranes and platelet cytosol. One day outdated human platelet concentrates (kindly donated by Dr Stute, Winterberg Kliniken, Saarbrücken, F.R.G.) were centrifuged for 5 min at 400 *g* at room temperature. The platelets of the supernatant were pelleted by centrifugation for 15 min at 2500 *g*. Then the platelets were washed twice with a buffer containing 140 mM NaCl, 5 mM EDTA and 10 mM triethanolamine-HCl (pH 7.4). The washed platelets were resuspended in the same volume of lysis buffer containing 5 mM EDTA and 10 mM triethanolamine-HCl (pH 7.4) and immediately frozen in liquid nitrogen. For preparation of the platelet membranes and cytosol fraction, the lysate was thawed and centrifuged for 15 min at 30,000 *g*. The supernatant was centrifuged for 60 min at 100,000 *g*, frozen in liquid nitrogen and stored in aliquots at −80°. Before use in the ADP-ribosylation assay, the cytosolic fraction was thawed and dialysed against 10 mM triethanolamine-HCl (pH 7.4) for 12 hr. The membrane pellet was washed twice with lysis buffer and once with 10 mM $MgCl_2$ and 10 mM triethanolamine-HCl (pH 7.4). The final pellet was resuspended in 10 mM $MgCl_2$ and 10 mM triethanolamine-HCl (pH 7.4), and frozen in liquid nitrogen. Before use the platelet membranes were washed once in 10 mM triethanolamine-HCl.

ADP-ribosylation assay. The ADP-ribosylation by C3 was performed essentially as described before [23, 25]. The rho protein preparations (5–10 pmol of rho determined by its GTP-binding activity) or the platelet membrane/cytosol preparations (50 μg of protein) were incubated in a buffer containing 50 mM triethanolamine-HCl (pH 7.5), 2 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 0.1 μM [^{32}P]-NAD (about 0.3 μCi), 300 μM GDP, 10 μg bovine serum albumin, 0.15 μg C3 and the indicated concentrations of SDS (%, w/v) for 10 min at 37°. The total volume was 100 μL. The reaction was terminated by the addition of 1 mL trichloroacetic acid (30%, w/v). The precipitates were filtered onto nitrocellulose membranes. The membranes were washed with 20 mL trichloroacetic acid (6%, w/v) and counted for radioactivity. The filter blank was 0.1–0.5% of added [^{32}P]NAD and was subtracted from retained radioactivity. For SDS–polyacrylamide gel electrophoresis (SDS–PAGE), the incubation was stopped by addition of 1 mL trichloroacetic acid (30%, w/v) and the mixture was placed on ice for 30 min. The pellet was collected by centrifugation (10 min, 13,000 *g*) washed with ether and finally dissolved in 25 μL sample buffer for SDS–PAGE according to Laemmli [26].

For studies on the effects of mastoparan on the C3-induced ADP-ribosylation of rhoA, the reaction mixture contained partially purified porcine brain rho protein (~10 pmol) [14] or recombinant rhoA protein (~10 pmol), 30 μM GTPγS, 1 mM EDTA, 2 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 50 mM triethanolamine-HCl (pH 7.4) and 0.1 μM [^{32}P]NAD (0.2 μCi/mL) in a total volume of 50 μL. The reaction was performed in the absence and presence of mastoparan (100 μM) and SDS (0.01%) and was initiated by the addition of C3 (0.25 μg/mL). After incubation for 10 min at 30°, the reaction was stopped by addition of sample buffer and the proteins were directly subjected to SDS–PAGE.

NAD-glycohydrolase assay. NAD-glycohydrolase activity of C3 was determined in a buffer containing 100 mM triethanolamine-HCl (pH 7.5), 1 mM $MgCl_2$, 1 μM [^{32}P]NAD, 25 μg/mL C3 exoenzyme and SDS as indicated. Total volume was 50 μL. After incubation for 4 hr at 37°, aliquots of the reaction mixture were spotted onto Silical Gel 60 TLC (Merck, Darmstadt) and developed in 60% isopropanol plus 0.3% ammonium sulfate. The NAD and ADP-ribose spots were detected by autoradiography, scraped off the TLC and counted for radioactivity.

For studies on the reversibility of the SDS-induced inhibition of the NAD-glycohydrolase activity, 750 μg/mL C3 was incubated in the absence and presence of 0.03% SDS for 2 hr at 37°. Thereafter, the samples were diluted to give a final concentration of 25 mg/mL C3 and 0.001% SDS. The control samples which were preincubated in the absence of SDS were supplemented with 0%, 0.01% and 0.03% SDS (w/v), respectively and the NAD glycohydrolase assay was performed for 4 hr at 37° as described.

GTP-binding assay. The rho protein preparation (5–10 pmol rho) was incubated in a buffer containing 50 mM triethanolamine-HCl (pH 7.5), 1 mM EDTA, 2 mM $MgCl_2$, 1 mM dithiothreitol, 100 μg bovine serum albumin, 1 mM phenylmethylsulfonyl fluoride, 5 mM sodium chloride and about 2 μCi [^{3}H]GTP (30.4 Ci/mmol) in the presence and absence of the indicated concentrations of SDS. The total volume was 1 mL. At the indicated times, two 100 μL aliquots were placed in 1 mL of ice-cold washing buffer containing 50 mM Tris–HCl (pH 7.5), 50 mM NaCl and 5 mM $MgCl_2$. The samples were immediately filtered onto nitrocellulose membranes, washed four times with 4 mL of washing buffer and counted for radioactivity. The filter blank was 0.1–0.5% of added [^{3}H]GTP and was subtracted from retained radioactivity. When the nucleotide release was studied, GTP, SDS or GTP plus SDS was added to the reaction mixture to give a final concentration of 1 mM GTP and 0.01% SDS, respectively. The release reaction was terminated at the indicated times as described for the nucleotide-binding assay.

All experiments were performed at least three times. The results shown are representative of a typical experiment performed in triplicate.

RESULTS

We studied the influence of various detergents on

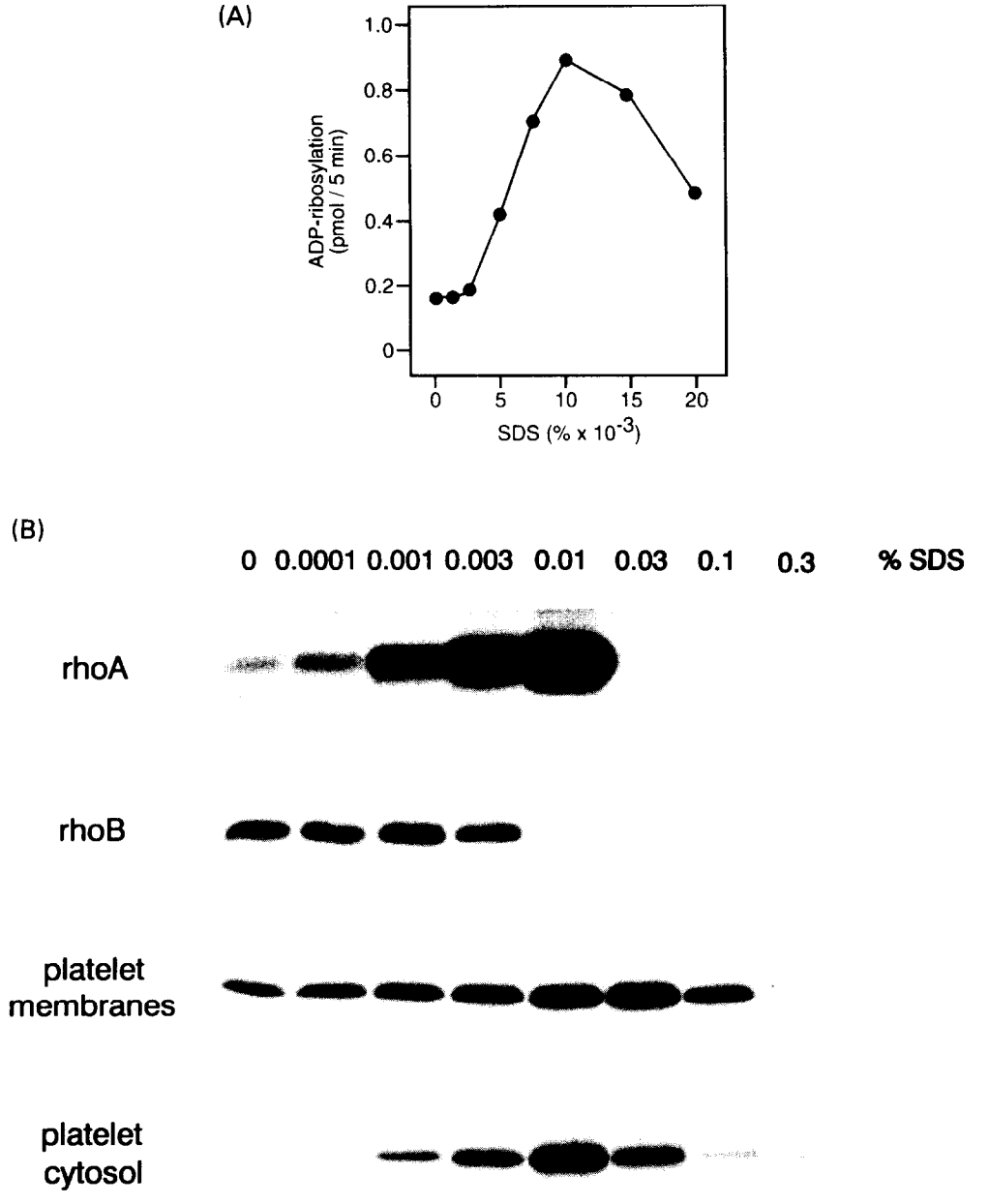

Fig. 1. Enhancement of C3-induced ADP-ribosylation by SDS. (A) Recombinant rhoA (about 5 pmol) was incubated in the presence of increasing concentrations of SDS (%, w/v) with 0.1 μM [^{32}P]NAD and 0.15 μg C3 for 5 min. The amount of incorporated [^{32}P]ADP-ribose was determined by the filter method. (B) Recombinant rhoA (5 pmol), rhoB (5 pmol), human platelet membranes (50 μg) and human platelet cytosol (50 μg) were ADP-ribosylated in the presence of increasing concentrations of SDS (%, w/v) with 0.1 μM [^{32}P]NAD and 0.15 μg C3 for 5 min. Thereafter, the labeled proteins were analysed by SDS–PAGE and autoradiography (shown).

the C3-induced ADP-ribosylation of rho. Whereas cholate (0.05–0.5%), deoxycholate (0.05–0.5%), Lubrol PX (0.005–0.05%) and Triton X-100 (0.005–0.05%) were only weak stimulators, SDS largely enhanced the ADP-ribosylation of recombinant rhoA by C3. In Fig. 1 the concentration dependence of the effect of SDS on the ADP-ribosylation of recombinant rhoA is depicted. The maximal stimulatory effect was observed at 0.01% SDS with about a 7-fold increase in [^{32}P]ADP-ribose incorporation. Higher concentrations decreased the ADP-ribosylation and at about 0.05% SDS the ADP-ribosylation reaction was completely blocked (Fig. 1). Similar results were obtained with either partially purified recombinant rhoA or with recombinant rhoA purified to more than 95% homogeneity from the rhoA-glutathione fusion protein (not shown).

In contrast to rhoA, the SDS-induced increase in ADP-ribosylation was not observed with rhoB (Fig. 1B). Moreover, SDS at concentrations (0.01%) that maximally stimulated ADP-ribosylation of rhoA,

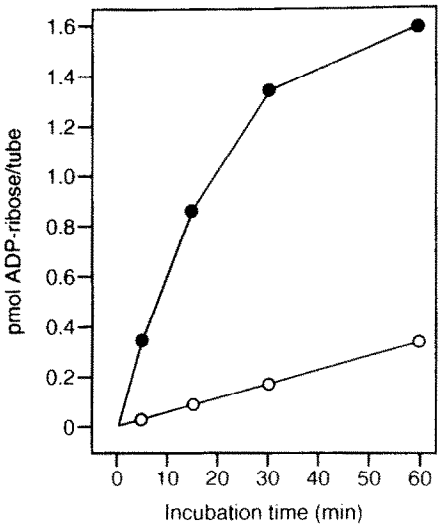

Fig. 2. Time course of the C3-induced ADP-ribosylation of recombinant rhoA in the absence and presence of SDS. Recombinant rhoA was ADP-ribosylated without (○) and with (●) SDS (0.01%, w/v) in the presence of 0.1 μM [^{32}P]-NAD and 0.15 μg C3 for the indicated periods of time. Thereafter, the amount of protein-incorporated [^{32}P]ADP-ribose was determined by the filter assay.

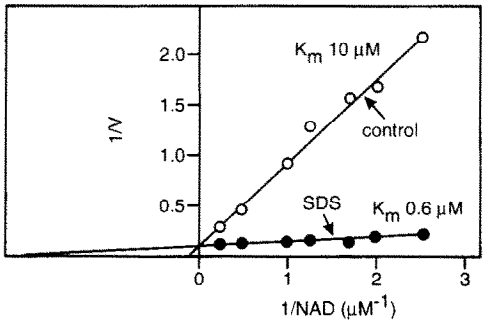

Fig. 3. Effects of SDS on the NAD dependence of the C3-induced ADP-ribosylation of recombinant rhoA. C3-induced ADP-ribosylation of recombinant rhoA was studied with increasing concentrations of NAD in the absence (○) and presence (●) of 0.01% SDS. The incorporation of [^{32}P]ADP-ribose was determined with the filter assay as described in Materials and Methods. The indicated K_m values were extrapolated from the Lineweaver–Burk plot (shown).

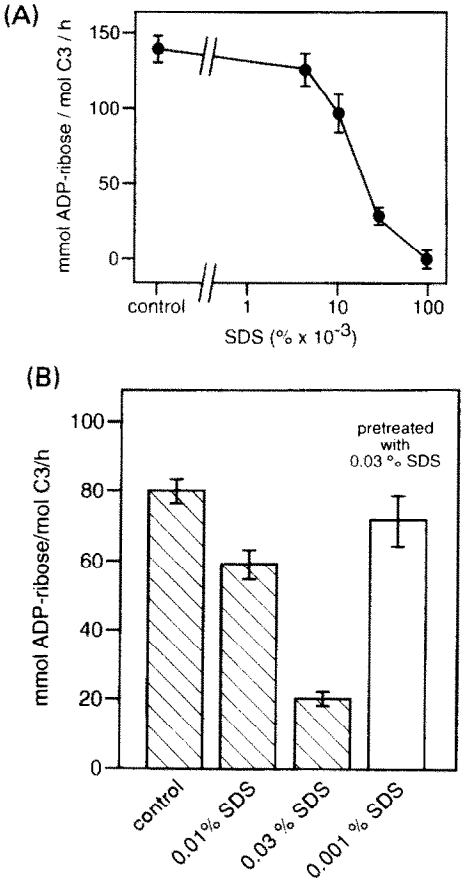

Fig. 4. Influence of SDS on the NAD glycohydrolase activity of C3. (A) 1 μM [^{32}P]NAD was incubated for 4 hr in the presence of 1 μM C3 with the indicated concentrations of SDS. (B) C3 was incubated for 2 hr in the absence and in the presence of 0.03% SDS. Thereafter, the samples were diluted 30-fold. The samples which were preincubated in the absence of SDS were supplemented to give 0 (control), 0.01 and 0.03% SDS and, thereafter, the NAD-glycohydrolase activity was determined as described in Materials and Methods.

decreased the incorporation of ADP-ribose into recombinant rhoB. Almost no stimulatory effect was observed when the ADP-ribosylation of human platelet membranes was performed in the presence of the detergent (Fig. 1B). However, SDS inhibited ADP-ribosylation of proteins in platelet membranes at concentrations higher than 0.3%. Similar to recombinant rhoA, ADP-ribosylation of platelet cytosolic proteins by C3 was increased in the presence of SDS with a maximal effect at 0.01% (Fig. 1B).

The time course of ADP-ribosylation in the presence and absence of SDS (0.01%) revealed that the detergent increased the initial rate of the ADP-ribosylation of rhoA (Fig. 2). In order to study the mechanism underlying the SDS-induced increase in ADP-ribosylation, we tested the influence of the detergent on the NAD dependence of the reaction. As shown in the Lineweaver–Burk plot (Fig. 3), the recombinant rhoA protein exhibited a K_m for NAD of about 10 μM. In the presence of SDS (0.01%), the K_m decreased to about 0.6 μM. Interestingly, a similarly low K_m for NAD is also observed for the ADP-ribosylation of C3 substrates in platelet membranes [10].

Since the observed effects could be due to an activation of C3 by SDS, we studied the influence of the detergent on the NAD glycohydrolase activity of the exoenzyme. SDS did not increase but rather inhibited the NAD glycohydrolase activity of C3. Figure 4A shows that half-maximal and maximal inhibition of C3-induced NAD hydrolysis occurred at about 0.013 and 0.1% SDS, respectively. Apparently, the inhibition of the NAD glycohydrolase activity of C3 was not caused by denaturation of the transferase by SDS. As shown in Fig. 4B, 0.01 and 0.03% SDS reduced NAD-glycohydrolase activity by about 25 and 75%, respectively. In contrast, when C3 was pretreated

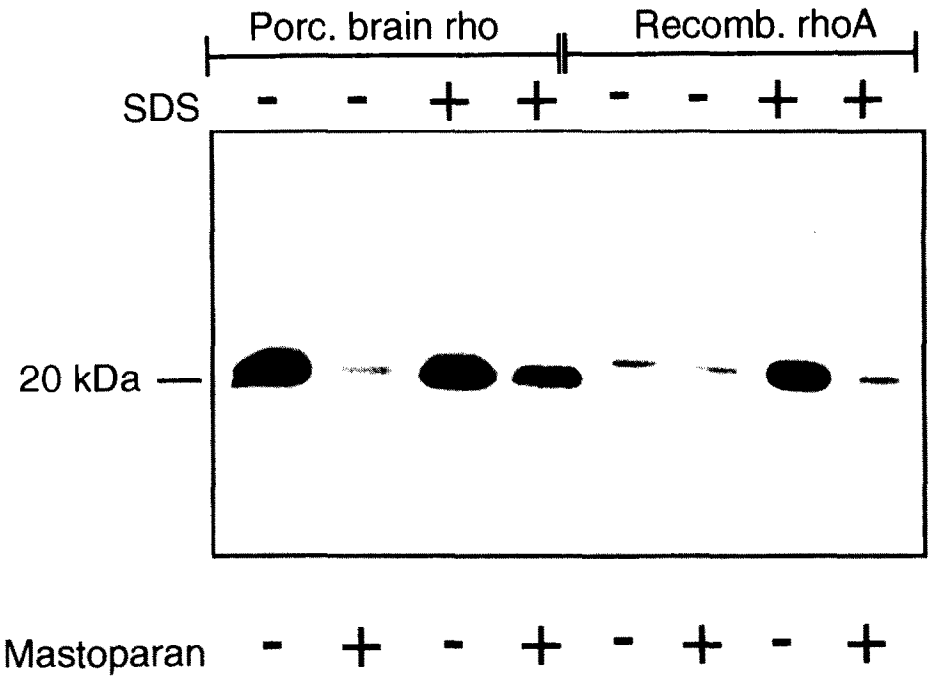

Fig. 5. Comparison of the effect of mastoparan on ADP-ribosylation of porcine brain rho protein or recombinant rhoA in the absence and presence of SDS. rho proteins were ADP-ribosylated without and with mastoparan (100 μM) in the absence and presence of SDS (0.01%, w/v) as described in Materials and Methods.

with 0.03% SDS for 2 hr and the reaction mixture was subsequently diluted to give a final concentration of 0.001% SDS, the NAD glycohydrolase activity was not significantly reduced. Furthermore, pretreatment of C3 with 0.03% SDS for 2 hr followed by dilution of SDS (final concentration 0.001%) did not decrease ADP-ribosyltransferase activity of C3 (not shown).

Recently, it has been shown that mastoparan, a basic amphiphilic tetradecapeptide from wasp venom, inhibits C3-induced ADP-ribosylation of endogenous rho proteins but not of recombinant rhoA protein [27]. We studied whether SDS treatment influences the effects of mastoparan on the C3-induced ADP-ribosylation of rho proteins. Figure 5 shows that mastoparan (100 μM) reduced the ADP-ribosylation of porcine brain rho by C3 in the absence and presence of 0.01% SDS. In contrast, ADP-ribosylation of recombinant rhoA was affected only very slightly by mastoparan in the absence of SDS. However, in the presence of 0.01% SDS, which increased ADP-ribosylation by C3, mastoparan largely inhibited the C3-catalysed incorporation of ADP-ribose into recombinant rhoA protein.

Next we studied whether SDS affects the binding of [^{3}H]GTP to recombinant rhoA protein (Fig. 6). Under the conditions used, maximal binding of [^{3}H]-GTP was observed after 60 min (Fig. 6A). However, in the presence of SDS at a concentration which maximally increased ADP-ribosylation (0.01% SDS), the binding of [^{3}H]GTP was completely inhibited. Figure 6B shows the concentration dependency of the detergent's effects on [^{3}H]GTP-binding. The binding of [^{3}H]GTP to recombinant rhoA was determined after 60 min in the absence and presence of increasing concentrations of SDS. SDS affected GTP binding in the same concentration range which was found to enhance ADP-ribosylation with half-maximal inhibition of GTP binding at about 0.017% SDS. The influence of SDS on the release of [^{3}H]GDP/GTP from rhoA is depicted in Fig. 6C. After maximal binding of [^{3}H]GTP, the time course of the GDP/GTP release was studied in the absence and presence of SDS. Whereas under control conditions the release of [^{3}H]GTP was initiated by the addition of a high concentration of unlabeled GTP (1 mM), in the presence of SDS (0.01%) the release of [^{3}H]GDP/GTP was observed even without addition of unlabeled GTP. Interestingly, the combination of GTP (1 mM) plus SDS (0.01%) did not enhance the release of prebound labeled GTP/ GDP.

As it is known that nucleotide-free small GTP binding proteins are rather unstable, we were prompted to study the influence of prolonged SDS treatment of rho protein on its ability to serve as a substrate for C3. Figure 7 shows that after pretreatment of the recombinant rho protein with SDS (0.01%) for 60 min, subsequent ADP-ribosylation by C3 was largely inhibited. The decrease in ADP-ribosylation and the release of GTP from rhoA followed a similar time course.

DISCUSSION

Here we studied the influence of SDS on the C3-induced ADP-ribosylation of rho proteins. The detergent affected the ADP-ribosylation of recombinant rhoA protein in a biphasic manner with

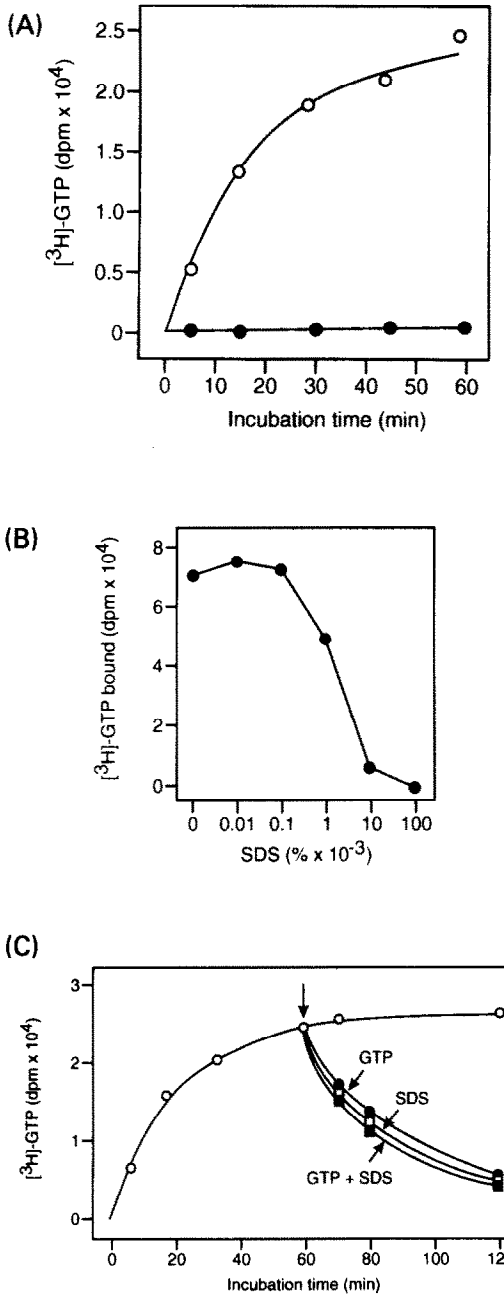

Fig. 6. Effect of SDS on the [^{3}H]GTP binding of recombinant rhoA. The binding of [^{3}H]GTP to rhoA and the release of prebound [^{3}H]GTP was studied as described in Materials and Methods. (A) Time course of the binding of [^{3}H]GTP onto recombinant rhoA in the absence and presence of SDS. Recombinant rhoA (0.4 pmol) was incubated with 66 nM [^{3}H]GTP in the presence (●) and absence (○) of 0.01% SDS for the indicated period of time. (B) Concentration dependency of inhibition of [^{3}H]-GTP binding by SDS. Recombinant rhoA (1.2 pmol) was incubated for 60 min in the presence of increasing concentrations of SDS. (C) Time course of [^{3}H]GTP binding to and release of [^{3}H]GTP/GDP from rhoA. Recombinant rhoA (0.4 pmol) was incubated with [^{3}H]-GTP (66 nM). The nucleotide release was initiated by addition of 1 mM GTP (●), 0.01% SDS (□) or GTP plus SDS (■) and determined after the indicated periods of time.

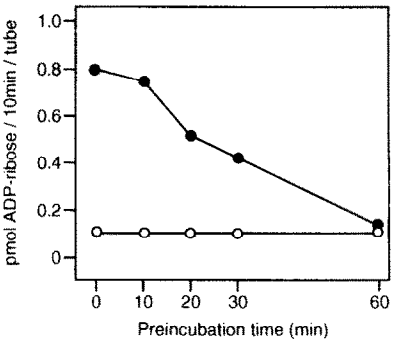

Fig. 7. Inhibition of ADP-ribosylation after preincubation of rhoA with SDS. Recombinant rhoA (5 pmol) was pretreated without (○) and with 0.01% SDS (●) for the indicated periods. Thereafter, ADP-ribosylation was initiated by the addition of 0.15 μg C3 and 0.1 μM [^{32}P]-NAD, and the reaction was continued for 10 min. Incorporated [^{32}P]ADP-ribose was determined by the filter assay.

increase at low (0.01%) and decrease at higher (>0.01%) concentrations of SDS. An increase in the C3-catalysed ADP-ribosylation has been reported for other detergents like cholate, deoxycholate, Chaps and Lubrol-PX [22]. In our hands, SDS was the most effective detergent in enhancing the C3-catalysed ADP-ribosylation of recombinant rhoA.

At concentrations which increased ADP-ribosylation, SDS inhibited C3-associated NAD glycohydrolase activity. This inhibition was not caused by SDS-induced denaturing of C3 because the NAD-glycohydrolase activity of C3 was restored after reducing the SDS concentration. As C3-associated NAD glycohydrolase was inhibited rather than stimulated, the enhancement of ADP-ribosylation by SDS is probably not caused by the activation of C3. It is more likely that SDS favors the formation of a ternary complex of C3, rho and NAD, which results in the enhanced ADP-ribosylation of rho protein. In line with this hypothesis is the observation that the initial rate of the ADP-ribosylation reaction was increased and that SDS treatment was accompanied by about a 20-fold decrease in the apparent K_m value for NAD to about 0.6 μM without affecting the V_{max} of the reaction. Interestingly, we determined a similar low K_m for NAD for the ADP-ribosylation of the C3 substrate(s) in platelet membranes in the absence of SDS [10]. One can speculate that SDS provides a microenvironment that favors a conformation of the recombinant rhoA protein that is similar to endogenous rho. In this respect, our finding that SDS renders recombinant rho protein sensitive towards mastoparan is of relevance. Recently, it was shown in our laboratory that the amphiphilic agent mastoparan facilitates the guanine nucleotide exchange and inhibits C3-induced ADP-ribosylation of endogenous rho proteins but not that of recombinant rho [27, 28]. In the presence of SDS, however, mastoparan, effectively inhibited the ADP-ribosylation of recombinant rhoA as found for endogenous rho protein.

The ADP-ribosylation of recombinant rhoB protein was not enhanced but rather decreased by SDS. Both rho proteins are about 85% homologous

and differ mainly at their C-terminus [29, 30]. Whereas rhoB possesses several cysteine residues at the C-terminus, rhoA is characterized by polybasic regions at the C-terminus which may be important for interaction with the anionic detergent.

The reason for the differences in the effects of SDS on platelet membrane and platelet cytosolic proteins is not clear. The rho proteins expressed in eukaryotic cells differ from recombinant proteins in their post-translational modification including poly-isoprenylation [25, 31] and (probably in the case of rhoB) acylation at the C-terminal end as known for the H- and N-ras proteins [32]. These post-translational modifications increase the hydrophobicity of the GTP-binding proteins and are important for their interactions with regulatory proteins like the guanine nucleotide dissociation inhibitor (GDI) [33, 34]. Similarly, poly-isoprenylation may be relevant for the interaction of rho proteins with the transferase and/or with detergent and can explain why the ADP-ribosylation of the endogenous membrane bound rho was not affected by SDS. At least two explanations are feasible for the SDS-induced increase in the ADP-ribosylation of platelet cytosolic rho proteins. Firstly, a considerable amount of the cytosolic rho proteins may lack the post-translational modification and, secondly, the cytosolic rho proteins which form complexes with other regulatory proteins are ADP-ribosylated only after disruption of the complex by SDS. The latter view is favored by the recent finding by Kikuchi *et al.* [35] that the rho–GDI complex is a poor substrate for ADP-ribosylation.

SDS treatment largely affected the binding of [^{3}H]GTP to the rhoA protein. In fact, at low concentrations of SDS ($IC_{50} \sim 0.017\%$), which increased the ADP-ribosylation by C3, the binding of GTP to recombinant rhoA was blocked. In contrast, the dissociation rate of previously bound nucleotide was affected only very slightly by the detergent at similar concentrations, which inhibited binding. Thus, SDS apparently did not increase the release of nucleotide, but completely blocked the binding of GTP to rho protein. This inhibition of nucleotide binding may be the reason for the SDS-induced inhibition of ADP-ribosylation of recombinant rho after prolonged treatment of the protein with the detergent for up to 60 min. The time course of the decrease in ADP-ribosylation and the release of nucleotide from rhoA protein were comparable. One can speculate that the release of the nucleotide renders the rhoA protein sensitive towards inactivation by SDS.

The mechanism of the action of SDS is not clear. The SDS concentration (0.01% = 350 μM) used was below the critical micelle concentration which is 2–10 mM depending on salt and temperature [36]. These low concentrations have been shown to inhibit protein kinase C activity [37] or to stimulate NADPH-oxidase in cell-free leukocyte extracts [38]. Furthermore, SDS at concentrations of 50–100 μM stimulates activation of cholera toxin by the ADP-ribosylation factor (ARF) [39–41]. In the latter case SDS decreased the K_m for NAD and also for the protein substrate [39]. Furthermore, the detergent largely affected the GTP-binding activity of ARF by inhibiting the high affinity binding of GTP to ARF [40, 41].

Taken together, the data presented indicate that SDS markedly increases the ADP-ribosylation of recombinant rhoA and affects its ability to bind nucleotides. These findings support the view that changes in the lipophilic/hydrophilic properties of rho are involved in the regulation of this small GTP binding protein.

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