Dinitrogenase Reductase-Activating Glycohydrolase Can Be Released from Chromatophores of *Rhodospirillum rubrum* by Treatment with MgGDP

AGNETA NORÉN AND STEFAN NORDLUND*

Department of Biochemistry, Arhenius Laboratories for Natural Sciences, Stockholm University, S-106 91 Stockholm, Sweden

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In the nitrogen-fixing phototroph *Rhodospirillum rubrum*, some other phototrophs, and some *Azospirillum* species, nitrogenase, the enzyme catalyzing the reduction of dinitrogen to ammonia (2), is regulated on both the genetic and the metabolic levels. In phototrophs this metabolic control is manifested as a reversible decrease in nitrogenase activity, the “switch-off” effect (14), when effectors such as ammonium, asparagine, or glutamine are added or when cells are transferred to the dark (5). At the molecular level it has been established that the switch-off effect is due to mono-ADP-ribosylation of dinitrogenase reductase (11), one of the two proteins comprising the nitrogenase complex. This reaction is catalyzed by dinitrogenase reductase ADP-ribosyltransferase (DRAT), whereas the activation, i.e., the removal of the ADP-ribosylation by dinitrogenase reductase (11), one of the two proteins comprising the nitrogenase complex. This reaction is catalyzed by dinitrogenase reductase ADP-ribosyltransferase (DRAT), whereas the activation, i.e., the removal of the ADP-ribosylation by dinitrogenase reductase (DRAT). Upon addition of switch-off effectors, DRAT is activated and DRAG is inactivated (5). We have previously suggested that an increase in NAD+ concentration could be the internal signal for DRAT activation, leading to modification (inactivation) of dinitrogenase reductase (8, 9, 12). The identity of the internal signal regulating DRAG activity is, however, still an unresolved question. When cells are disrupted, the DRAG protein is found to be associated with the chromatophore membrane fraction, but it can be released by the addition of 0.5 M NaCl (4, 7). To investigate if this association is of physiological significance, we have studied the effect of nucleotides on the association of DRAG with the membranes.

*R. rubrum* cells were grown under nitrogen-fixing conditions with N₂ as the nitrogen source (10) and subjected to switch-off by the addition of 2 mM ammonium chloride 30 min prior to harvest. After being harvested, the cells were resuspended in 50 mM Tris-Cl (pH 7.6). One-milliliter aliquots of this chromatophore suspension were transferred to anaerobic vials and incubated (143,000 × g, 60 min), and the supernatants obtained were examined for DRAG activity. As shown in Table 1, incubation with 10 mM MgGDP led to the release of DRAG activity into the supernatant (about 40% of the activity obtained when chromatophores were treated with 0.5 M NaCl). The total recovery of DRAG activity in the 0.5 M NaCl supernatant was about 50% of that in the chromatophore suspension. The activity obtained with the other nucleotides studied, ATP, ADP, and GTP, was no higher than that obtained when only buffer was added. Furthermore, magnesium ions alone had no effect, whereas the addition of only GDP in some experiments led to detectable but much lower release of activity (data not shown), which could be due to the presence of manganese in the buffer or possibly to magnesium ions bound to the chromatophores. If the release of DRAG was due merely to the increase in ionic strength caused by the

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* Corresponding author. Mailing address: Department of Biochemistry, Arhenius Laboratories for Natural Sciences, Stockholm University, S-106 91 Stockholm, Sweden. Phone: 46 8 16 29 32. Fax: 46 8 15 77 94. E-mail: stefan@biokemi.su.se.
additions, it would be expected that ADP and GDP would have similar effects and that ATP and GTP would be more efficient, neither of which is the case. There was also no effect by NAD$^+$ or NADH (data not shown).

Since DRAG activity is measured indirectly as the capability to activate inactive dinitrogenase reductase, the effect of MgGDP on the in vitro assay was investigated. At concentrations higher than 1 mM, which is the highest concentration of GDP that was present during activity measurements due to carryover from the incubation mixture, GDP inhibited the in vitro activity (Table 2). To investigate if the stimulatory effect of GDP was due to interaction with some other protein(s) solubilized from the chromatophores, GDP was added to the supernatant after centrifugation instead of before. An acetylene reduction assay was run for 30 min with 1.2 mg of nitrogenase (DEAE pool), and nitrogenase activity (nanomoles of C$_2$H$_4$ formed) was as follows: with no additions, 26 nmol; with 50 mM Tris-Cl (pH 7.8), 43 nmol; with 10 mM MgGDP added to the supernatant after centrifugation, 36 nmol; and with 10 mM MgGDP added before centrifugation, 532 nmol. As can be seen, no DRAG activity was obtained, indicating that the effect of GDP can be attributed to solubilization of DRAG from the chromatophores. Taken together these results suggest that DRAG was released from the membranes upon addition of GDP. To verify that the DRAG protein was indeed released, the supernatants were subjected to SDS-PAGE and DRAG was identified by Western blotting. As shown in Fig. 1, a clear band was detected in the supernatant from GDP-treated chromatophores, although it was not as strong as that in the sample from chromatophores treated with 0.5 M NaCl, the standard procedure for solubilization of DRAG. No DRAG signal was obtained with ATP or ADP. In some experiments a faint band was also detected when GTP was used, which could possibly be due to hydrolysis of GTP or contamination by GDP in the GTP solution. The major slower-migrating band and the other faint bands (Fig. 1, lanes a to d) have not been identified and were not present to the same degree in all experiments. Most likely they are due to nonspecific reactions with the antibodies, which were raised against a synthesized N-terminal peptide of DRAG and not the purified protein. The results shown in Fig. 1 are in good agreement with the activities shown in Table 1. Our results show for the first time that DRAG can be released by treatment with a low-molecular-weight compound of physiological significance. Furthermore, the fact that neither GTP, ADP, nor ATP has the same effect shows that the release of DRAG is not due to a nonspecific effect, e.g., an increase in ionic strength. It is thus possible that the association of DRAG with the chromatophores is a physiological phenomenon and not an artifact resulting from breaking the cells. It could be hypothesized that the association of DRAG with the membranes is in fact the mode by which DRAG is rendered inactive during switch-off conditions. In this context it is interesting that the specific effect of GDP was not as clear when chromatophores produced in the presence of reducing agents, i.e., dithionite and dithiothreitol, were used or when the solubilization was done under aerobic conditions. This could indicate that the redox status of the membranes is part of the mechanism regulating the association of DRAG. The role of GTP- and GDP-binding proteins in the regulation of metabolic processes in eukaryotic cells is well documented (1); however, a similar possible role for GDP in the switch-off regulation of nitrogenase in phototrophic bacteria remains to be demonstrated.

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REFERENCES


