Demonstration and partial characterization of ADP-ribosylation in *Pseudomonas maltophilia*

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ADP-ribosylation of proteins occurs in many eukaryotes, and it is also the mechanism of action of a growing number of important bacterial toxins. To date, however, there is only one well-characterized ADP-ribosylation system where the ADP-ribosyltransferase and the substrate protein are both bacterial in origin, namely within the nitrogen-fixing bacterium *Rhodospirillum rubrum*. The present paper demonstrates the endogenous ADP-ribosylation of two proteins of M_r 32000 and 20000 within *Pseudomonas maltophilia*, a Gram-negative aerobe. The proteins have been partially purified: two apparently separate species of modified protein can be separated by ion-exchange chromatography and gel filtration (V_0 and M_r 158000– V_1). The substrate protein(s) either has, or is co-eluted with, NAD^+ glycohydrolase activity. The modification is mono-ADP-ribosyl in nature. The linkage between the acceptor amino acid and the ADP-ribose moiety is alkali-labile and stable to hydroxylamine, possibly indicating an *S*-glycosidic bond. The activity appears to be a true ADP-ribosylation reaction and not an NAD^+ glycohydrolase activity followed by non-enzymic addition of ADP-ribose to protein. The results presented here indicate that ADP-ribosylation may have a wider significance within prokaryotic systems than previously thought.

INTRODUCTION

Pseudomonas maltophilia is the second most commonly isolated Pseudomonad from human material (Pedersen *et al.*, 1970). Reports of involvement of the bacterium in human disease are increasing, and a recent review of the expanding clinical spectrum is given by Muder *et al.* (1987), including reports of neurosurgical meningitis and endocarditis. Zuravleff & Yu (1982) note that disease where *P. maltophilia* is implicated as the aetiological agent is seen most commonly as a consequence of infection from the hospital environment. They also document the wide-ranging resistance of the organism to antibiotics. It is therefore clear that *P. maltophilia* has considerable pathogenic importance. A survey of the extracellular enzymes of the bacterium was undertaken by O'Brien & Davis (1982), and Bjorn *et al.* (1977) reported that *P. maltophilia* did not produce the ADP-ribosylating exotoxin A of *Pseudomonas aeruginosa*; there have been no other biochemical studies reported in the literature.

ADP-ribosylation is a post-translational modification of proteins that has considerable importance in the regulation of enzyme activity (Gaal & Pearson, 1986). Substrate proteins may be poly- or mono-ADP-ribosylated. Poly-ADP-ribosylation appears to be confined to the eukaryotic nucleus; it may be involved in DNA repair, cellular differentiation, transformation and some processes requiring gene rearrangements (Williams & Johnstone, 1983; Gaal & Pearson, 1985; Ueda & Hayaishi, 1985). Mono-ADP-ribosylation occurs throughout the eukaryotic cell and appears to be im-

portant in several diverse systems (Ueda & Hayaishi, 1985; Gaal & Pearson, 1986; Adamietz *et al.*, 1981). In addition, many bacterial toxins are ADP-ribosyltransferases (ADPRTs) and apparently exert their toxic effect by mono-ADP-ribosylating host-cell proteins (Gemmell, 1984; Middlebrook & Dorland, 1984; Foster & Kinney, 1985). Some bacteriophages code for ADPRTs that act on the proteins of the host bacterium (Pesce *et al.*, 1976; Zillig *et al.*, 1977). Only one well-characterized ADP-ribosylation system has been reported where all the enzymes and substrate proteins involved are internal to the bacterium, namely the system in *Rhodospirillum rubrum*, an anaerobic nitrogen-fixing bacterium. The Fe-protein of the nitrogenase complex in this bacterium is reversibly inactivated by ADP-ribosylation in response to the changing metabolic state of the bacterium (Lowery *et al.*, 1986).

The present study demonstrates that ADP-ribosylation occurs within *P. maltophilia*, an aerobic, non-nitrogen-fixing bacterium. This suggests that ADP-ribosylation may have a wider significance within prokaryotic systems than previously thought.

MATERIALS AND METHODS

Growth of *P. maltophilia*

Strain 10499 was obtained from the National Collection of Type Cultures (N.C.T.C.), Colindale, London N.W.9, U.K., and cultured aerobically in L-broth [1% (w/v) tryptone/0.5% (w/v) yeast extract/0.5% (w/v) NaCl] or on agar-containing L-broth at 25 °C.

Abbreviations used: ADPRT, ADP-ribosyltransferase.

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***P. maltophilia* whole-bacterium ADPRT assay**

Each assay tube contained 7.5×10^6 bacteria and 0.2 μCi of radioactive NAD^+ either $[^3\text{H}]\text{NAD}^+$ (prepared by Dr. W. J. D. Whish, Department of Biological Sciences, University of Bath, Claverton Down, Bath, U.K.; 26 Ci/mmol) or $[^{32}\text{P}]\text{NAD}^+$ (NEN; 30 Ci/mmol) similarly diluted to 10 μl . Rat liver membrane protein (200 μg , or 100 μg if the samples were to be electrophoresed) or L-broth (final concn. 50%, v/v) was added as appropriate. The content of each tube was adjusted to 150 μl by the addition of phosphate reaction buffer [400 mM-potassium phosphate buffer (pH 7.0)/20 mM-dithiothreitol]. The tubes were incubated at 30 °C for 2 h and then 50 μl of stop solution (9 M-urea/48 mM-nicotinamide) was added to each. The reaction mix was harvested on to GF/C filters pre-soaked in 10% (w/v) trichloroacetic acid, which were then washed and counted for radioactivity by liquid scintillation. If the tube contents were to be subjected to gel electrophoresis, 150 μl of 20% (w/v) trichloroacetic acid was added immediately after incubation, without addition of stop mix. The precipitates were then washed in ethanol before solubilization in sample loading buffer.

SDS/polyacrylamide-gel electrophoresis

Slab gels (13 cm $\times$ 13 cm; final acrylamide concn. 10%, w/v) in Tris/Bicine were used (final concns: 100 mM-Tris, 100 mM-Bicine and 0.1% SDS; Johnstone & Thorpe, 1987). The electrode buffers were 20 mM-Tris, 20 mM-Bicine and 0.1% SDS. Gels were run at 110 V for approx. 3 h before drying and subjection to autoradiography.

Snake-venom phosphodiesterase digestion

This method was adapted from that described by Lehmann *et al.* (1974). Radiolabelled substrate sample (20 μl ; approx. 2000 c.p.m.) was incubated in 1.5 ml Eppendorf tubes with 5 μl of snake-venom phosphodiesterase (Lorne Diagnostics Ltd., Bury St. Edmunds, Suffolk, U.K.) in 80 mM-Tris/HCl (pH 7.2)/10 mM-MgCl₂ (final vol. 100 μl) for 2 h at 37 °C. The samples were then prepared for t.l.c.

T.l.c.

This method was adapted from Lehmann *et al.* (1974) and Williams (1979). After digestion, an equal volume of ice-cold ethanol was added to the samples, which were mixed and kept on solid CO₂ for 10 min. The samples were then spun at 10000 *g* at -20 °C in a Sorvall RC-50 centrifuge (HB4 rotor) to pellet the precipitated material. The supernatants were freeze-dried, re-dissolved in 10 μl of 50% (v/v) ethanol and applied to spots 2 cm apart on a polyethyleneimine-cellulose plate (Camlab Ltd., Cambridge, U.K.) using capillary tubes. The plates were developed by using, first, 1 M-acetic acid, until the solvent front was 2 cm above the origin and then 0.9 M-acetic acid/0.3 M-LiCl until the solvent was within 0.5 cm of the end of the plate. Internal standards of ADP-ribose, AMP and NAD^+ (20 nmol each) were run with each sample, and these were revealed under u.v. light (254 nm) after the plate had been dried. After marking the position of these standards, each lane (2 cm wide) was cut into 1 cm strips. The cellulose matrix was scraped off the backing plastic of each strip into a scintillation vial, and 0.5 ml of 1.6 M-LiCl added to elute the nucleotides. Scintillation

fluid (4 ml; Beckman ReadyValue, Beckman-Riic Ltd., High Wycombe, Bucks., U.K.) was added, and the vials counted for radioactivity on an LKB Rackbeta instrument.

Stability of the ADP-ribose-protein bond in hydroxylamine and NaOH

These methods were modified from those of Hsia *et al.* (1985) and Bredehorst *et al.* (1978). ADP-ribosylated proteins (either radiolabelled histones after treatment with cholera toxin, or partially purified radiolabelled *P. maltophilia* substrate) were adjusted to 37.5 μl with 10 mM-phosphate buffer (pH 7.0)/0.02% (w/v) NaN₃. For assessment of stability to hydroxylamine, 37.5 μl of 1 M-NaCl, pH 7.5 (for control incubations) or 1 M-hydroxylamine, pH 7.5, was added to the sample and timed incubations at 37 °C carried out. For assessment of stability to alkali, 12.5 μl of 4 M-NaOH or distilled water (control) was added to the samples, which were incubated at 56 °C for 2 h and then neutralized with 25 μl of 2 M-HCl. Bovine serum albumin (10 μg) was added to each tube to act as a carrier protein, and the samples were precipitated as described for the ADPRT assay.

Purification schemes

Gel filtration. A column of Sephacryl S-300 (Superfine) of 1.5 cm $\times$ 90 cm was packed and used at 4 °C. *V*₀ volume was 64 ml. The flow rate was 10 ml/h and the volume of fractions collected was 2 ml. The running buffer was Dulbecco's phosphate-buffered saline (pH 7.2) (Dulbecco & Vogt, 1954)/0.02% (w/v) NaN₃.

Ion-exchange chromatography. A column of DEAE-Sephacrose CL-6B (1 cm $\times$ 15 cm) was packed and used at room temperature. The flow rate was 24 ml/h, and 4 ml fractions were collected. The running buffer was 50 mM-Tris/HCl, pH 7.5, and elution of bound protein was carried out by increasing concentrations of NaCl in the running buffer. The final elution buffer of each run was 2 M-NaCl/50 mM-Tris/HCl, pH 7.5. This appeared sufficient to remove all bound protein; however, the column was regenerated periodically as described in the literature provided by the manufacturer (Pharmacia).

Radiolabelled-substrate preparation. Bacteria (2×10^8 /ml) were incubated with labelled NAD^+ (2 $\mu\text{Ci}/\text{ml}$) and rat liver membrane protein (1.35 mg/ml) in phosphate reaction buffer [400 mM-potassium phosphate buffer (pH 7.0)/20 mM-dithiothreitol] containing 10 μM unlabelled NAD^+ at 30 °C for 20 h. The cells were centrifuged, resuspended in 0.3 M-NaCl in 50 mM-Tris/HCl, pH 7.5, and sonicated for 3 min. The debris was removed by centrifugation at 10 000 *g* for 10 min. (Sorvall RC-5B instrument, SS-34 rotor) and the membrane was pelleted in a Beckman TL-100 centrifuge at 250 000 *g* for 15 min. The supernatant was desalted on a Sephadex G-50 column in 0.3 M-NaCl/50 mM-Tris/HCl, pH 7.5, and then applied to the ion-exchange column with a 0.3 M-NaCl wash, a 0.3–0.8 M gradient, followed by a 2 M wash. As described in the text, selected fractions eluted from the ion-exchange column were subsequently subjected to gel filtration.

Preparation of ADP-ribosylated histones

Histones (1 mg) were incubated overnight at 30 °C with 60 μg of activated cholera toxin and 2 μCi of labelled

NAD⁺ in phosphate reaction buffer containing 10 μ M unlabelled NAD⁺ (final vol. 450 μ l). The reaction mixture was then applied to a Sephadex G-50 desalting column equilibrated with 10 mM-phosphate buffer (pH 7.0)/0.02% (w/v) NaN₃, and the fractions in the void volume pooled, and stored in portions at -20°C .

RESULTS AND DISCUSSION

Demonstration of endogenous ADP-ribosylation

Linear incorporation of radiolabel from [³H]NAD⁺ into whole *P. maltophilia* was observed over a 2 h period (up to 25000 c.p.m./ 7.5×10^6 cells). The level of incorporation was found to be dependent on the amino acid source for the bacteria in the assay, with eukaryotic cell membrane preparations proving the most efficient.

Fig. 1. Radiolabelled polypeptides after incubation with [³²P]NAD⁺

Acid-precipitable material from *P. maltophilia* and *E. coli* incubated with [³²P]NAD⁺ in the presence of various amino acid sources was analysed by SDS/polyacrylamide (10%-polyacrylamide/Tris/Bicine gels)-gel electrophoresis, followed by autoradiography. Each assay tube containing 100 μ g of protein of L-broth (50% final concn.). *E. coli* non-toxicogenic laboratory strain RW 1053 was used under the same conditions as *P. maltophilia*. Abbreviations: P.m., *P. maltophilia*; E.c., *E. coli*; R.L.M., rat liver membrane; L-b., L-broth. The positions of molecular-mass (*M*) markers are indicated on the left-hand side, and the two polypeptides attributable to *P. maltophilia* are indicated with stars.

Boiled bacteria were totally ineffective in the assay; bacteria taken from exponential-phase culture or stationary-phase culture were equally active. Differential centrifugation showed that the acid-precipitable label co-sedimented with the bacteria and not with the added protein. Hence the bacteria were not producing a toxin with specificity for the membrane proteins. Addition of L-broth, which contains no acid-precipitable material, instead of the membrane, also stimulated the activity, clearly demonstrating that the radiolabelling activity, and the substrate for this activity, are both within the bacterium.

Fig. 1 shows the autoradiograph of an SDS/polyacrylamide-gel-electrophoretic separation of the acid-precipitable material from the reaction. Although some automodification of the membrane occurred, it is clear that there are two proteins (starred), of approximate *M_r* 32000 and 20000, that are radiolabelled regardless of the amino acid source; indeed, these are radiolabelled to a certain extent even in the absence of amino acid source. The labelling was not observed with either *Escherichia coli* (Fig. 1) or *Pseudomonas aeruginosa* (not shown).

Inhibitor studies

The response of the *P. maltophilia* radiolabelling activity was tested with a variety of inhibitors developed for use with the eukaryotic poly-ADP-ribosyltransferase system (Table 1). The almost total inhibition observed

Table 1. Inhibition by different compounds of the activity of eukaryotic nuclear ADPRT compared with the *P. maltophilia* ADPRT

Inhibition was measured by the change in incorporation of radiolabel from [³H]NAD⁺ into acid-precipitable material. The cumulative mean ($\pm$ S.E.M.) of each individual experimental determination, expressed as a percentage, is shown. The numbers of determinations and the concentrations of the inhibitor are given in parentheses. The error thus expressed cannot take account of the intra-experimental variation; therefore it is valid to use more stringent levels of significance when attempting to interpret these results. Significance tested: **P* < 0.01; ***P* < 0.001.

Inhibitor	Inhibition of ADPRT activity (%)	
	Nuclear ADPRT	<i>P. maltophilia</i>
3-Methoxybenzamide	89.4 $\pm$ 3.3** (<i>n</i> = 9, 250 μ M)	46.0 $\pm$ 2.6** (<i>n</i> = 12, 2.5 mM)
3-Aminobenzamide	88.1 $\pm$ 4.9** (<i>n</i> = 9, 1 mM)	21.9 $\pm$ 2.4** (<i>n</i> = 12, 10 mM)
Thymidine	76.5 $\pm$ 4.2** (<i>n</i> = 9, 1 mM)	3.2 $\pm$ 2.9 (<i>n</i> = 15, 1 mM)
ADP-ribose	-51.2 $\pm$ 14.8 (<i>n</i> = 12, 1 mM)	93.3 $\pm$ 2.5** (<i>n</i> = 15, 1 mM)
Ribose 5-phosphate	11.8 $\pm$ 5.5 (<i>n</i> = 12, 10 mM)	17.3 $\pm$ 3.2 (<i>n</i> = 12, 10 mM)
Ribose	12.5 $\pm$ 9.9 (<i>n</i> = 12, 10 mM)	-3.0 $\pm$ 3.2 (<i>n</i> = 15, 10 mM)
Nicotinamide	86.1 $\pm$ 1.8** (<i>n</i> = 9, 1 mM)	7.1 $\pm$ 2.5 (<i>n</i> = 15, 10 mM)
Isonicotinamide	65.6 $\pm$ 5.3** (<i>n</i> = 12, 1 mM)	19.8 $\pm$ 2.6* (<i>n</i> = 12, 10 mM)
Isonicotinic acid hydrazide	17.6 $\pm$ 6.5 (<i>n</i> = 18, 5 mM)	30.1 $\pm$ 4.4* (<i>n</i> = 15, 5 mM)

Fig. 2. Partial purification of *P. maltophilia* material that incorporates label from $[^3\text{H}]\text{NAD}^+$

(a) Elution profile from DEAE-Sepharose. Radiolabelled material from bacteria preincubated with $[^3\text{H}]\text{NAD}^+$ and prepared as described in the Materials and methods section was applied to the ion-exchanger and bound protein was eluted with a gradient of increasing concentration of NaCl. The molarity of the eluent was calculated from calibrated conductivity measurements (●). The radioactivity in 100 μl samples from 4 ml fractions was measured (—). (b) and (c), The indicated peaks from (a) were then separately applied to a Sephacryl S-300 column: (b) second peak from (a); (c) first peak from (a). The radioactivity in 300 μl (b) or 150 μl (c) samples from 2 ml fractions is shown. The column void volume, V_0 , the total column volume, V_i , and the elution positions of standard molecular-mass markers (kDa) are arrowed.

with ADP-ribose was unexpected, initially suggesting that non-enzymic addition of free ADP-ribose is the mechanism of radiolabelling, as opposed to the one-step transferase reaction. However, such an addition occurs through the aldehydic carbohydrate, and ribose, and in particular ribose 5-phosphate (neither of which inhibited the *P. maltophilia* activity), have been found to be even more sterically suitable for this addition than ADP-ribose (Kun *et al.*, 1976). Hence a one-step transferase reaction remains the most likely mechanism. It is possible that the ADP group competitively inhibits the entry of the NAD^+ into the cell, by being preferentially bound by

the appropriate transport proteins, and thus indirectly inhibits the radiolabelling reaction.

The response to the other compounds, within the limitations of current knowledge, is consistent with a mono-ADP-ribosylation reaction. Thymidine clearly distinguished between the eukaryotic nuclear reaction and that of *P. maltophilia*. The lack of significant inhibition by nicotinamide is surprising; we have also observed that cholera-toxin activity is similarly unaffected (C. Edmonds, G. E. Griffin & A. P. Johnstone, unpublished work). The benzamidine derivatives are reported to be specific inhibitors of ADP-ribosylation (Purnell &

Whish, 1980; Hunting *et al.*, 1985), although Milam & Cleaver (1984) have described some other effects. The significant inhibition of *P. maltophilia* activity by these nicotinamide analogues is a strong indicator of ADP-ribosylating activity.

Partial purification of the substrate for ADP-ribosylation

On application of the extract from prelabelled bacteria to DEAE-Sepharose, two peaks of material were eluted at 0.55 M- and 0.6 M-NaCl (Fig. 2a). The first peak always contained most of the material, although the exact proportion was variable (60–85%). On re-chromatography of these peaks separately on Sephacryl S-300, the second peak was found to be eluted consistently with the void volume (Fig. 2b), whereas the first peak was eluted as a series of peaks, between the M_r -158 000 and -25 000 markers, most material being eluted at M_r 100 000–150 000 (Fig. 2c). The two peaks from the ion-exchanger did not interconvert on sequential elutions, nor after storage at -20°C for several weeks. Between the void elution at M_r greater than 1 000 000 and the start of the series of peaks at approx. M_r 150 000 radiolabelled material was never observed, suggesting that the relationship between the peaks was not simply one of aggregation or degradation.

The very-high- M_r material, being the minor component, was not studied further. We attempted to purify the substrate(s) from the lower- M_r material (first peak, Figs. 2a and 2c; representing 0.4% of total bacterial protein), but repeated attempts were unsuccessful, because various co-purifying enzyme activities (particularly an NAD^+ glycohydrolase, phosphodiesterases and proteinases) prevented reproducible assays.

Characterization of the ADP-ribosylation modification

In order to determine whether the modification was mono-ADP-ribosyl or poly-ADP-ribosyl in nature, snake-venom phosphodiesterase digestion of the radiolabelled substrate (first peak, Fig. 2a) was carried out. Digestion of a poly-ADP-ribosyl chain with this enzyme produces one AMP molecule per chain or branch as the terminal residue is released; each internal residue will be released as a phosphoribosyl-AMP molecule. Digestion of a mono-ADP-ribosyl modification only produces AMP.

T.l.c. was used to characterize the digestion products (Fig. 3). Under the conditions used, phosphoribosyl-AMP co-migrates with the ADP-ribose marker (Williams, 1979). No radiolabelled material co-migrated with this marker, whereas substantial amounts of radiolabel co-migrated with the AMP marker. However approximately half of the recovered radiolabel was in a fast-migrating form that, on the basis of its migration, is likely to be adenine or adenosine. Lee & Iglewski (1984) reported that their snake-venom phosphodiesterase preparation contained a contaminating activity that converted most of the released AMP into adenosine during the digestion. Since the major product of the digestion was AMP, most of the modifications of the *P. maltophilia* protein are mono-ADP-ribosyl in nature. The discrete bands obtained by SDS/polyacrylamide-gel electrophoresis (Fig. 1) confirm this conclusion, since poly-ADP-ribosylated proteins electrophorese as a diffuse smear, owing to the heterogeneous M_r values attributable to different-length chains.

Fig. 3. Action of snake-venom phosphodiesterase on radiolabelled material from *P. maltophilia*

Samples were incubated in either the absence (a) or presence (b) of phosphodiesterase for 2 h as described in the Materials and methods section and the supernatants from ethanol precipitation were analysed by t.l.c. Radioactivity is expressed as a percentage of the total radioactivity per incubation with the total percentage radioactivity released stated. The positions of unlabelled standards are shown by bars; abbreviations: ADPR, ADP-ribose; Ado, adenosine; F, solvent front.

Five types of linkage between the ADP-ribosyl residue and protein have been described: (1) glutamic acid forms a carboxylate ester (poly-ADP-ribosylation only); (2) arginine forms an *N*-glycosidic bond resembling a secondary amine; (3) cysteine forms an *S*-glycosidic bond resembling a thioether; (4) diphthamide forms an *N*-glycosidic bond (diphtheria toxin) resembling a tertiary amine; (5) lysine can form a Schiff base by the non-enzymic addition of the aldehydic ribose.

Table 2. Stability of ADP-ribosylated protein to NaOH and hydroxylamine

The radiolabelled *P. maltophilia* substrate or ADPR-histone was incubated with 1 M-NaOH or 0.5 M neutral hydroxylamine, as described in the Materials and methods section. The radioactivity remaining acid-precipitable in triplicate samples was then assessed by liquid-scintillation counting. Results are expressed as the amount removed by the treatment compared with the total radioactivity remaining in control incubations under identical conditions but without the appropriate agent: [(c.p.m. in control - c.p.m. in treated)/c.p.m. in control] $\times 100\%$ (mean $\pm$ S.E.M.). Significance between control and treated: * $P < 0.05$; ** $P < 0.01$; (†, adjusted to account for unequal variances).

Treatment	Period (h)	Proportion of radiolabel released (%)	
		ADPR-histones	<i>P. maltophilia</i> substrate
NaOH	2	48.3 $\pm$ 5.6*	77.9 $\pm$ 20.8**
Hydroxylamine	2	71.7 $\pm$ 4.5**	25.3 $\pm$ 15.0
	2	66.3 $\pm$ 13.3*†	-3.8 $\pm$ 8.1
	6	87.9 $\pm$ 9.8*†	-36.6 $\pm$ 13.2†

All the bonds formed by enzymic ADP-ribosylation are alkali-labile (1M-NaOH) over a 2 h incubation; the *P. maltophilia* modification was labile under these conditions (Table 2). From the stability of ADP-ribosylated proteins to hydroxylamine treatment, the nature of the amino acid modified can be inferred. The carboxylate ester linkage is the most labile to hydroxylamine, then the *N*-glycosidic arginine bond and the Schiff base; the *S*-glycosidic and diphthamide bonds appear to be completely stable (Hsia *et al.*, 1985; Payne *et al.*, 1985).

Table 2 shows that, under conditions where the *N*-glycosidic bond between ADP-ribose and arginine formed by the action of cholera toxin on histones is labile to hydroxylamine treatment, the ADP-ribosyl linkage of the radiolabelled *P. maltophilia* is completely stable. Because of the rarity of the diphthamide residue, it is tempting to speculate that the amino acid residue modified in *P. maltophilia* is cysteine; alternatively, a novel modification not described before could be involved.

The work presented here provides a strong basis for further investigation of the role of ADP-ribosylation in prokaryotes. The nitrogenase-regulation system in *Rhodospirillum rubrum* is very specialized; the bacterium is one of few that fixes dinitrogen anaerobically, and the whole process is inhibited by oxygen. The present study implies that reversible ADP-ribosylation within bacteria may be a more general phenomenon.

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