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Effects of Cadmium Stress on Growth, Morphology, and Protein Expression in *Rhodobacter capsulatus* B10

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The effects of cadmium stress on growth, morphology, and protein expression were investigated in *Rhodobacter capsulatus* B10 using two-dimensional polyacrylamide gel electrophoresis and a scanning electron microscope with an energy dispersive X-ray spectrometer. The bacterium grew in the presence of 150 μM $CdCl_2$ and highly induced heat-shock proteins (GroEL and Dnak), *S*-adenosylmethionine synthetase, ribosomal protein S1, aspartate aminotransferase, and phosphoglycerate kinase. Interestingly, the ribosomal protein S1 was proportionally expressed as the amount of cadmium in the medium, suggesting that S1 may be required for the repair of cadmium-mediated cellular damage. On the other hand, we identified five cadmium-binding proteins: 2-methylcitrate dehydratase, phosphate peripalsmic binding protein, inosine-5′-monophosphate dehydrogenase/guanosine-5′-monophosphate reductase, inositol monophosphatase, and lytic murein transglycosylase. The cadmium-treated cells had a filamentous structure and contained less phosphorous than the untreated cells. We propose that these characteristics of the cadmium-treated cells may be due to the inactivation of the phosphate peripalsmic binding protein and lytic murein transglycosylase by cadmium.

Key words: cadmium; cadmium-induced proteins; cadmium-binding proteins; *Rhodobacter capsulatus* B10

Cadmium has been used in a variety of industrial applications, such as electroplating and plastics manufacturing, resulting in terrestrial and aquatic environmental contamination. Although cadmium has no biological functions in bacteria, the heavy metal seems to be readily taken up by the Mn^{2+} uptake system or Mg transport system.[1,2] The heavy metals transported into the cytoplasm seem to inhibit DNA replication,[3,4] and make the DNA more susceptible to nucleolytic attack, resulting in single-strand DNA breaks.[5] Therefore, cadmium causes serious damage during the growth of bacteria present in polluted environments.

Recently, several approaches have been considered for heavy metal removal from polluted environments. For example, photosynthetic purple bacteria have been shown to be particularly resistant to various heavy metals and transition metal oxyanions. This resistance is attributed to the capacity of the organisms to reduce metal-oxyanions to their elemental ground state,[6] which is poorly soluble and thus less toxic than the initial oxyanions. Kobayashi and Kobayashi have developed very promising tools for purifying waste water using anoxygenic phototrophic bacteria.[7] A photosynthetic bacterium, *Rhodospirillum rubrum*, has also been used for cadmium bioaccumulation,[8] and inactivated *Rhodobacter sphaeroides* for cadmium biosorption as a basic bioremediation step,[9,10] but there are no reports regarding the effects of cadmium stress on protein expression in cadmium-resistant photosynthetic bacteria. In the present study, we investigated the growth, morphology, and protein expression of the photosynthetic bacterium *Rhodobacter capsulatus* B10 in response to cadmium stress. We discuss the roles of cadmium-induced proteins and cadmium-binding proteins.

Materials and Methods

Bacterial strain, medium, and growth. R. capsulatus strain B10 (ATCC 33303) was anaerobically cultivated in 500-ml screw cap medium glass bottles full of RÄH medium at 32 °C under light condition (continuous fluorescence intensity 8.5×10^3 Lux, 20 W/m^2) without shaking.[11,12] To maintain anaerobic conditions, the medium was bubbled with N_2 gas (99.95%) for 30 min before inoculation. The composition was as follows:

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Abbreviations: IMP dehydrogenase, inosine-5′-monophosphate dehydrogenase; GMP reductase, guanosine-5′-monophosphate reductase; RpS1, ribosomal protein S1

D,L-malic acid, 2.5 g; NH_4Cl_2, 1.2 g; $MgSO_47H_2O$, 0.2 g; $CaCl_2$, 0.07 g; K_2HPO_4, 0.9 g; KH_2PO_4, 0.6 g; yeast extract, 0.5 g; microelement solution, 40 ml; deionized water, 1,000 ml. To investigate the effects of cadmium on growth, the bacterial cells in the exponential phase were inoculated into bottles containing RÄH medium (500 ml) supplemented with 50, 100, 150, and 300 μM $CdCl_2$. To avoid cadmium precipitation, the pH of the medium was constantly adjusted to 6.2. Growth of bacteria was monitored spectrophotometrically by measuring the absorbance at 660 nm using a Klett-summerson photoelectric colorimeter.

Preparation of soluble cell-free extract. Cells (about 10 g wet weight) in the exponential phase were suspended in 40 ml of 10 mM Tris–HCl buffer (pH 8.0), and broken by three passages through a French pressure cell (100 MPa). After the unbroken cells were removed by centrifugation at $10{,}000 \times g$ for 15 min at 4 °C, the supernatant was further centrifuged at $100{,}000 \times g$ for 1 h at 4 °C. The supernatant obtained was used as a soluble cell-free extract.

Two-dimensional polyacrylamide gel electrophoresis. Two-dimensional polyacrylamide gel electrophoresis was performed according to method of O'Farrell (1975),[13] using Ampholine™ preblended pH 5.0–8.0 (Amersham Bioscience, Uppsala). A 10% Tris–glycine polyacrylamide/SDS slab gel electrophoresis was used as second dimension.[14] After second dimension electrophoresis, the gel was fixed for 3 h in 15% trichloroacetic acid, washed overnight in 25% methanol plus 8% acetic acid solution to remove ampholine, and then stained with Coomassie Brilliant Blue R-250.

Preparation of proteins for amino acid sequencing. For determination of the N-terminal amino acid sequences of the proteins on the gels, Tris–tricine SDS–PAGE was done as a second dimension of electrophoresis.[15] The proteins separated by the second dimension of electrophoresis or normal SDS–PAGE were transferred to a polyvinylidene fluoride membrane (Sequi-Blot™ PVDF membrane, Bio-Rad, Hercules, CA.) using a semidry-type electroblotting apparatus, and then stained with Coomassie Brilliant Blue R-250. Selected spots were cut from the membrane and then washed with methanol several times to remove Coomassie Brilliant Blue R-250, and sequenced in a Shimazu PPSQ-21A protein sequencer operating as recommended by the manufacturer. The sequence data in the present study were analyzed using the Blast program.[16]

Immunoblot assays. Proteins separated on SDS–PAGE were transferred onto hybond-P membranes (Amersham Bioscience, Uppsala) by a semidry transfer system.[14] The membranes were blocked overnight at 4 °C in PBS containing 5% skim milk and 0.1% (v/v) Tween 20 and incubated for 1 h at room temperature with the appropriate primary antibody diluted into PBS containing 0.1% (v/v) Tween 20 (PBS-T). After an extensive washing in PBS-T, the membranes were incubated for at least 1 h at room temperature with antisheep IgG (whole molecule)-peroxidase antibody produced in donkey (Sigma, St. Louis, MO) diluted in PBST. After further washing, the immunocomplexes were revealed using ECL plus a western blotting detection system (Amersham Bioscience, Uppsala). Antibody against ribosomal S1 protein was kindly gifted by Professor Richard Brimacombe (Max Planck Institute for Molecular Genetics).

Metal-chelating affinity column chromatography. A Mg-binding resin column and a Cd-binding resin column were prepared by the following method: IDA agarose resin (His-Bind Resin, Novagen, Tokyo) was washed with 3 volumes of sterile deionized water, charged with 5 volumes of 50 mM $MgCl_2$ or 50 mM $CdCl_2$, and equilibrated with 3 volumes of 10 mM Tris–HCl buffer (pH 8.0). The soluble cell-free extract was applied onto the column at a flow rate 0.4 ml/min. The column was completely washed with 10 mM Tris–HCl buffer (pH 8.0) until no proteins were eluted. The adsorbed proteins were subsequently eluted with 10 mM Tris–HCl buffer (pH 8.0) containing 0.5 M NaCl. The chelating metals were finally eluted with 100 mM Na–EDTA.

Scanning electron microscope. A scanning electron microscope equipped with an energy dispersive X-ray spectrometer (SEM-EDX) was used to observe the morphology of the cells and to analyze the contents of cadmium and phosphorus in the cells, as follows: The cells cultivated with 150 μM $CdCl_2$ in the exponential phase were collected by centrifugation at $8{,}000 \times g$ for 10 min and fixed with glutaraldehyde (2.5%) in 10 mM Tris–HCl buffer (pH 8.0) for 1 h at room temperature, washed with 10 mM Tris–HCl buffer (pH 8.0) for 20 min × 2 times, dehydrated in a series of ethanol-alcohol (50–99.5%) 10 min for each one, washed in *t*-butyl alcohol for 10 min in a water bath 30 °C (two times), pipette-drawn, mounted on a carbon tape attached sample holder (aluminium holder), subsequently frozen in liquid nitrogen, and dried with low-vacuum SEM. After they were freeze-dried completely, the samples were coated with carbon and then observed with a scanning electron microscope (JEOL JSM-5200 LV), equipped with an energy dispersive X-ray spectrometer (Phillips EDAX PV 9800 EX).

Results and Discussion

Effect of cadmium on growth and morphology of R. capsulatus *B10*

To study the effect of cadmium on the growth of *R. capsulatus* B10, the cells were cultivated in the

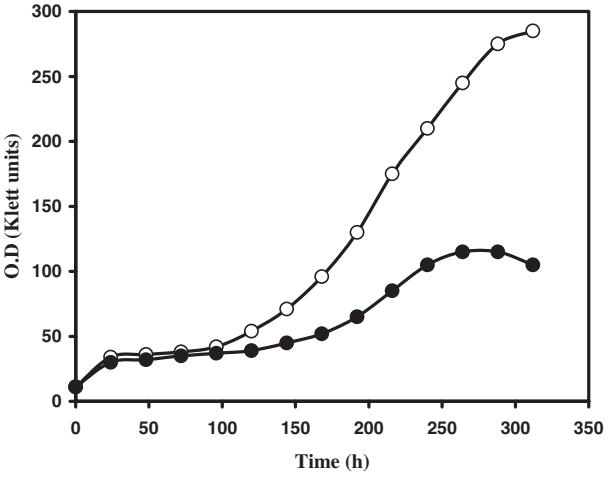

Fig. 1. Growth Curves of *R. capsulatus* B10 in the Absence (○) or Presence (●) of 150 μM $CdCl_2$.

Cadmium was added at the beginning of growth. *R. capsulatus* B10 was phototrophically cultivated by the method described in "Materials and Methods."

presence of various concentrations of $CdCl_2$. Concentrations of 50 μM and 100 μM $CdCl_2$ scarcely inhibited the growth of *R. capsulatus* B10. It should be noted that when *Esherichia coli* was cultivated with $CdCl_2$, 10 μM was lethal.[17] Therefore, *R. capsulatus* seems to have high resistance to cadmium. Figure 1 shows the growth curves of the bacterium in the absence and presence of 150 μM $CdCl_2$. Cadmium inhibited cell proliferation and extended the lag phase. In the presence of 300 μM $CdCl_2$, bacterial growth was completely inhibited (data not shown). Extension of the lag phase was also observed under nickel stress in *Rhodospirillum photometricum*.[18] At low cadmium concentrations, the cells are able to adapt and resume growth after a long lag phase or a period of stasis. This period appears to involve repair of cadmium-mediated cellular damage and adjustment of the cell physiology to limit the distribution of toxic ions in the cell.

Bacteria change their shapes in response to heavy-metal stress. The marine bacterium *Vibrio fischeri* produces very small vesicles on the surface when exposed to cadmium.[19] The phototrophic bacterium *Rhodobacter* spp. elongates the cell in the presence of chromate, arsenate, or selenate.[20] In the present study, we investigated the effect of cadmium on the morphology of *R. capsulatus* B10 using a scanning electron microscope. As shown in Fig. 2, cells cultivated in the presence of 150 μM $CdCl_2$ had filamentous shapes. Furthermore, EDAX analyses indicated that a significant amount of cadmium was taken up by the filamentous cells, while the phosphorus content decreased in the cadmium-treated cells (Fig. 2, insert).

Identification of the cadmium-induced proteins and cadmium-binding proteins in R. capsulatus *B10*

We investigated the global stress response generated by cadmium to identify potentially important proteins involved in cadmium detoxification and metabolism. The protein extract prepared from the cells in the mid-exponential phase were analyzed by two-dimensional polyacrylamide gel electrophoresis and visualized by CBB staining. Figure 3 shows the profiles of the two-dimensional polyacrylamide gel electrophoreses of the extracts prepared from (A) non-treated cells, and (B) cadmium-treated cells. Interestingly, some proteins were highly expressed in the cadmium-treated cells. In order to characterize these cadmium-induced proteins, the larger spots (nos. 1, 2, 3, 4, 5, and 6) were excised from the gel and identified on the basis of their N-terminal amino acid sequence, as described in "Materials and Methods" (Table 1).

Spots nos. 1 and 2 appear to be heat shock proteins, GroEL2 and DnaK respectively. This is in agreement with earlier studies showing the up-regulation of heat shock proteins that are induced by cadmium.[17,21] High expression of the heat-shock proteins in the cadmium-treated cells may be essential for overcoming changes that involve protein denaturation induced by cadmium. Spot no. 3 appears to be *S*-adenosylmethionine synthetase. The enzyme catalyses the formation of *S*-adeno-

Table 1. N-Terminal Amino Acid Sequences of the Cadmium-Induced Proteins of *Rhodobacter capsulatus* B10

Protein no.	Sequence determined	Machs characteristics
1	SAKEVKFGVDARDRMLRGVD	100% identity with GroEL2 of *Rhodopseudomonas palustris* CGA009 with 60 kDa, and pI = 5.23.
2	TKVIGIDLGTTNAAVAVMAA	94% identity with DnaK of *Lactobacillus sanfranciscensis* with 70 kDa, and pI = 4.61.
3	MRASYQFTSESVDEGHPDKV	90% identity with *S*-adenosylmethionine synthetase of *Rhodopseudomonas palustris* CGA009 with 43 kDa, and pI = 5.33.
4	AQTYNPXRDDFAAMLDEEFA	88% identity with ribosomal protein S1 of *Rhodopseudomonas palustris* CGA009 with 63 kDa, and pI = 5.16.
5	QFLATALDRVKPSATIAVSD	94% identity with aspartate aminotransferase A of *Rhodopseudomonas palustris* CGA009 with 44 kDa, and pI = 5.92.
6	TKTFRTLDDADLKGKRVLLR	90% identity with phosphoglycerate kinase of *Rhodopseudomonas palustris* CGA009 with 42 kDa, and pI = 5.95.

Proteins are numbered according to Fig. 3.

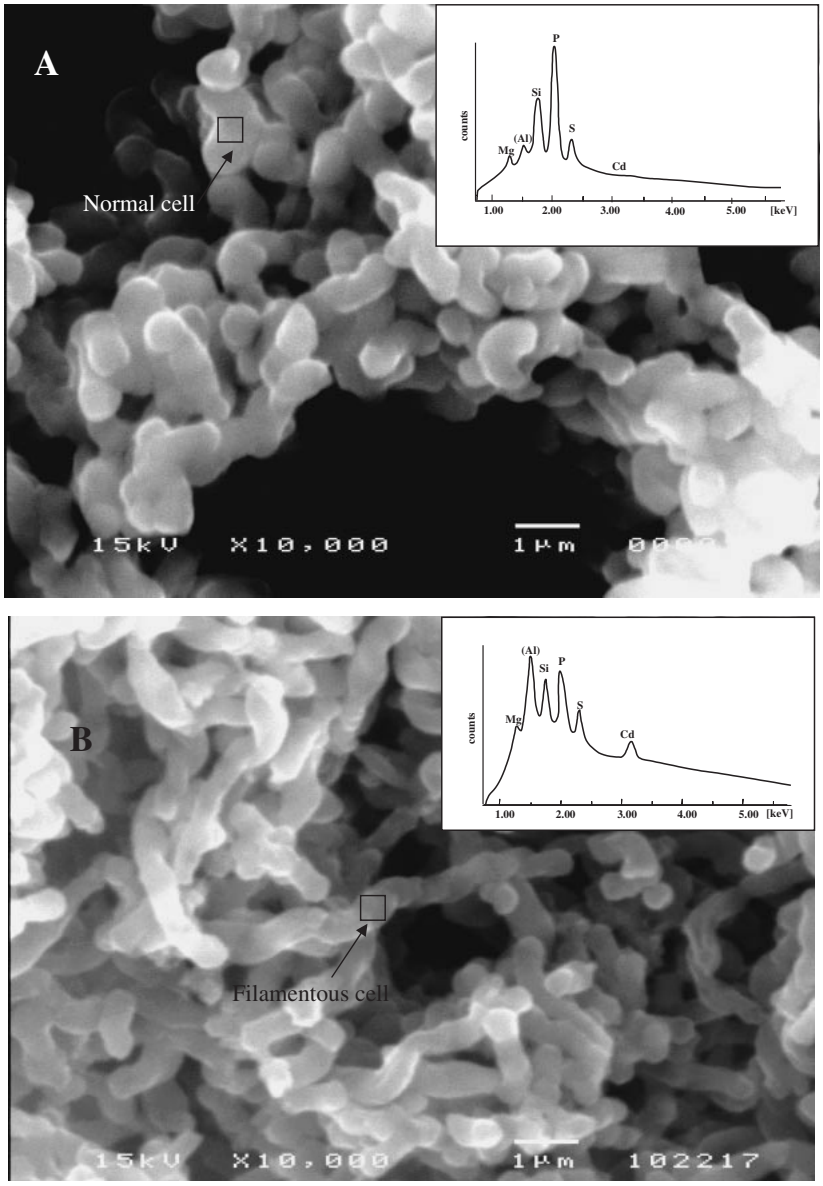

Fig. 2. SEM of *R. capsulatus* B10 Cultivated in the Absence (A) or the Presence (B) of 150 μM $CdCl_2$.

R. capsulatus B10 was phototrophically cultivated by the method described in "Materials and Methods," and collected in the mid-exponential phase by centrifugation at 10,000 × *g* for 15 min. EDAX analyses (inside the figure) show phosphorus level and cadmium level in the square parts of the cell.

sylmethionine from methionine and ATP. *S*-adenosylmethionine is one of the most important cellular biochemical cofactors, and it plays a role in a large number of essential metabolic pathways. Therefore, it represents a crucial checkpoint for numerous functions required for cell growth and division, such as biological methylation and polyamine biosynthesis. This latter function is particularly important in rapidly growing cells, which must continually synthesize polyamines in order to replicate their DNA.[22] Spot no. 4 appears to be the ribosomal protein S1 (RpS1). RpS1 is a prominent component of the ribosome, and is most probably required for the translation of most if not all natural mRNAs.[23] The expression of RpS1 in response to cadmium stress was further demonstrated by western blot analyses. As shown in Fig. 4, RpS1 was expressed in the cadmium-treated cells in proportion to the cadmium concentrations in the medium. It should be noted that RpS1 was not induced by heat stress (a shift from 32 to 42 °C). These results strongly suggest that although induction of heat shock proteins is common with various stresses, including heavy-metal stress, high induction of RpS1 is specific to cadmium stress. Spot no. 5 appears to be aspartate aminotransferase. Aspartate aminotransferase is important for the metabolism of amino acids. Spot no. 6 appears to be phosphoglycerate kinase. Phosphoglycerate kinase catalyses the reversible conversion of 1,3-diphospho-D-glycerate and ADP to 3-

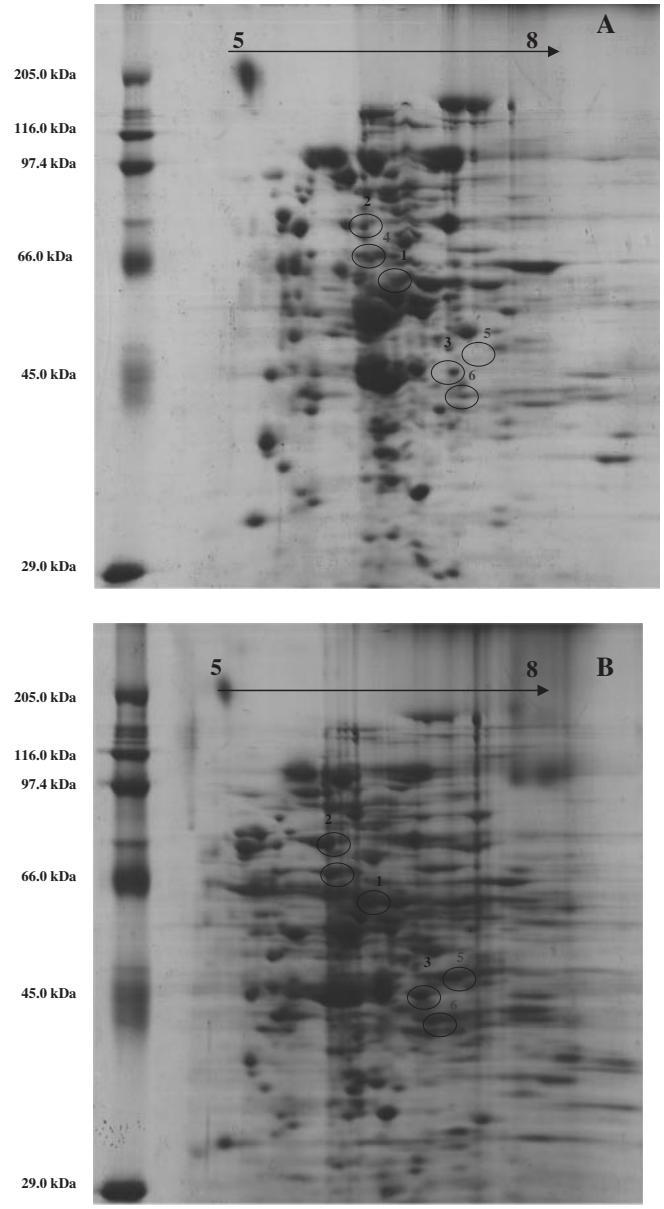

Fig. 3. Two-Dimensional Polyacrylamide Gel Electrophoreses of the Extracts Prepared from Cells Cultivated in the Absence and Presence of Cadmium (150 μM).

A, control cells; B, the cadmium-treated cells. The cells were collected in the mid-exponential phase by centrifugation at 10,000 × *g* for 15 min. The circles spots are proteins which are highly induced in the cadmium-treated cells. Spot numbers correspond to the protein numbers in Table 1.

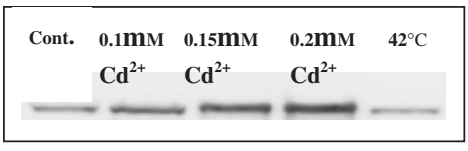

Fig. 4. Western Blot Analysis of Ribosomal Protein S1 in Response to Cadmium Stress, and Heat Stress (a shift from 32 to 42 °C).

Cell extracts prepared from *R. capsulatus* B10 (about 5 μg protein) was separated by SDS–PAGE on mini-gel and transferred to hybond-P membranes by the method described in "Materials and Methods." The protein was immunodetected using antiserum specific for ribosomal protein S1. The bacterium was phototrophically cultivated in the absence and in the presence of $CdCl_2$ (100 μM, 150 μM, and 200 μM).

phospho-D-glycerate and ATP, and is required for ATP generation in the glycolytic pathway.[24)]

It has been proposed that Cd causes damage to cells primarily by the generation of reactive oxygen species,[25)] which causes single-strand DNA damage and disrupts the synthesis of nucleic acids and proteins.[26,27)] Therefore, *R. capsulatus* B10 may respond to exposure to cadmium by the induction of proteins that are required for protein synthesis, such as ribosomal protein S1, and energy generation, such as aspartate aminotransferase and phosphoglycerate kinase.

In the present study, we tried to identify the cadmium-binding proteins in *R. capsulatus* B10 using cadmium-

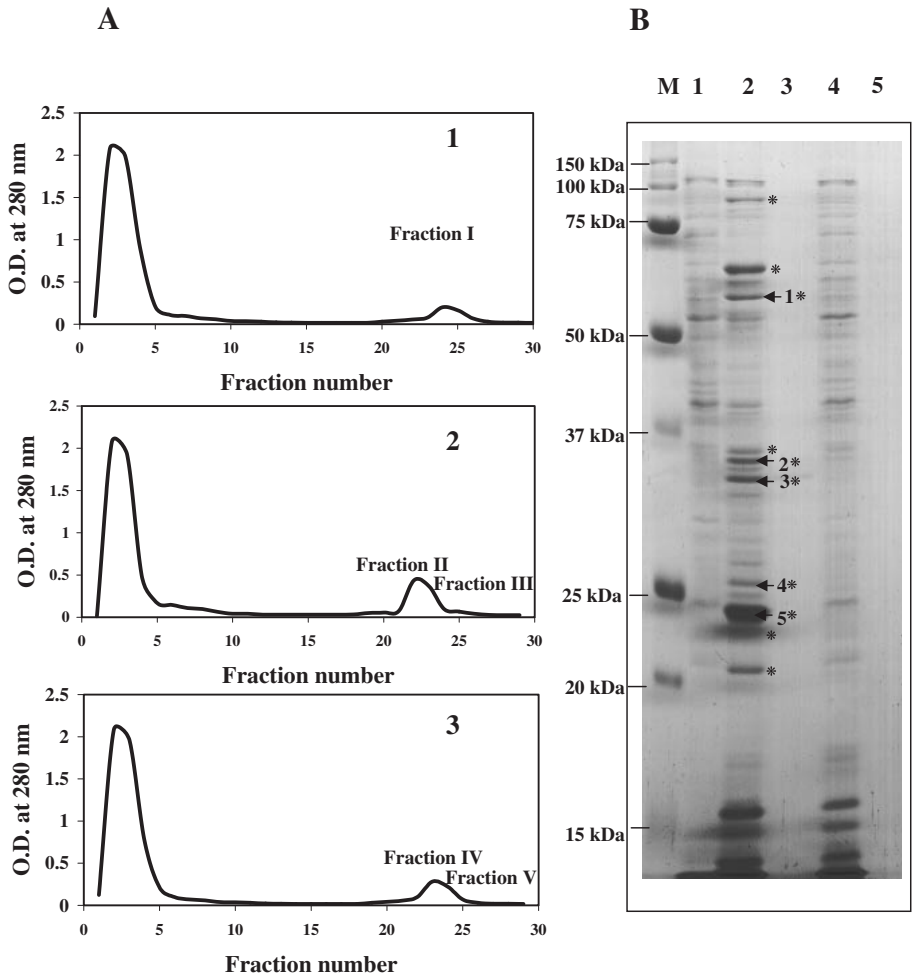

Fig. 5. Cadmium-Binding Proteins in *R. capsulatus* B10.

A, Metal chelate affinity chromatography of the soluble cell-free extract of *R. capsulatus* B10. (1) no metal-binding column, (2) cadmium-binding column, (3) magnesium-binding column. Ten mL of the soluble cell-free extract prepared from the untreated cells (1.5 g wet weight) was applied to each of the columns. After the column was washed with 10 mM Tris–HCl (pH 8.0), the adsorbed proteins were eluted with 10 mM Tris–HCl (pH 8.0) containing 0.5 M NaCl. Finally the column was washed with 10 mM Tris–HCl (pH 8.0) containing 0.1 M Na–EDTA. All steps were performed at 4 °C. Fraction I (nos. 23–26) was eluted with 0.5 M NaCl from the no metal-binding column; fraction II (nos. 21–24) was eluted with 0.5 M NaCl from the cadmium-binding column; fraction III (nos. 27–29) was eluted with 0.1 M Na–EDTA from the cadmium-binding column; fraction IV (nos. 23–25) was eluted with 0.5 M NaCl from the magnesium-binding column; and fraction V (nos. 27–29), eluted with 0.1 M Na–EDTA from magnesium-binding column. B, SDS–PAGE of fractions I–V. Lane 1, fraction I; lane 2, fraction II; lane 3, fraction III; lane 4, fFraction IV; lane 5, fraction V; lane M, markers. Protein bands marked with an asterisk are specifically found in fraction II. They were eluted with 0.5 M NaCl from the cadmium-binding column. The protein band numbers in the gel correspond to the protein numbers in Table 2.

chelating affinity column chromatography. Soluble cell-free extracts were prepared from untreated cells and applied to the no-metal binding column, magnesium-binding column, and cadmium-binding column, respectively. As shown in Fig. 5A, most of the proteins passed through the columns. The adsorbed proteins on the columns were eluted with buffer containing 0.5 M NaCl. We obtained fraction I from the no-metal binding column, fraction II from the cadmium-binding column, and fraction IV from the magnesium-binding column. Figure 5B shows the SDS polyacrylamide gel electrophoreses of fractions I–V. Fractions III and V, which were eluted with 100 mM Na–EDTA, scarcely contained the proteins, indicating that the proteins adsorbed on the columns were completely eluted with 0.5 NaCl. Some proteins, marked with asterisk in Fig. 5B, were specifically found in fraction II, suggesting that these may be the cadmium-binding proteins. Hence, we extracted all cadmium-binding proteins from the gel and determined their N-terminal amino acid sequences by the method described in "Materials and Methods." As summarized in Table 2, we identified proteins nos. 1, 2, 3, 4, and 5, although the molecular weights of the proteins of R. capsulatus B10 did not exactly correspond to those of the proteins for identification. The N-terminal amino acid sequences of other cadmium-binding proteins have not been determined yet.

The N-terminal amino acid sequence of protein no. 1

Table 2. N-Terminal Amino Acid Sequences of the Cadmium-Binding Proteins of *Rhodobacter capsulatus* B10

Protein no.	Sequence determined	Machs characteristics
1	MKLHSVRTRKSADHLP	75% identified with 2-methylcitrate dehydratase of *Rhodospirillum rubrum* ATCC 11170 with 58 kDa, and pI = 6.06.
2	ATSLTGAGATFPAPVYAKWA	100% identity with Phosphate-binding periplasmic rotein precursor PstS of *Photorhabdus luminescens* subsp. *laumondii* TTO1 with 35 kDa, and pI = 9.4.
3	TKAVQVHKVGGPEALVYEAI	95% identified with IMP dehydrogenase/GMP reductase:Zinc-containing alcohol dehydrogenase superfamily of *Rhodopseudomonas palustris* BisA53 with 33 kDa, and pI = 7.8.
4	MLQSALINVMVKAARRAGRS	95% identity with inositol monophosphatase of *Rhodopseudomonas palustris* BisB5 with 25.5 kDa, and pI = 6.4.
5	ARCGGDFQGFVAA?SQWA	100% identity with Lytic murein transglycosylase of *Rhodopseudomonas palustris* BisA53 with 23.5 kDa, and pI = 9.1.

Proteins are numbered according to Fig. 5.

is similar to that of 2-methylcitrate dehydratase of *Rhodospirillum rubrum*. Protein no. 2 appears to be the periplasmic phosphate-binding protein precursor, PstS. The phosphate assimilation system, Pst (phosphate-specific transport), is best understood in *E. coli*. The Pst system is composed of four distinct subunits encoded by the *pstS*, *pstA*, *pstC*, and *pstB* genes arranged in an operon, and are induced when the cells undergo starvation for Pi.[28] In the present study, we found that cadmium specifically binds the periplasmic PstS, suggesting that the phosphate uptake may be inhibited. As shown in Fig. 2B, the phosphorus level decreased in the cadmium-treated cells. Therefore, the growth inhibition by cadmium may be partially caused by inactivation of the Pst system. Protein no. 3 appears to be the IMP dehydrogenase/GMP reductase. IMP dehydrogenase (IMPDH) is an essential enzyme that catalyzes the first step unique to GTP synthesis. Bacteria, yeast, and mammalian cells are all dependent on an adequate supply of guanylates to maintain proliferation. Depletion of the intracellular guanylates, especially by inhibition of *de novo* synthesis *via* the IMP dehydrogenase pathway, is a potent signal for inhibition of proliferation.[29] Protein no. 4 appears to be inositol monophosphatase, which hydrolyzes myo-inositol 1-monophosphate to myo-inositol and phosphate. The genes have been identified in *Escherichia coli*,[30] *Mycobacterium smegmatis*,[31] *Methanococcus jannaschii*,[32] *Rhizobium leguminosarum* bv. *trifolii*,[33] and *Rhizobium leguminosarum* bv. *viciae*.[34] In *M. smegmats*, this enzyme is important in the synthesis of phosphatidylinositol, which is essential for growth,[35] but the existence of phosphatidylinositol in *R. capsulatus* membranes has not been proven. Protein no. 5 appears to be lytic murein transglycosylase, which catalyses the cleavage of the $\beta(1,4)$-glycosidic bond between *N*-acetylmuramic acid and *N*-acetylglucosamine residues in peptidoglycan with the concomitant formation of a 1,6-anhydro bond between the C1 and O6 atoms of the *N*-acetylmuramic acid residue. Höltje and Tuomanen proposed that the enzyme acts as cell-wall zippers during cell division.[36] As shown in Figs. 1 and 2, cadmium inhibits growth and produces filamentous cells. Therefore, it seems likely that cadmium specifically binds the lytic murein transglycosylase, resulting in inhibition of cell division.

In the present study, we identified six cadmium-induced proteins and five cadmium-binding proteins of *R. capsulatus* B10. Based on the elevated production of GroEL2, DnaK, and the ribosomal protein S1, it appears that cells growing in the presence of cadmium have an increased demand for the protein repair system and protein synthesis. In addition, increased synthesis of aspartate aminotransferase and phosphoglycerate kinase may be required for high energy production in the presence of cadmium. On the other hand, some bacterial species such as *Escherichia coli* produce intracellular cadmium-binding proteins, including alternative metal transporters, metal-detoxifying enzymes, and metallothioneins, to protect themselves.[26,37,38] Analysis of the SDS–PAGE of the proteins adsorbed on the metal-chelating column indicates that *R. capsulatus* has several cadmium-binding proteins. Among the cadmium-binding proteins, 2-methylcitrate dehydratase, the periplasmic phosphate-binding protein precursor, and lytic transglycosylase were highly induced in the cadmium-treated cells (data not shown). It is of interest that the major cadmium-binding protein (protein no. 5) is the lytic murein transglycosylase. The *E. coli* lytic transglycosylase contains a single metal ion-binding site that resembles the EF-hand calcium-binding sites.[39] Therefore, cadmium may specifically bind the metal-binding site of lytic transglycosylase and partially inhibit the growth and cell division of *R. capsulatus*. Further study on the lytic transglycosylase should give insight into growth inhibition by cadmium stress in *R. capsulatus* B10.

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