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Induction of stress proteins in the phototrophic bacterium *Rhodobacter sphaeroides*

Bernadette B. Nepple*, Reinhard Bachofen

University of Zürich, Institute of Plant Biology, Department of Microbiology, Zollikerstr. 107, CH-8008 Zürich, Switzerland

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Abstract

The stress response of the phototrophic bacterium *Rhodobacter sphaeroides* was investigated by two-dimensional gel electrophoresis. Oxygen, 0.5% and 4% ethanol, UV radiation, heat shock at 42°C and 0.01% phenanthrene were tested as stress factors. The protein pattern on two-dimensional gels of stressed as compared to control cells revealed that all stress factors applied caused modifications in the protein pattern of *R. sphaeroides*. The intensity of particular spots increased or decreased as a consequence of altered protein synthesis. Specific and general stress responses were observed.

Keywords: *Rhodobacter sphaeroides*; Stress protein; Heat shock; UV stress; Two-dimensional gel electrophoresis

1. Introduction

In nature microorganisms live most of the time in an environment that does not provide optimal growth conditions. They have to cope with nutrient starvation, temperature changes and other growth-restrictive conditions, generally called stress [1,2]. Bacteria need adaptational strategies to survive or even to grow in such adverse conditions and to repair damages resulting from high stress situations. When reacting to different stress factors the cells may change the rate of synthesis of certain cell components. Proteins whose expression is increased due to environmental adversity are called stress proteins. Studies with *Escherichia coli* [3], *Bacillus subtilis* [4] and other microorganisms reveal two kinds of stress proteins, general stress proteins and specific stress proteins. Proteins whose concentration is altered by two or more stress factors are called general stress proteins. Nyström and Neidhardt [5] described such a universal stress protein for *E. coli* named UspA. Most stress proteins, like the heat shock chaperonin proteins, are constitutively synthesised at a low level because they possess functions also required under normal growth conditions. They facilitate the correct folding of nascent proteins, prevent them from aggregation and furthermore renature aggregated proteins.

Rhodobacter sphaeroides is a facultative phototrophic, Gram-negative bacterium found in many aquatic ecosystems, that is able to grow either anaerobically in the light or aerobically in the dark. Being a purple non-sulfur bacterium, it belongs to the α group of the Proteobacteria [6]. So far not much

* Corresponding author. Tel.: +41 (1) 385 42 82; Fax: +41 (1) 385 42 04; E-mail: bnepple@botinst.unizh.ch

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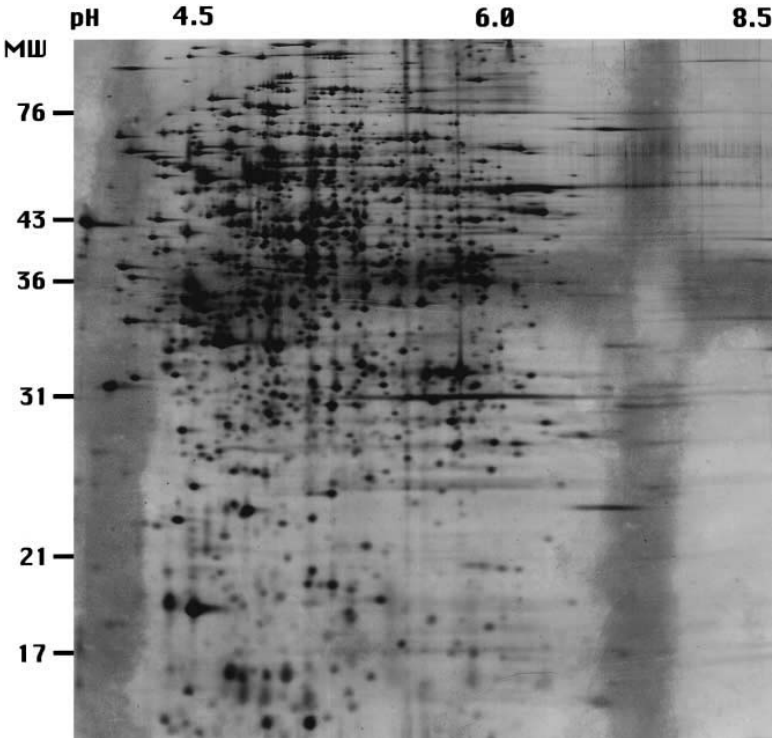

Fig. 1. Two-dimensional gel protein pattern of control cells of *Rhodobacter sphaeroides*. Cells were collected in the mid-exponential phase at OD_{660} of about 0.550. For growth conditions see Section 2.

is known about the stress response in phototrophic bacteria. The chaperonin 10 and 60 proteins, two heat shock proteins of *R. sphaeroides*, have been isolated and characterised [7]. They resemble the *E. coli* chaperonin proteins in their N-terminal amino acid sequences. General changes in the protein pattern as a reaction of stress have not been examined in phototrophic purple bacteria so far.

In this work we investigated the induction of stress proteins in *R. sphaeroides* as an example for the stress response in phototrophic bacteria. Protein samples from stressed and control cells were separated by two-dimensional gel electrophoresis and the protein patterns compared. As stress factors we used those which have been applied before for *E. coli* and other organisms, namely heat shock, UV radiation, ethanol and oxygen. Additionally we tested the pollutant phenanthrene.

2. Materials and methods

2.1. Bacterial strain and culture conditions

Rhodobacter sphaeroides 158 (ATCC 17023) was used for this study. The bacteria were grown at 30°C anaerobically in the light with 14 W m^{-2} in 100 ml screw cap bottles as batch cultures in Sistrom medium [8] containing 0.24% succinic acid as carbon source. Colour change from greenish brown to red indicating aerobic conditions in the light [6] occurred at an oxygen concentration of 0.25–0.3 mg l^{-1}. Therefore the cultures grown with oxygen in the light were bubbled with sterile air until 0.3 mg l^{-1} oxygen were reached, as measured with a WTW OXI 92 oxygen electrode.

2.2. Stress conditions

When the bacteria reached mid-exponential phase (OD_{660} 0.5–0.55), they were exposed to the various

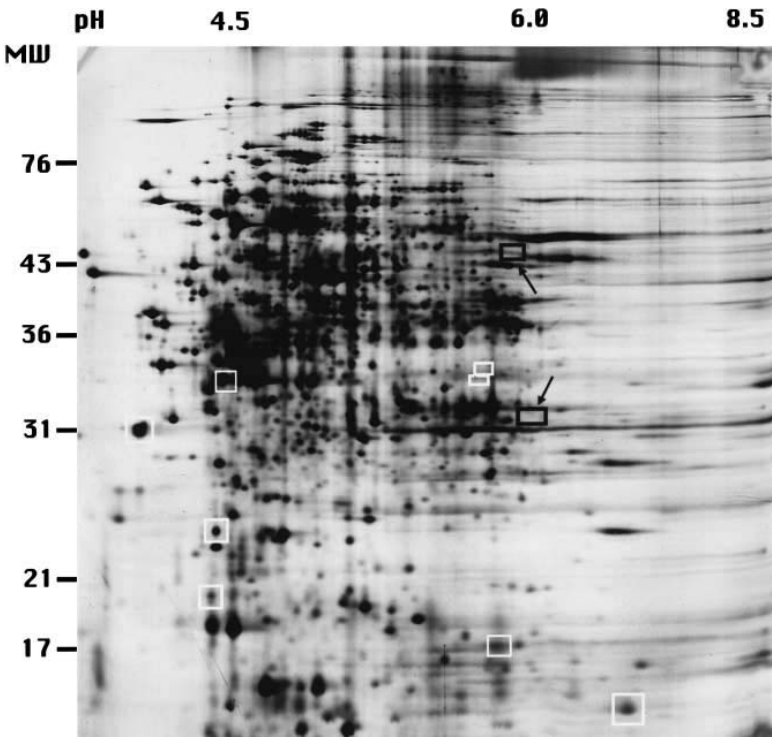

Fig. 2. Two-dimensional gel protein pattern of *R. sphaeroides* cells grown with air in the light. Samples were collected in the mid-exponential phase. The proteins newly induced are indicated by white squares, the ones with reduced expression by dark squares. The spots mentioned in the text are marked with an arrow.

stress factors by adding 0.5% ethanol, 4% ethanol, 0.01% phenanthrene (dissolved in ethanol, yielding a final concentration of 0.5% ethanol in the culture), respectively and samples taken after 30 min. Cultures that grew from the beginning with 0.3 mg l^{-1} oxygen in the light were taken for protein analysis when an OD_{660} of 0.5–0.55 was reached. Other cultures were exposed to UV radiation (short wave, 0.06 W m^{-2}) for 10 min. For heat shock, the cultures were exposed to 42°C for 30 min. Samples were taken after 10, 20 and 30 min. A control sample was taken before stress treatment. Each experiment was repeated at least three times.

2.3. Sample preparation and two-dimensional gel electrophoresis

The cell suspensions sampled were collected by centrifugation, washed with 50 mM Tris buffer pH 6.8 and resuspended in distilled water. Protein concentration was determined according to Bradford, with bovine serum albumin as standard. For electrophoresis 0.1 M Tris buffer pH 6.8, 1% DTT and 2% SDS were added to the cell suspensions. After shaking for 1 min, the mixtures were treated with DNase and RNase (50 and 10 μg ml^{-1}) for 30 min at room temperature. The solutions were then incubated for 1 h with 20 mM urea, 0.44% CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-propanesulfonate), 0.04% 3/10 and 0.01% 5/7 carrier ampholytes (Bio-Rad) and 0.7% DTT at room temperature. The solubilised protein was separated from insoluble material by centrifugation (modified after [9]). 2-D electrophoresis was done according to O'Farrell [10]. Equivalent amounts of protein were loaded onto isoelectric focusing gels (4% capillary gels, inner diameter 1.5 mm). For the second dimension the capillary gels were transformed to a 12% SDS gel after 8 min incubation in equilibration buffer [11]. For staining of the proteins a sensitive method of silver staining

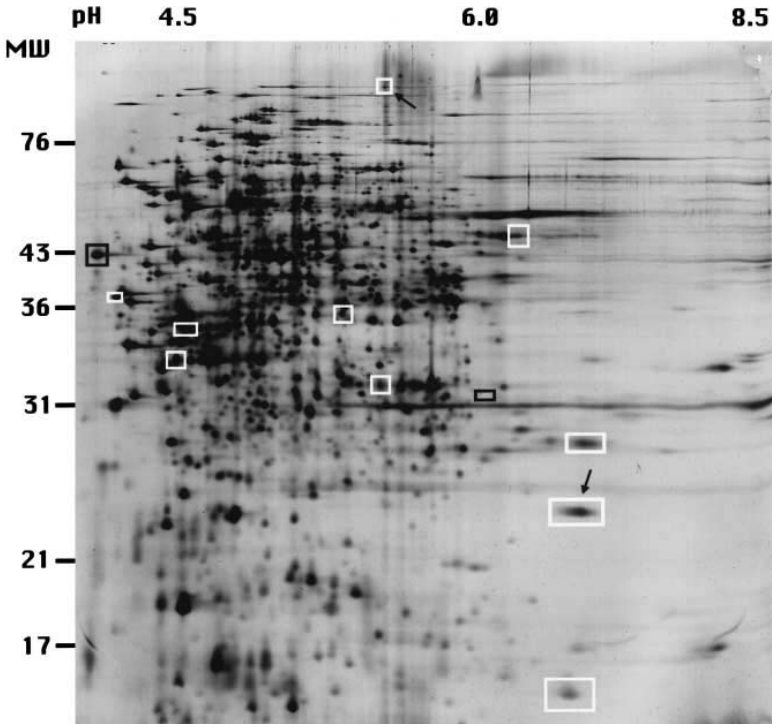

Fig. 3. Two-dimensional gel protein pattern of *R. sphaeroides* cells treated with 0.01% phenanthrene in the mid-exponential phase. Cells were harvested 30 min after the addition of phenanthrene. Symbols used are as in Fig. 2. The two proteins specific for phenanthrene stress are indicated by arrows.

was used (modified from [12]). Commercially available standard proteins allowed to define all proteins on a 2-D gel by their isoelectric point and molecular mass. Comparison of protein patterns from stressed cells and controls was done with at least three gels each. For publication the optically best gels were chosen.

3. Results and discussion

All stress factors used, with the exception of phenanthrene, had no inhibitory effects in the concentrations applied on the growth of the bacteria. When phenanthrene was added, the stationary phase was reached at a lower optical density compared with other cultures (0.7 instead of 1.6). None of the stress factors had a lethal effect on the bacteria.

In the presence of oxygen the culture (normally greenish brown) turned red caused by the conversion of spheroidene and hydroxyspheroidene into their corresponding ketocarotenoids [6]. Protein analysis showed that increased oxygen concentration led to a stress response. In comparison to the control culture (Fig. 1), oxygen stress (Fig. 2) led to reduced intensity of two spots, corresponding to molecular masses of 30 and 55 kDa. The 30 kDa protein showed furthermore reduced expression in 0.5% or 4% ethanol and 0.01% phenanthrene treated cells.

Phenanthrene was used as a stress factor because of its environmental impact. Phenanthrene represents one of 16 polyaromatic hydrocarbons of the Environmental Protection Agency (EPA) list. Only two of the 12 proteins with altered expression were specific for phenanthrene stress (Fig. 3). Thus, this toxic compound caused a rather general stress response. As phenanthrene was dissolved in ethanol, some stress response of 0.5% ethanol alone was expected due to the ethanol added (final concentration 0.5%). However, in the presence of phenanthrene five alterations in protein expression were identical with those caused by ethanol (Fig. 4). The other proteins in-

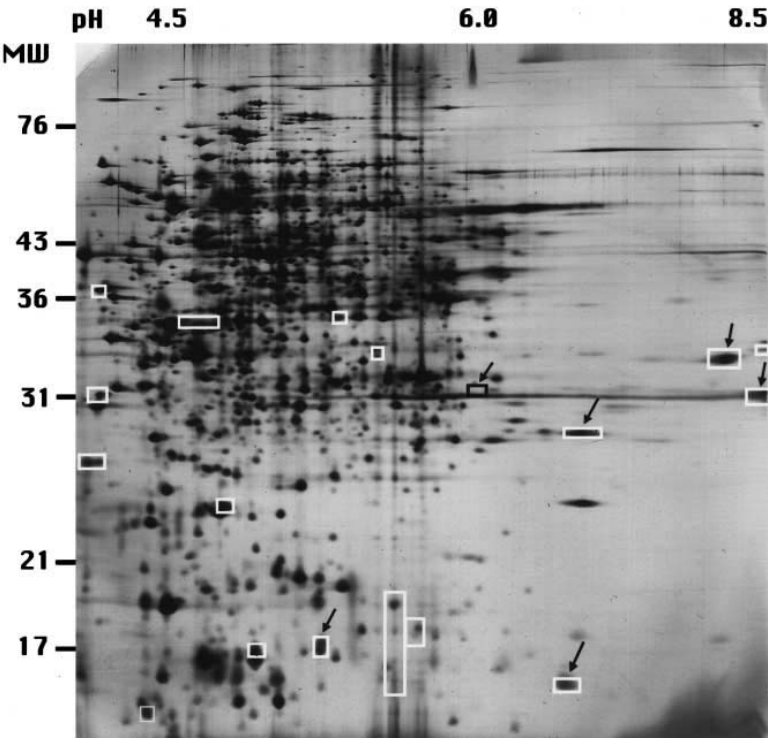

Fig. 4. Two-dimensional gel protein pattern of *R. sphaeroides* cells treated with 0.5% ethanol in the mid-exponential phase. Cells were harvested 30 min after the addition of ethanol. Symbols used are as in Fig. 2. The spots that are more intensive after 4% ethanol treatment are indicated with arrows.

duced matched with those induced by UV, 4% ethanol, oxygen or heat shock. This result indicates that the stress response of a combination of two stress factors is not simply the addition of the effects of both, but leads to a new adaptation with a specific pattern. Comparing the stress responses of two different ethanol concentrations (0.5% and 4%, respectively) revealed that five altered spots were identical for both concentrations. Different proteins were induced specifically at each concentration. A similar result was obtained applying cold shock at different temperatures for *B. subtilis* [13]. Several of the proteins induced were common to all temperatures, while others were specific to a particular shock temperature.

Treatment with UV radiation resulted in the induction of at least 25 proteins (Fig. 5). None of the control proteins was inhibited in its expression. UV radiation causes an enhanced synthesis of distinct proteins that may help the cell to survive this special stress situation. This might indicate an SOS-like response as UV radiation damages DNA. In *E. coli* the expression of the SOS response proteins is regulated by RecA and LexA proteins [14]. The *recA* gene from *R. sphaeroides* is known [15] and for its gene product an approximate molecular mass of 39 kDa was estimated. One of the two 41 kDa proteins induced by UV stress in *R. sphaeroides* might be identical with the RecA protein. Three proteins with enhanced expression match with those after heat shock. A connection between the heat shock regulon and the SOS response was suggested by Hartke and co-workers [14]. UV and heat shock appear to be severe stresses as they result in more pronounced modifications in the protein pattern than the other stress factors tested.

A total of 34 polypeptides were induced by heat shock (Fig. 6). This change was already visible 10 min after exposure to the higher temperature and did not alter afterwards. When the cells were trans-

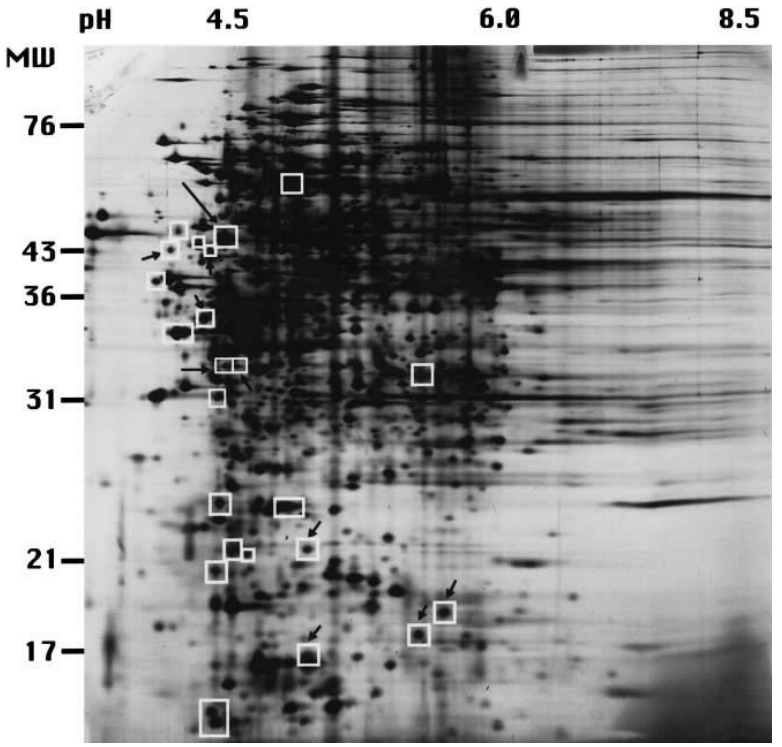

Fig. 5. Two-dimensional gel protein pattern of *R. sphaeroides* cells exposed to UV radiation for 10 min in the mid-exponential phase. Symbols used are as in Fig. 2. The proteins induced by heat shock as well are indicated with arrows.

ferred back to 30°C after 30 min, the analysis of the protein pattern 10, 20 and 30 min afterwards presented no change. Thus, readaptation to the original growth temperature induced no additional change in the protein composition. Only seven of the proteins induced by heat shock were induced also by other stress factors, and these may therefore belong to the group of so-called general stress proteins. The majority of the newly induced proteins, however, appear to be heat shock specific, similar to the result found in *B. subtilis* [2]. A protein of low molecular mass (18 kDa) and an isoelectric point of about 6.1 was induced by heat shock. This protein (marked with a big arrow in Fig. 6) was not detected in the control sample and induced only slightly by 4% ethanol and oxygen. Therefore it may contain a function specially needed after exposition of the cells to elevated temperatures. In *B. subtilis* a protein with similar molecular mass and isoelectric point was induced very strongly by heat shock, strongly by salt stress and glucose limitation and slightly by oxidative stress and oxygen limitation [16]. Recently we found that the *R. sphaeroides* 18 kDa protein is also induced weakly by cadmium and very strongly by copper. It will be interesting to know more on the function and structure of this protein.

In summary, all stress factors used caused modifications in the protein pattern of *R. sphaeroides*. For UV radiation stress and heat shock most of the observed changes were specific for this very stress. For oxygen, ethanol and phenanthrene only 10–30% of the changed proteins were stress specific. Thus, the concept of differentiation into specific and general stress proteins, as postulated for *E. coli* [5], *B. subtilis* [4], *Listeria* [1] and other organisms, may apply to *R. sphaeroides* too.

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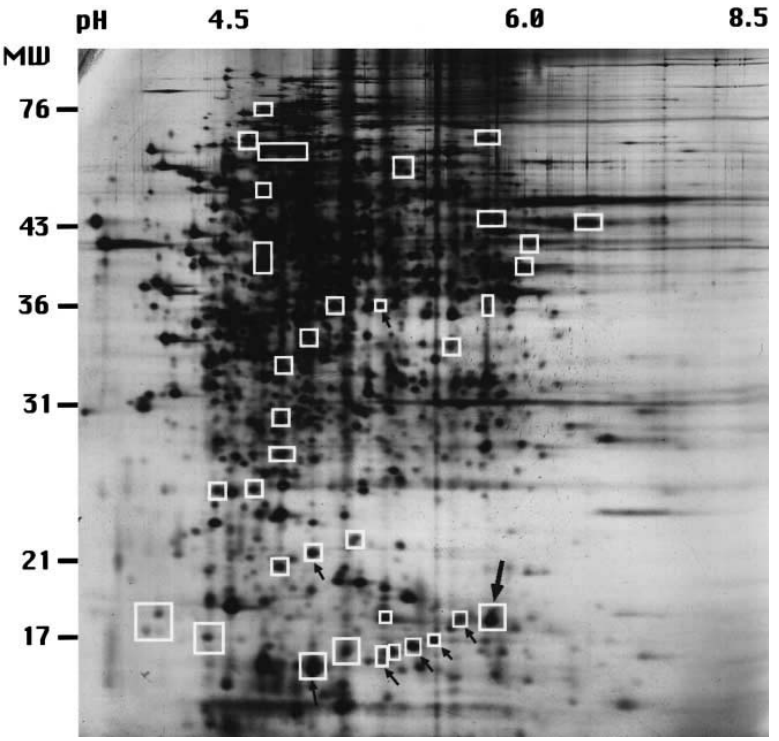

Fig. 6. Two-dimensional gel protein pattern of *R. sphaeroides* cells incubated at 42°C for 30 min in the mid-exponential phase. Cells were harvested 30 min after the heat treatment. Symbols used are as in Fig. 2. The proteins that are induced by other stress factors as well are indicated with arrows.

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