A High Potential Iron Sulfur Protein of the Purple Sulfur Bacterium *Thiocapsa roseopersicina*

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Thiocapsa roseopersicina, High Potential Iron Sulfur Protein (HIPIP), Phototrophic Bacteria

High potential iron sulfur protein (HIPIP) from the purple sulfur bacterium *Thiocapsa roseopersicina* was isolated in the oxidized and reduced form by DEAE- and Sephadex column chromatography (best purity index *A* 282 nm red./*A* 385 nm red. = 3.78). The enzyme contains non-heme iron and acid-labile sulfide in an equimolar ratio. The enzyme shows no aconitase activity.

Introduction

During our work on dissimilatory sulfur metabolism in the purple sulfur bacterium *Thiocapsa roseopersicina* (Chromatiaceae) besides a thermostable, soluble cytochrome c-550, which is involved in sulfide oxidation, we found large amounts of a high potential iron sulfur protein (HIPIP) [1]. HIPIP had been found in some phototrophic bacteria, *e. g.*, in *Chromatium vinosum* strain D [2], *Rhodopseudomonas gelatinosa* [3] and *Thiocapsa pfennigii* [4]. Both, the HIPIPs of *Chromatium vinosum* and of *Thiocapsa pfennigii* possess 4Fe–4S per protein molecule [5]. The function of HIPIP in the electron transport system or in sulfur metabolism is still unknown. Recently Ruzicka and Beinert [6] reported that the soluble mitochondrial HIPIP from beef heart is identical with aconitase, an enzyme of the tricarboxylic acid cycle. Here we present some properties of the HIPIP of *Thiocapsa roseopersicina.*

Materials and Methods

T. roseopersicina strain DSM 219 was grown photolithoautotrophically at 30 °C and approximately 1000 lx, in Pfennig's medium [7], using the trace element solution of Pfennig and Lippert [8]. The cells were harvested by continuous-flow centrifugation at 10500 rpm. Wet cells were suspended and homogenized in 200 mM Tris-HCl, pH 7.5 (w/v 1:1), broken up by sonification (1 min/ml cell suspension) in a cooled vessel (−4 °C) in a Schoellerschall TG 250 (Schoeller and Co., Frankfurt, FRG), and then extracted according to Bartsch [9]. After the second DEAE-cellulose column (2 mM Tris-HCl, pH 8.0) the fractions containing most of the cytochromes and the HIPIP were concentrated by ultrafiltration using a PM 10 membrane (Amicon Corporation). Besides cytochrome c-550, oxidized and reduced HIPIP was eluted from the second DEAE-cellulose column, with 500 ml of 20 mM Tris-HCl, pH 8.0, with a linear NaCl gradient of 0–0.2 M. Protein was determined following the method of Lowry *et al.* [10] modified as described by Fischer and Amrhein [11].

Non-heme iron was determined by the bathophenanthroline method (Boehringer test combination Nr. 124214) in 1 cm cells at 546 nm. Acid-labile sulfide was determined by the method of King and Morris [12] with the following modification: 2 ml enzyme solution; 2.5 ml of 2% zinc acetate solution and 0.25 ml of 5 N NaOH were vigorously shaken for 1 min in a small test tube stoppered with a serum stopper. 1 ml of 0.2% N,N-dimethyl-*p*-phenylenediamine in 20% of H_2SO_4 was added with a syringe, mixed, and then 0.05 ml of 10% ammonium iron sulfate in 2% of H_2SO_4 was added by a syringe, too. The precipitated protein was then centrifuged for 10 min at 4500 rpm and the absorbancy of the supernatant fluid was read at 670 nm against a blank without protein. Sulfide concentration was taken out of a standard curve.

For flat bed electrofocusing the center fractions of the reduced HIPIP of the second DEAE-cellulose column were combined and dialysed twice against 2 l of 1 mM Tris-HCl, pH 8.0 over night at 4 °C and then concentrated by ultrafiltration to a total volume of 3 ml.

Flat bed electrofocusing was carried out in a LKB Multiphor 2117 on "Ampholine PAGplate, pH range 3.5 to 9.5" by the method described by Winter *et al.* [13]. Instead of 500 ml of distilled water, a mixture of 150 ml methanol and 350 ml distilled water was used for the preparation of the fixing solution, otherwise method 1 [13] was used for fixing and staining the gel. 1–2 cm broad destained gel stripes were re-

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corded in a Beckman Acta M VI gel scanner at 550 nm.

Aconitase activity was measured by the reduction of NADP at 340 nm in aqueous solution in a total volume of 3 ml, by the method described by Rose and O'Connell [14]. The reaction was started by adding isocitratedehydrogenase.

Absorption spectra were recorded in a Beckman Acta M VI spectrophotometer using 1 cm quartz cells.

All standard chemicals (analytical grade) were obtained from Merck, Darmstadt; biochemicals from Boehringer Mannheim GMBH and DEAE-cellulose from Whatman Biochemicals Ltd., Maidstone, England.

Results and Discussion

While looking for cytochromes involved in dissimilatory sulfur metabolism in the soluble fraction of *Thiocapsa roseopersicina* we also identified a high potential iron sulfur protein (HIPIP) [1]. This was isolated from the second DEAE-cellulose column as a small band of the oxidized purple form and a large band of the reduced green form (Fig. 1).

When we used the combined and dialysed center fractions of the reduced green form of the HIPIP for flat bed electrofocusing we observed one major band (HIPIP) and two minor bands (Fig. 2). At the end of the electrofocusing procedure HIPIP can immediately be detected in the unstained gel by its green coulour. On the basis of a control curve for Ampholine PAGplate pH range 3.5 to 9.5 (given in the LKB instruction manual for electrofocusing) an isoelectric point for the reduced HIPIP was calculated between pH 4 and 5.

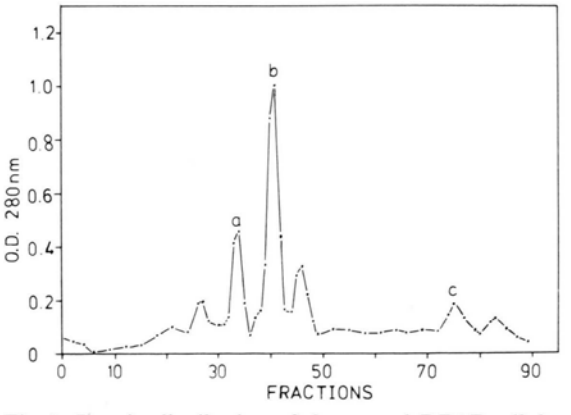

Fig. 1. Protein distribution of the second DEAE-cellulose column. Column properties: diameter 1.6 cm, bed volume 40 ml. Proteins were eluted using a linear gradient of 0–0.2 M NaCl in 20 mM Tris-HCl, pH 8.0, and measured spectrophotometrically at 280 nm in 1 cm cells against buffer. Fraction volume: 3.7 ml. a = ox. HIPIP; b = red. HIPIP; c = cytochrome c-550.

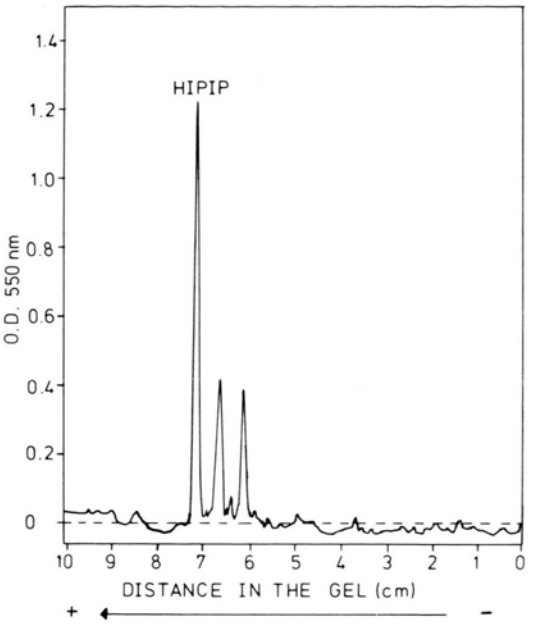

Fig. 2. Flat bed electrofocusing of the HIPIP of *T. roseopersicina* on Ampholine PAGplate pH range 3.5 to 9.5. Gel scanning was carried out at 550 nm. The sample (15 µl) contained 10.2 µg protein. Electrofocusing conditions: voltage and current: initial 200 (V), 50 (mA); final 1150 (V), 22 (mA). Temperature 7 °C; operating time: 90 min.

The absorption spectra (Fig. 3) revealed the typical wavelength maxima in the ultraviolet range: 278 nm and 282 nm and a shoulder at 291 nm. The best ratio for the reduced HIPIP was *A* 282 nm/*A* 385 nm = 3.78. Except for minor differences in this region, the data found are nearly identical with those of *Thiocapsa pfennigii* (277 nm, 283 nm, 291 nm; best ratio *A* 280 nm/*A* 375 nm red. = 2.8) [4] and *Rhodopseudomonas gelatinosa* (277 nm, 283 nm, 292 nm; best ratio *A* 283 nm red./*A* 388 nm red. = 2.35) [3]. When 0.1 ml of 10 mM mercaptoethanol was added to the oxidized form there appeared a small shoulder between 350–400 nm (Fig. 3A). This shoulder was also present in the originally eluted reduced form (Fig. 3B).

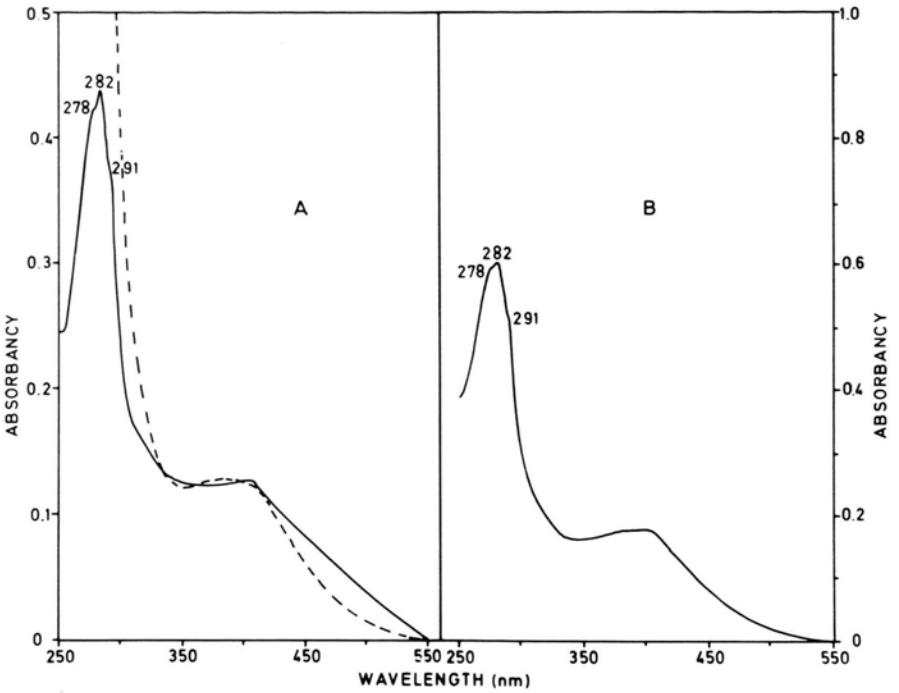

Fig. 3A. Oxidized and reduced absorption spectra of *T. roseopersicina* HIPIP (0.3 mg protein/ml) in 20 mM Tris-HCl, pH 8.0; total volume: 1 ml. ——— Oxidized form (purple band); – – – – reduced by the addition of 0.1 ml of 10 mM mercaptoethanol.
Fig. 3B. Absorption spectra from the originally eluted reduced form (green band) of the HIPIP of *T. roseopersicina* (0.3 mg protein/ml) in 20 mM Tris-HCl, pH 8.0; total volume: 1 ml, reference cell: 1 ml buffer.

It is known that the HIPIPs of *Chromatium vinosum* and *Thiocapsa pfennigii* possess 4 iron atoms and 4 labile sulfur atoms per molecule and are able to transfer a maximum of one electron per molecule [5]. When we combined the center fractions of the green reduced HIPIP (0.5 mg protein/ml) we determined 115 nmol non-heme iron and 120 nmol of acid labile sulfide per mg protein. From this result we conclude that in the HIPIP of *Thiocapsa roseopersicina* non-heme iron and acid-labile sulfide are in an equimolar ratio (1 : 1) as it is required for other well examined HIPIPs [5]. Until now little is known about the natural function of this electron carrier protein. Ruzicka and Beinert [6] were able to demonstrate that the soluble HIPIP from beef heart mitochondria is identical with aconitase, on the other hand these authors could not observe aconitase activity with the HIPIP of *Rhodopseudomonas gelatinosa.* This last report is in accordance with our results. We could not observe aconitase activity with the HIPIP from *Thiocapsa roseopersicina* in the described standard assay.

Another possible function of HIPIP was reported by Wood and Cusanovich [15]. The authors could show that cytochrome c-552 of the green alga *Euglena viridis* is reversibly reduced and oxidized by HIPIP of *Chromatium vinosum.* Complementary to this finding, Mizrahi *et al.* [16] confirmed that cytochrome c_2 of *Rhodospirillum rubrum* could be reduced or oxidized by the HIPIP of *Chromatium vinosum.* These results are of great interest, because Tedro *et al.* [5] postulated that the high potential iron sulfur protein of the photosynthetic bacteria may function as a soluble high potential electron transport poising agent, operating between substrate oxidizing pathways and the particulate photosystem.

From this point of view Fischer [17] suggested that HIPIP may perhaps act in the electron transport processes between the soluble cytochromes involved in dissimilatory sulfur metabolism of *Thiocapsa roseopersicina* and the membrane bound bacteriochlorophyll. Further studies should help to clear the real function of HIPIP.

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