

# Molecular Properties of High Potential Iron Sulfur Protein of *Chromatium warmingii*

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*Chromatium warmingii*, High Potential Iron Sulfur Protein (HIPIP), Phototrophic Bacteria

High potential iron sulfur protein (HIPIP) of the purple sulfur bacterium *Chromatium warmingii* was purified to homogeneity by ion exchange chromatography, gel filtration and ammonium sulfate fractionation. The acidic protein was isolated in the reduced form. The best purity index ( $A_{280}/A_{388}$ ) obtained was 2.52, and 3.8  $\mu\text{mol}$  of the protein was isolated out of 100 g wet cell material. The molecular weights estimated by sodium dodecylsulfate polyacrylamide gel electrophoresis and gel filtration through Sephacryl S-200 were 8900 and 10500, respectively. The protein has an isoelectric point at pH 3.6 for the reduced form and at pH 3.8 for the oxidized form, and a midpoint redox potential of +355 mV. One mol of HIPIP contains 4 mol nonheme iron and 4 mol acid-labile sulfur.

## Introduction

In the preceding paper, we reported that the purple sulfur bacterium *Chromatium warmingii* contains two soluble acidic cytochromes – *c*-552 and *c'* – in addition to a membrane-bound cytochrome *c*-552 [1]. During the purification procedure, we also found large amounts of a high potential iron sulfur protein (HIPIP) in addition to a very labile bacterial ferredoxin. HIPIP is now also included as a subgroup of the (4Fe-4S) bacterial ferredoxins with very positive redox potentials [2]. Among phototrophic bacteria, HIPIP is found in Chromatiaceae and Rhodospirillaceae [2], whereas rubredoxin seems to be a characteristic iron-containing protein of Chlorobiaceae [3, 4]. HIPIP's isolated from the purple sulfur bacteria *Chromatium vinosum* and *Thiocapsa pfennigii* both possess single (4Fe-4S) clusters [5].

Little is known about the function of HIPIP. While the soluble mitochondrial HIPIP from beef heart shows aconitase activity [6], HIPIP of *Thiocapsa roseopersicina* does not [7]. Until now, there has been only one report of the involvement of HIPIP in sulfur metabolism. Fukumori and Yamana [8] reported that soluble HIPIP of *Chromatium*

*vinosum* was the most effective electron acceptor for the partially purified thiosulfate-oxidizing enzyme.

Here we present the molecular properties of *Chromatium warmingii* HIPIP and compare them with the corresponding properties of other phototrophic bacteria.

## Materials and Methods

*Chromatium warmingii* strain 6512 (DSM Number 173) was cultivated and harvested as described by Wermter and Fischer [1].

Cells of *Chromatium warmingii* were disrupted by sonification [1], and HIPIP was isolated by the method described by Bartsch [2]. HIPIP precipitates between 75% and 100% ammonium sulfate saturation.

Nonheme iron was determined by the batho-phenanthroline method (Boehringer test combination No. 124214) in 1 cm cells at 450 nm. Acid-labile sulfur was determined by the method of King and Morris [9] modified after Fischer [7].

If not otherwise indicated, all standard methods (spectrophotometric determinations, estimation of protein, redox potential, isoelectric point and molecular weight) were carried out as previously described by Wermter and Fischer [1] and Steinmetz and Fischer [4].

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Chemicals were purchased as described by Wermter and Fischer [1].

## Results and Discussion

High potential iron sulfur protein – an acidic electron carrier protein – was isolated in its reduced form as a broad green band from a DEAE-52 cellulose column with 20 mM Tris-HCl, pH 7.8 containing 60 mM NaCl. Oxidized red-brown HIPIP was not found. This is in contrast to the findings on HIPIP from *Thiocapsa roseopersicina* [7], from which both forms have been isolated. A typical spectrum of *Chr. warmingii* HIPIP is illustrated in Fig. 1. The reduced form of HIPIP was obtained by adding several crystals of sodium dithionite to the sample, while the oxidized state of the protein was brought about by adding potassium ferricyanide. The absorption spectrum obtained for reduced HIPIP of *Chr. warmingii* is very similar to those found in *Thiocapsa pfennigii* [10], *Thiocapsa roseopersicina* [7], and *Rhodospseudomonas gelatinosa* [11].

The best purity index ( $A_{280}/A_{388}$ ) obtained for the reduced HIPIP was 2.52. This value lies in the range

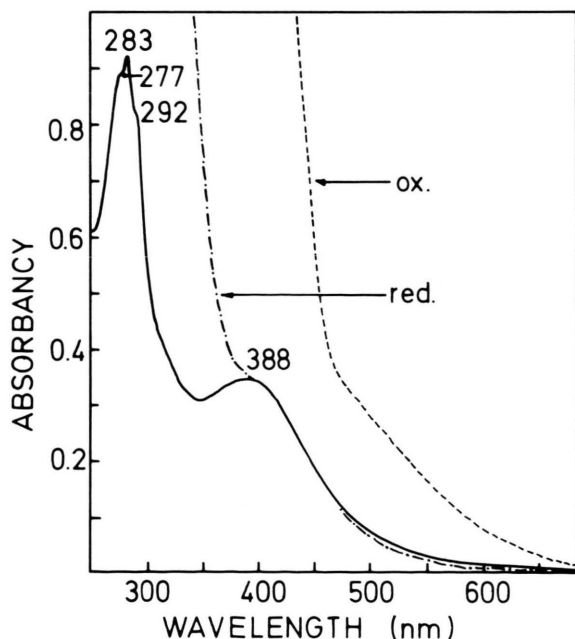


Fig. 1. Absorption spectra of *Chr. warmingii* HIPIP. 1 cm cells contained 0.63 mg protein in 1 ml 100 mM potassium phosphate buffer, pH 7.5. HIPIP was oxidized by adding a few crystals of potassium ferricyanide and reduced by adding sodium dithionite.

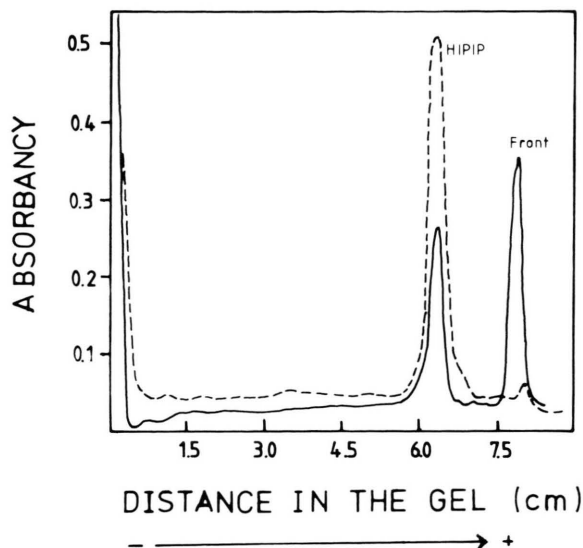


Fig. 2. Densitogram of purified *Chr. warmingii* HIPIP obtained by gel electrophoresis in 7.5% polyacrylamide gel with bromophenol blue as marker. Dotted line: after staining with Coomassie blue R-150 (0.25%), dissolved in a mixture of acetic acid:methanol:water = 1:5:5. Dashed line: unstained gel. Gel scanning was carried out in an Acta M VI double beam spectrophotometer at 580 nm (---) and 390 nm (—).

of the results described for *T. pfennigii* (Purity index 2.94; ref. [10]), *Chr. vinosum* (2.56; ref. [12]) and *Thiocystis violacea* (2.46; Fischer, unpublished results). To determine whether the HIPIP of *Chr. warmingii* with the purity index described above was purified to homogeneity, the protein was applied to polyacrylamide gel electrophoresis (7.5% gel). As can be seen in Fig. 2, only one protein band, representing HIPIP, was detected in the gel. This indicated that HIPIP isolated from *Chr. warmingii* was pure.

The molecular weight of *Chr. warmingii* HIPIP was determined by gel filtration through Sephacryl S-200 (with marker proteins of known molecular weight) and by polyacrylamide gel electrophoresis (7.5% gel) with 0.1% sodium dodecylsulfate. With the former method, we obtained a molecular weight of 10500, while a molecular weight of 8900 was calculated with the latter method (Fig. 3). The molecular weight of 8900 is closer to the values of *Chr. vinosum* (MW: 9258) and *Thiocapsa pfennigii* (MW: 9267) HIPIP's, calculated from amino acid sequence data [10, 12]. We therefore believe that 8900 value is closer to the actual molecular weight

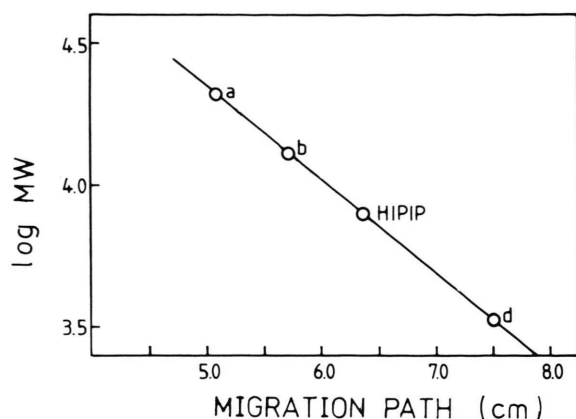


Fig. 3. Estimation of molecular weight of *Chr. warmingii* HIPIP by SDS-electrophoresis (7.5% gel). The migration path of HIPIP was compared with those of the following calibration proteins (Boehringer Combithek No. 236292): a, trypsin inhibitor from soy bean (MW: 21 500); b, horse heart cytochrome *c* (MW: 12 500); d, insulin chain B (MW: 3 400).

of the protein than is the value calculated by gel filtration. Only amino acid sequence studies of the protein can confirm this.

The isoelectric point of *Chr. warmingii* HIPIP was determined by flat bed electrofocusing on PAG-plates in the range of pH 3.5 to 9.5. Oxidized HIPIP

had an isoelectric point at pH 3.8 and reduced HIPIP at pH 3.6. The two isoelectric points differ by only 0.2 pH units. Similar observations have also been made for the acidic *Chr. vinosum* HIPIP (Table I). In contrast to the acidic HIPIP's of the purple sulfur bacteria, the HIPIP found in *Rhodopseudomonas gelatinosa* is basic (Table I and Ref. [2]).

The midpoint redox potential at pH 7.0 was +355 mV for *Chr. warmingii* HIPIP. Fig. 4 shows that the values of the ferricyanide titrated redox potential were identical to the theoretical values for  $n=1$  calculated from the Nernst equation and a redox potential of +355 mV. The high positive redox potential found for *Chr. warmingii* HIPIP is very similar to HIPIP's from other phototrophic bacteria (Table I and Ref. [2]).

In Table I, we summarize the main molecular properties of the HIPIP isolated from *Chr. warmingii* and we compare them with the corresponding properties of *Chr. vinosum*, *T. pfennigii* and *Rhodopseudomonas gelatinosa*. As can be seen in Table I, all results obtained for *Chr. warmingii* HIPIP are comparable and very similar to the data already described for HIPIP's from other organisms.

*Chr. warmingii* HIPIP, like *T. pfennigii* HIPIP, possesses four iron atoms and four labile sulfur

Table I. Comparison of molecular properties of *Chromatium warmingii* HIPIP with those of *Chromatium vinosum*, *Thiocapsa pfennigii* and *Rhodopseudomonas gelatinosa*.

Organism Properties	<i>Chromatium vinosum</i> <sup>a</sup>	<i>Thiocapsa pfennigii</i> <sup>a</sup>	<i>Rhodopseudomonas gelatinosa</i> <sup>a</sup>	<i>Chromatium warmingii</i> <sup>b</sup>
Molecular weight <sup>c</sup>	9 258 (A)	9 267 (A)	7 972 (A)	8 900 (P) 10 500 (S)
Redox potential ( $E_{m7}$ )	+ 350 mV	+ 340 mV	+ 330 mV	+ 355 mV
Isoelectric point				
– oxidized form	3.9	acidic	> 9.3	3.8
– reduced form	3.7	acidic	9.3	3.6
Purity index ( $A_{UVmax}/A_{visible max}$ )				
– Reduced	$\frac{283}{388} = 2.56$	$\frac{283}{375} = 2.94$	$\frac{283}{388} = 2.3$	$\frac{280}{388} = 2.52$
– Oxidized	$\frac{283}{375} = 2.2$	$\frac{283}{375} = 2.26$	$\frac{283}{380} = 1.84$	$\frac{280}{375} = 2.07$
Yield of HIPIP (μmol) per 100 g cell material	4	5	6	3.8

<sup>a</sup> Data taken from Bartsch [2].

<sup>b</sup> Own results.

<sup>c</sup> Molecular weights were determined by: amino acid sequence data (A); gel filtration (S); polyacrylamide gel electrophoresis with sodium dodecylsulfate (P).

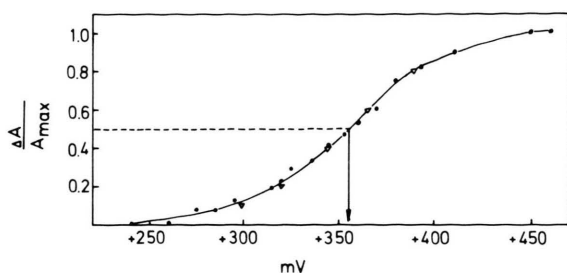


Fig. 4. Ferricyanide titration curve of *Chr. warmingii* HIPIP. The redox potential was varied by the stepwise addition of several  $\mu$ l of an anaerobic solution of 100 mM dithionite and 50 mM ferricyanide, and was measured with a calomel and a platinum electrode. (●—●) Ferricyanide titration curve of *Chr. warmingii* HIPIP. (▲—▲) Theoretical curve according to the Nernst equation for  $n = 1$  and a midpoint potential of +355 mV.

$$\frac{\Delta A}{A_{\max}} = \frac{\Delta A (510 \text{ nm} - 700 \text{ nm})}{A (510)_{\max}}$$

atoms per protein molecule [5]. The amounts of nonheme iron and acid-labile sulfide found in *Chr. warmingii* HIPIP were 341 nmol Fe and 360 nmol S per mg protein. This indicates that the HIPIP contains 4Fe and 4S atoms per protein molecule, calculated from a molecular weight of 10 500.

Only little is known about the function of HIPIP as an electron carrier. Mitochondrial beef heart HIPIP possessed aconitase activity [6], while HIPIP of *T. roseopersicina* did not [7]. Hori [13] reported that a "brown HIPIP-containing fraction" from the

halophilic *Paracoccus* is a very effective electron donor to nitrite reductase and terminal oxidase(s). No specific or unique function has been described for HIPIP of phototrophic bacteria.

Mizrahi and coworkers [14] could show that cytochrome  $c_2$  of *Rhodospirillum rubrum* was reduced or oxidized by *Chr. vinosum* HIPIP. Similar observations have been made by Wood and Cusanovich [15] with cytochrome  $c$ -552 of *Euglena viridis*, which was reduced or oxidized by HIPIP from *Chr. vinosum*.

Concerning the sulfur metabolism of phototrophic bacteria, an interesting result regarding the function of HIPIP was reported by Fukumori and Yamanaka [8]. These authors could show that soluble HIPIP of *Chr. vinosum* was the most effective electron acceptor for the thiosulfate oxidizing enzyme. Cytochromes ( $c$ -552;  $c'$ ; and  $c$ -553 (550)) from the same organism did not act as electron acceptors.

Since *Chr. warmingii* cannot utilize thiosulfate, it seems unlikely that HIPIP from this organism would take over the same function described above for the protein of *Chr. vinosum*. Further studies must be done to clarify the role of HIPIP in the sulfur metabolism of purple sulfur bacteria which cannot utilize thiosulfate.

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