Primary Structure of a High Potential Iron-Sulfur Protein from the Purple Non-sulfur Photosynthetic Bacterium

*Rhodopseudomonas gelatinosa*

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The third amino acid sequence of a high potential iron-sulfur protein, that of the non-sulfur purple photosynthetic bacterium *Rhodopseudomonas gelatinosa*, has been determined. It consists of a single polypeptide chain of 74 amino acid residues, which is slightly smaller than the high potential iron-sulfur proteins from the sulfur purple bacteria *Chromatium vinosum* (85 residues) and *Thiocapsa pfennigii* (81 residues). The sequence of the *gelatinosa* protein is similar to the *C. vinosum* and *T. pfennigii* proteins with 38% and 37% identically matching residues, although six gaps are proposed for the comparison (the *C. vinosum* and *T. pfennigii* proteins have 44% identically matching residues out of 73 positions compared with only one 4-residue gap). Only 17 residues, including the 4 cysteine residues essential for binding the four-iron-sulfur chromophore, are invariant in the three known sequences. A discussion of the role of conserved residues in maintenance of the three-dimensional structure and in electron transport is presented.

The ferredoxins, or electron transport iron-sulfur proteins, comprise a rather large and diverse group serving a number of essential roles in cellular metabolism (see Orme-Johnson for a review (1)). Nitrogenase (2), NADH dehydrogenase (3), succinate dehydrogenase (4), adenine phosphosulfate reductase (5), nitrate reductase (6), glutamate synthase (7), hydrogenase (8), and xanthine oxidase (9) are prominent examples. However, only the smaller, simpler ferredoxins have been well characterized structurally and these have been found to fall into four groups: the one-iron protein rubredoxin (10, 11), the two-iron-sulfur proteins, including "plant" (12) and adrenal (13) ferredoxins; and the four-iron-sulfur proteins of two types, "bacterial" ferredoxin (14, 15) and high potential iron-sulfur protein (16, 17).

Bacterial ferredoxin and HiPIP have virtually identical four-iron-sulfur cubane chromophores, which have been proposed capable of existing in three oxidation states (18). However, only two of these three oxidation states are observed in either protein under nondenaturing conditions. In bacterial ferredoxin, the pair of chromophores can each take up an electron at low oxidation-reduction potential (about +350 mv) (19), whereas the HiPIP chromophore can be reversibly oxidized at high oxidation-reduction potential (about +350 mv) (20). The peptide chains, responsible for the marked difference in oxidation reduction properties of structurally identical chromophores, are apparently unrelated in amino acid sequence and three-dimensional structure.

The observation that two unrelated peptide chains bind structurally identical four-iron-sulfur clusters with different resultant oxidation-reduction potentials poses some questions which may be answered through additional primary and tertiary structure determinations: e.g. (a) how many other unrelated peptide chains bind the same type of chromophore? (b) are there three oxidation states also available to the two-iron-sulfur centers, i.e. might one find a high oxidation-reduction potential two-iron-sulfur protein? (c) what is the extent and type of variability allowed in each peptide chain without appreciably altering the properties of the chromophore?

The HiPIP class of ferredoxin constitutes a family of soluble, monomeric proteins with molecular weights about 9000 which are physicochemically similar (20). The protein was, until recently, isolated from a very limited number of organisms. It was first reported in the photosynthetic purple sulfur bacterium *Chromatium vinosum* (21), then in the non-sulfur purple bacterium *Rhodopseudomonas gelatinosa* (20, 22), and in another purple sulfur bacterium, *Thiocapsa pfennigii* (23). We have since confirmed an early report of a HiPIP-like protein in a halotolerant, denitrifying bacterium which was tentatively identified as a *Micrococcus* sp. (24) but which is probably a *Paracoccus* sp. according to the latest edition of Berger's *Manual of Determinative Bacteriology* (25), and have isolated HiPIP from two additional species of purple non-sulfur bacteria, *Rhodomicrobiurn vannielii* and *Rhodospirillum vinosum*. 

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The abbreviations used are: HiPIP, high potential iron-sulfur protein; dansyl, 5-dimethylaminonaphthalene-1-sulfonyl.
HiPIP has thus been found in both species of purple sulfur bacteria examined; it occurs occasionally in all three genera of non-sulfur purple bacteria, but in a minority of species, and is found in a nonphotosynthetic denitrifying bacterial species.

A protein bearing some similarity to HiPIP has been isolated from mitochondria (26); it contains approximately equimolar amounts of iron and inorganic sulfur, with an EPR spectrum similar to that of HiPIP and unlike that of either plant or bacterial ferredoxin, but differs from HiPIP in that it is autooxidizable and of large molecular weight (37,000).

The amino acid sequences of the high potential iron-sulfur proteins from the purple sulfur photosynthetic bacteria C. vinosum (16) and T. pfennigii (27) are similar, although there are only 44% identical residues of 73 positions compared and a 4-residue gap in the middle of the peptide chain has been proposed. R. gelatinosa, although photosynthetic, is a facultatively aerobic organonothroph (25), physiologically distinct from the two strictly anaerobic sulfur bacteria previously studied. R. gelatinosa HiPIP is a basic protein (pI 9.33) as opposed to the acidic proteins from the two purple sulfur bacteria, and the amino acid composition is markedly different (29). The amino acid sequence of R. gelatinosa HiPIP presented in this report indicates less similarity to C. vinosum and T. pfennigii HiPIP sequences than they show to one another.

### Experimental Procedure

**Materials**—L-1-Tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin, a-chymotrypsin, and diisopropylphosphorofluoridate-treated carboxypeptidase A were purchased from Worthington Biochemical Corp., carboxypeptidase C and aminopeptidase M from Henley and Co., Inc., New York. Dapsyl amine acids were purchased from Calbiochem, La Jolla, Cal., and dansyl-chloride from Regis Chemical Co., Chicago, Ill. Staphylococcus aureus protease was a gift from Dr. Richard P. Ambler. All other materials were obtained from commercial sources.

**Methods**—Cells of Rhodospseudomonas gelatinosa C. B. Van Niel ATCC 2.2.1 (ATCC 17011) were grown according to De Klerk and Kamen (22). One kilo of frozen cells was suspended in 4 liters of 0.1 M Tris-Cl (pH 7.3) and disrupted in a Ribi-Sorvall Cell Fractionator at 20,000 p.s.i. and 20-30°C. A few milligrams of DNase were added and broken cells were centrifuged in a Sorvall SS34 rotor at 30,000 x g for 1 hour and then in a Spino 35 rotor at 140,000 x g for 4 hours. The supernatant solution was desalted on Sephadex G-25-C, the pH was adjusted to 6.0, 1 ml of 2-mercaptoethanol was added, and the solution was adsorbed on a column of CM-cellulose (7 x 15 cm). A stepwise salt gradient between 10 mM Tris-Cl (pH 7.3) and 10 mM Tris plus 40 mM NaCl, followed by cytochrome c-550 at 60 to 80 mM NaCl. At this stage of purification, there were 64 pmol of HiPIP with a A414/A280 of 4, about 20 pmol of cytochrome c', and 2 amol of cytochrome c-550. The HiPIP was fractionated with ammonium sulfate, the major portion precipitating between 60 and 90% saturation. The bout fractions were pooled (purity index 2.4) and then chromatographed on a column of Sephadex G-100 (6 x 120 cm). The final purification step involved CM-cellulose chromatography using a linear gradient between 10 mM Tris-Cl (pH 7.3) and 10 mM Tris plus 40 mM NaCl. The final purity index was 2.3, the same as found previously (22). A description of the isolation and purification of “halotolerant Micrococcus,” R. tense, and R. marinus HiPIPs is deferred to a future publication.

The protein was modified before enzymic digestion by either performic acid oxidation of the native protein or S-β-aminoethylthiolation of apoprotein as previously described (27).

Enzymic digestion and isolation of peptides was carried out as follows: L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin or α-chymotrypsin was added in ratios of 1/25 (w/w) to the modified protein dissolved in 0.5% ammonium bicarbonate (pH 8.5) at a concentration of 10 mg/ml. Digests were carried out at 4°C for 5 hours and terminated by lyophilization. Peptides were separated by Sephadex G-25-F chromatography and high voltage paper electrophoresis at pH 8.5 or pH 7.0 as described by Ambler (28) and the electrophoresis was repeated when required to further purify the peptides. Subdigestion of peptides by Staphylococcus aureus protease was performed at 40°C for 16 hours in 50 mM ammonium bicarbonate (pH 6.8), at an enzyme to substrate ratio of 1/30 (w/w), essentially as described by Houmard and Drapeau (29).

### Results

The amino acid composition of R. gelatinosa high potential iron-sulfur protein obtained from 24-, 48- and 72-hour hydrolysates of both S-β-aminoethylthiolated and performic acid-oxidized protein is presented in Table I and agrees very well with the composition derived from the sequence. The composition reported previously (20) is 16 residues high, but if normalized to 74 residues, agrees within experimental error.

NH₂-terminal degradation of the protein by the Edman procedure and digestion with carboxypeptidase A for 1 to 60 min yielded the following NH₂- and COOH-terminal sequence:

\[ \text{NH₂-Ala-Pro-Val-Asp-Glu-Lys-Asn-Pro-Gln-Ala-Val...} \]

### Enzyme Digestion and Isolation of Peptides

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### Letter Designations for Peptides

Letter designations for peptides to indicate protein modification and enzyme digestion are as follows: As for S-β-aminoethylthiolation, Ox for performic acid oxidation, T, C, and P for trypsin, chymotryptic, and Staphylococcus aureus protease digestion. Peptides are numbered in the order they occur in the sequence. Peptide mobility at pH 6.5 was measured relative to the mobility of lysine (+), aspartic acid (−), and neutral amino acids (0).

Amino acid analyses were performed as previously described (27). To minimize destruction of S-β-aminoethylcysteine and tyrosine during acid hydrolysis, 0.05% thioglycolic acid was routinely added to the tubes and 2% was added prior to hydrolysis of tryptic-containing peptides (30). Asparagine and glutamine were identified according to Benson et al. (31).

Peptides were sequenced by the dansyl method of Gray (32), but direct identification of phenylthiohydantoin-derivatives was found to give more satisfactory results for sequencing the NH₂-terminus of the whole protein. Edman degradation (33) of the denatured protein as described by Edman and Begg (34) was performed as described by Doolittle (35). The phenylthiohydantoin-derivatives were identified by thin layer chromatography as described by Dus et al. (36). COOH-terminal degradation of peptides and protein was accomplished using carboxypeptidase A (27) and carboxypeptidase C (36).

### Amino Acid Sequence of R. gelatinosa HiPIP

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Amino Acid Sequence of R. gelatinosa HiPIP

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Rhodospirillum gelatinosus</th>
<th>Chromatium okeni (18)</th>
<th>Thiocapsa pfennigii (27)</th>
<th>Haloarcula &quot;Micrococcus&quot;</th>
<th>Rhodobacter sphaeroides a</th>
<th>Rhodovulum vannielii b</th>
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<tr>
<td>Aspartic acid</td>
<td>7.0 ± 0.30</td>
<td>4</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>14</td>
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<td>Asparagine</td>
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<td>4</td>
<td>6</td>
<td>2</td>
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<tr>
<td>Threonine</td>
<td>1.0 ± 0.4</td>
<td>1</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Serine</td>
<td>2.7 ± 0.12</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>9</td>
</tr>
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<td>Glutamic acid</td>
<td>5.0 ± 0.35</td>
<td>1</td>
<td>9</td>
<td>9</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Glutamine</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Proline</td>
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<td>4</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Cystine</td>
<td>3.0 ± 0.10</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Glycine</td>
<td>8.0 ± 0.2</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>7</td>
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<tr>
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<td>9</td>
<td>10</td>
<td>13</td>
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<tr>
<td>Valine</td>
<td>5.9 ± 0.10</td>
<td>6</td>
<td>3</td>
<td>3</td>
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<td>3</td>
</tr>
<tr>
<td>Methionine</td>
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<td>0</td>
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<td>1</td>
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<tr>
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<td>3.0 ± 0.00</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
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<td>1.8 ± 0.19</td>
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<td>1</td>
<td>3</td>
<td>1</td>
<td>4</td>
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<tr>
<td>Phenylalanine</td>
<td>2.9 ± 0.35</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.0 ± 0.06</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Lysine</td>
<td>9.5 ± 0.32</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Arginine</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>12</td>
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<tr>
<td>Tryptophan</td>
<td>1.6 ± 0.26</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total residues</td>
<td>74</td>
<td>85</td>
<td>81</td>
<td>(70)</td>
<td>(85)</td>
<td>(85)</td>
</tr>
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</table>

* Average of 17 hydrolyses.
* Tentative results based upon 21 and 72 hour hydrolyses.
* Determined as cysteic acid after performic acid oxidation.
* Average of three hydrolyses.

Seven peptides were isolated from the tryptic digest of performic acid-oxidized protein. Their amino acid compositions are listed in Table III. As suspected from previous experience with oxidized protein, no peptide(s) containing tryptophan could be isolated. Peptide Ox1 3 (Ala,Lys) was recovered in a yield of 112% if regarded as a heterogeneous dipeptide, but Edman degradation gave the same result as with peptide AeT 3, further confirming that this was a mixture of two dipeptides with identical compositions, but different sequences.

**Chymotryptic Digests**—The 2.5 µmol of S-β-aminoethylated protein and 1.5 µmol of oxidized protein were digested by α-chymotrypsin. The amino acid compositions of the isolated peptides are given in Tables IV and V. The sum of the compositions of the AeC peptides agreed completely with the protein composition. Peptides AeC 12 and OxC 12 both had equimolar concentrations of alanine and lysine but had mobilities at pH 6.5 of +0.95 to +1.00 compared with +0.83 to +0.85 for AeT 3 and OxT 3. The sequence of AeC 12 was found to be Ala-Lys-Lys-Ala by the Edman degradation, and alanine and lysine were recovered in 70% and 10% yield, respectively, by 1-min digestion of the peptide with carboxypeptidase A.

**Identification of Amides**—Amides were determined in four ways. (a) electrophoretic mobility at pH 6.5, (b) Edman degradation with direct identification of phenylthiohydantoin-derivatives, (c) carboxypeptidase C digestion followed by quantitative analysis, and (d) specificity of Staphylococcus aureus protease for glutamic acid bonds.

Electrophoretic mobilities provided the best evidence for amides in the small peptides, but amides in the large peptides could not be determined in this manner alone. The acidic nature of peptide AeC 1 indicated the presence of at least two free acids. Initial indication of glutamine and glutamic acid residues was provided by Staphylococcus protease cleavage of OxC 1 (Table VI) at Glu 5-Lys 6, but not at Gln 9-Ala 10. The position of amides was confirmed by Edman degradation of AeC 1 with direct identification of phenylthiohydantoin-derivatives yielding: Ala-Pro-Val-Asp-Glu-Lys-Asn-Pro...

The electrophoretic mobility of the pentapeptide P1 was anomalously low, but carboxypeptidase C released 1.0 Glu, 0.58 Asp, and 0.40 Val, further confirming absence of amides at positions 4 and 5.

**Lack of Tryptic Cleavage at Lysine 6**—There was no tryptic cleavage observed at the Lys 6-Asn 7 bond in either oxidized or reduced protein. Edman degradation of P2 was repeated to be certain that the proline had been properly assigned. The peptide was also examined for methylation of the lysine. A standard mixture of methylated lysines was separated on the amino acid analyzer (dimethyllysine could not be separated from lysine, but trimethyllysine was eluted 5 min before and monomethyllysine 3 min after lysine). Hydrolyzed P2 showed only one peak corresponding to lysine. dansyl lysine and dansyl dimethyllysine standards were used on thin layer chromatography in pyridine/acetic acid/water/ethanol (30/60/500/147), but P2 yielded only dansyllysine. Aminopeptidase M (37) released lysine from P2, apparently...
also ruling out a derivatization of the lysine which might have been labile to acid hydrolysis. In situations where there is an aspartic acid residue adjacent to a lysine, the peptide bond formed by that lysine is often partially resistant to tryptic hydrolysis (38), but in the case of lysine 6 in R. gelatinosa HiPIP, the proximity of two acidic residues must render it totally unreactive under the experimental conditions employed.

The amino acid sequence of the protein derived from sequence data from four enzyme digests is presented in Table VII. The order of the tryptic peptides was established by overlapping chymotryptic peptides and the NH2 and COOH-terminal sequence of the protein. Single residue overlaps at cysteine 67, and lysines 28 and 64 introduce an element of weakness in the proposed sequence by providing sites for insertions of potentially missing segments. However, this possibility is unlikely because no peptides were isolated which were incompatible with the proposed sequence and the amino acid composition for the protein is in excellent agreement with the sequence. In order to avoid confusion all amides are listed as such in the peptide sequences, although they are identified as their respective acid by the dansyl method; also, the S-pyroglutamyl C. vinosum HiPIP is most similar (Table XI) to that of C. vinosum (16) and T. pfennigii (27) HiPIPs (Table IX), although six gaps are earlier along the peptide chain, at residues 53 to 55 and 53 to 56. Preliminary sequence results with Micrococcus HiPIP indicate that the deletion between cysteines 46 and 63 could extend to 7 residues.

A detailed discussion of the essential features of the HiPIP structure likely to be conserved in light of the three known amino acid sequences has been presented by Carter et al. (39). Foremost of the essential features appears to be the antiparallel β-structure, and the nonpolar interactions, which define the cavity in which the iron-sulfur cubane is bound to 4 cysteine residues.

The cavity in which the iron-sulfur cluster is situated is lined with 0 nonpolar residues. Of these residues, 5 are strictly invariant: leucines 17 and 65, tyrosine 19, and tryptophans 76 and 80. The remaining 4 residues are conservatively substituted with nonpolar side chains at positions 48, 49, 66, and 71. The aromatic residues have received special attention because of proposals developed with respect to the structures and mechanisms of action of cytochromes c (40) and c1 (41) and bacterial ferredoxin (15).

Conserved aromatic residues were proposed (40) to provide a route for electron flow in cytochrome c until cytochromes were found which had substituted residues in critical positions. In cytochrome c1 (41), a hydrogen bond network involving a tyrosine residue was suggested as a means of stabilizing the positive charge produced on oxidation of the heme and to explain the dependence of oxidation-reduction potential on pH. A weak point of this proposal was found to be nonconservation of some residues involved in the hydrogen bond network, although there could be a different network in each cytochrome. Aromatic residue involvement was also proposed in mechanisms of action of HiPIP (39) and bacterial ferredoxin (15), which had conserved aromatic residues near each iron-sulfur cluster. However, a clostridial ferredoxin was then found, which had only 1 aromatic residue, a tyrosine adjacent to the NH2 terminus, which could be chemically substituted by leucine without impairment of its enzymatic activity in the phosphoroclastic assay, although its stability was reduced (42). HiPIPs have 5 aromatic residues near the iron-sulfur cubane and in view of the reduced stability of the bacterial ferredoxin lacking aromatics (42), these residues, by analogy, might contribute to the remarkable stability of HiPIP.

In the mechanism of oxidation-reduction of HiPIP, a critical role has been ascribed to tyrosine 19, which would provide the route of electron transport and stabilize the oxidized form of the chromophore by ion pair formation, with hydrogen-bonding to a bound water molecule at the surface of the protein (39). Tyrosine 19 is conserved in the three known HiPIP sequences and appears to be present in Micrococcus HiPIP. However, it has been found* that the oxidation-reduction potential of C. vinosum HiPIP is constant between pH 6.5 and 11. If HiPIP tyrosine 19 does form an ion pair with the iron-sulfur cluster, one would expect the ionization constant for the tyrosine hydroxyl to be lowered and reflected in the oxidation-reduction potential measurements, which it is not. Furthermore, the rejection of mechanisms of electron transport mediated by endogenous tyrosine or other aromatics in cytochromes and bacterial ferredoxin casts some doubt on implication of such a role in HiPIP.

The surface charge in HiPIP was concluded to be unimportant because of its high variability (39). The total number of

* R. P. Ambler, personal communication.
* Dr. M. A. Cusanovich, personal communication.

DISCUSSION

The proposed amino acid sequence of R. gelatinosa HiPIP is similar to the published sequences of C. vinosum (16) and T. pfennigii (27) HiPIPs (Table IX), although six gaps are indicated in the sequence comparison. R. gelatinosa HiPIP appears to be equally similar to C. vinosum and T. pfennigii HiPIPs, with 38 and 37% identical residues, respectively, whereas the latter two proteins are identical at 44% of the 73 residue positions compared (Table X). The genetic distance between R. gelatinosa and the purple sulfur bacterial HiPIPs may be greater than indicated by Table X in view of the additional mutations required to produce gaps at six possible positions in the sequence.

Comparison of the newly determined amino acid sequence of R. gelatinosa HiPIP to those of C. vinosum and T. pfennigii has reduced the number of apparently invariant residues from 92 to 17 out of 85. The amino acid compositions of three new HiPIPs (Table I) also show a number of unique features, which should make their sequence determination most interesting, especially with respect to the number of conserved residues. *Dr. M. A. Cusanovich, personal communication.

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### Table VII

**Amino acid sequence of HiPIP from *R. gelatinosa* and peptide sequence as obtained by dansyl-Edman method**

Parentheses indicate when the degradation was terminated or the amino acid could not be identified.

<table>
<thead>
<tr>
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* See table VIII for COOH-terminal degradation by carboxypeptidase C.
Amino Acid Sequence of R. gelatinosa HiPIP

### Table IX

<table>
<thead>
<tr>
<th>C. vinosum</th>
<th>T. pfennigii</th>
<th>K. gelatinosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser Ala Pro Ala Asp Ala Val Ala Ala Asp Ala Thr Ala Ile Ala Leu Tyr Asn Gin Asp Ala Thr Lys Ser</td>
<td>Glu Asp Leu Pro His Val Asp Ala Thr Asn Pro Ile Ala Gin Ser Leu His Tyr Ile Glu Asp Ala Asn Ala Ser</td>
<td>Glu Lys Asp Pro Gin Ala Val Ala Leu Gly Val Val Ser Asp Ala Lys Ala</td>
</tr>
<tr>
<td>Cys Ala Ser</td>
<td>Asp Lys Thr Val Phe Ala Gly Ser Glu Thr</td>
<td>Cys Ala Ser</td>
</tr>
<tr>
<td>Glu Arg Asp Pro Val Thr Lys Thr Glu Leu Pro Gly Ser Glu Gin Phe His Asn Ser</td>
<td>Asp Lys - Ala Lys Tyr Lys Gin Phe Val Ala Gly Ser - - His Cys Gly Asn Ala Leu Phe Gin Gly Lys -</td>
<td></td>
</tr>
<tr>
<td>Glu Arg Asp Pro Val Thr Lys Thr Glu Leu Pro Gly Ser Glu Gin Phe His Asn Ser</td>
<td>Asp Lys - Ala Lys Tyr Lys Gin Phe Val Ala Gly Ser - - His Cys Gly Asn Ala Leu Phe Gin Gly Lys -</td>
<td></td>
</tr>
</tbody>
</table>

### Table X

<table>
<thead>
<tr>
<th>Chromatium vinosum</th>
<th>Thiocapsa pfennigii</th>
<th>Rhodopseudomonas gelatinosa</th>
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<tbody>
<tr>
<td>44</td>
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### Table XI

<table>
<thead>
<tr>
<th>Rhodobacter sphaeroides</th>
<th>Rhodopseudomonas capsulata</th>
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<tbody>
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</tbody>
</table>

charged residues, excluding histidine, in the three HiPIPs are similar (15 to 16 residues), but the difference between positive and negative charge leaves a net -7 charge on T. pfennigii, a -2 charge on C. vinosum, and a +5 charge on R. gelatinosa HiPIPs, consistent with their chromatographic and isoelectric behavior. However, in cytochrome c, localized positive surface charge (40), and in cytochrome f, localized negative charge (43), are involved in oxidation-reduction reactions. There is as yet no enzymatic assay for HiPIP, but kinetics of oxidation by ferricyanide ion suggest that a minus-minus charge interaction is involved. The only invariant charged residues in the three HiPIPs, of known sequence, are Asp 22 and Lys 83. Position 27 can be either glutamic or aspartic acid, and positions 28 and 33 can be either lysine or arginine. Tentative results of the Micrococcus HiPIP sequence work indicate that Asp 22 may be a constant feature, whereas none of the basic residues are likely to be conserved. It appears to be more than coincidence that the only charged residue likely to be conserved in HiPIP is Asp 22, that a negative charge is involved in the oxidation of C. vinosum HiPIP, and that Asp 22 is near the Tyr 19 suggested to be the key element in the oxidation-reduction mechanism. However, further kinetic experiments are required before participation of charged residues in the oxidation-reduction mechanism of HiPIP is to be considered general. Because the HiPIPs have been found in three physiologically distinct bacterial families, their functional roles need not necessarily be identical and might even be unrelated. Thus, different functional constraints, including surface charge, may be imposed on those HiPIPs having different physiological roles.

There is a small degree of similarity between the amino acid sequences of HiPIP and plant ferredoxin, viz. the proteins are comparable in size and three of the four chromophore-binding cysteines are similarly distributed. The 2-Fe-S cluster-binding cysteines in the 97-residue plant ferredoxins (44) are at positions 39, 44, 47, and 77, and the 4-Fe-S cluster-binding cysteines in the 85-residue HiPIP are at positions 43, 46, 63, and 77. Furthermore, no helix was found in spinach ferredoxin by circular dichroism measurements (45), consistent with the near absence of helix in HiPIP. X-ray diffraction analysis of plant ferredoxin in progress should provide a test of the proposed similarity to HiPIP. The degree of similarity proposed between plant ferredoxin and HiPIP is analogous to that observed (46) between Pseudomonas cytochromes c-551 and c-552. These cytochromes are of comparable size and the heme-binding residues (cysteine, histidine, methionine) are similarly situated.

A postulated alignment of bacterial and plant ferredoxin sequences has provided a tentative basis for construction of evolutionary trees (47), although the three-dimensional structural test of relatedness has not yet been applied. There is no similarity in the manner of peptide chain folding in HiPIP and bacterial ferredoxin, which would suggest that one or both of

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1 T. Pouls and J. Krut, personal communication.
Amino Acid Sequence of R. gelatinosa HiPIP

the above hypotheses relating plant ferredoxin to either HiPIP or bacterial ferredoxin is incorrect.

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REFERENCES

47. Dayhoff, M. O. (1972) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation. Silver Spring, Maryland
Amino Acid Sequence of R. gelatinosa HbPiP
Primary structure of a high potential iron-sulfur protein from the purple non-sulfur photosynthetic bacterium Rhodopseudomonas gelatinosa.

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