MOLECULAR WEIGHT AND AMINO ACID COMPOSITION OF CHROMATIUM FERREDOXIN*

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Arnon (1965a) has divided the ferredoxins into bacterial and plant types which have certain properties in common but are distinct in other characteristics. The amino acid sequences of two ferredoxins isolated from clostridial species and that of spinach ferredoxin have been described (Tanaka et al., 1964, 1966; Benson et al., 1966; Matsubara et al., 1967). It was suggested that the two different types had similar amino acid sequences extending through 19 consecutive residues, indicating their origin from a common archetype during evolution (Matsubara et al., 1967).

The molecular weight and some chemical properties of a ferredoxin isolated from the photosynthetic anaerobic bacteria, Chromatium, have been described in several reports, but some discrepancies exist in these descriptions (Bachofen and Arnon, 1966; Arnon, 1965b; Bearden et al., 1965). The primary structure of Chromatium ferredoxin is of interest, since it might be intermediate between those of the ferredoxins of nonphotosynthetic bacteria and of green plants.

As a first step, we determined the molecular weight and amino acid composition of Chromatium ferredoxin, and identified the amino-terminal amino acid residue. The results suggested that the molecular weight and the amino acid composition of Chromatium ferredoxin were intermediate between those of the clostridial and plant types.

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MATERIALS AND METHODS

The cells of Chromatium, strain D, were grown as described by Arnon et al. (1963). Ferredoxin was purified essentially by the method of Mortenson (1964) using acetone extraction, DEAE treatment, gel filtration and ammonium sulfate fractionation. After crystallization, the material was further purified by a dextran column, Bio-Gel P-10.

The molecular weight was determined by a gel filtration method according to Whitaker (1963). A Sephadex G-75 column (0.8 x 104 cm) was used at 5°C with a buffer composed of 0.1 M Na-acetate and 0.1 M NaCl at pH 7.0. Each fraction contained 5 drops of the effluent. The fractions were monitored by a spectrophotometer either at 280 mμ or at the specific wavelength for the reference materials, which were bovine cytochrome c (Hagihara et al., 1958), bovine α-chymotrypsin and bovine hemoglobin (Worthington Biochem. Corp.). Blue dextran 2000 (Pharmacia) was used for the determination of the void volume.

The amino acid composition was determined according to the method of Spackman et al. (1958) using Beckman-Spinco analyzers with an accelerated system, sensitive cuvettes and recorder (Hubbard, 1965). Twenty and 44 hr hydrolyses were employed. The values for threonine, serine and tyrosine were calculated after extrapolation to zero hydrolysis time. Half-cystine and methionine values were obtained from the 20 hr hydrolysate of a performic acid-oxidized sample. Tryptophan was determined by a spectrophotometric method (Bencze and Schmid, 1957) and also by the Ehrlich reaction (Smith, 1953).

The amino-terminal amino acid residue was determined by a modified Edman's phenylisothiocyanate method (Doolittle, 1965). The phenylthiohydantoin derivative of the amino acid was identified both by paper chromatography (Sjöquist, 1953) and thin layer chromatography (Brenner et al., 1961). The hydantoin derivative was also confirmed by the analyzer after regeneration (Africa and Carpenter, 1966).

RESULTS AND DISCUSSION

The purification procedure gave a rather low recovery of ferredoxin, but

crystalline material was easily obtained. The purified material resembled the clostridial type of ferredoxin in its absorption spectrum as shown in Fig. 1. There were a flat peak between 278 mμ and 282 mμ, a shoulder at 310 mμ and a broad peak at around 385 mμ. The sample showed a ratio, A_{385}/A_{280}, of 0.71 which was slightly lower than that reported, 0.74, by Bachofen and Arnon (1966)

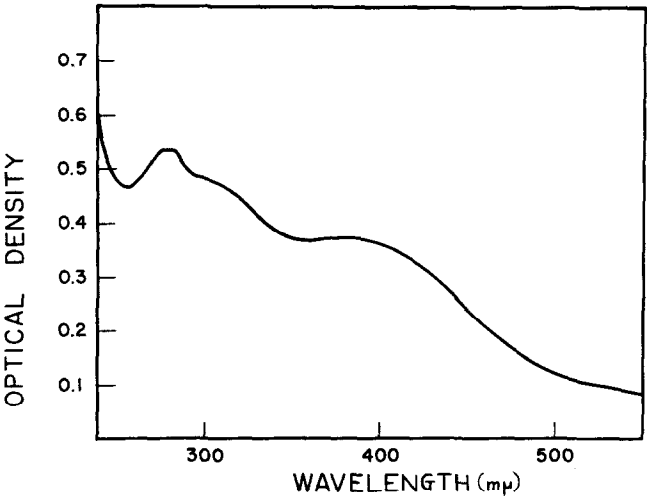

Fig. 1 Absorption spectrum of Chromatium ferredoxin. Ferredoxin was dissolved in 0.005 M phosphate buffer, pH 7.6.

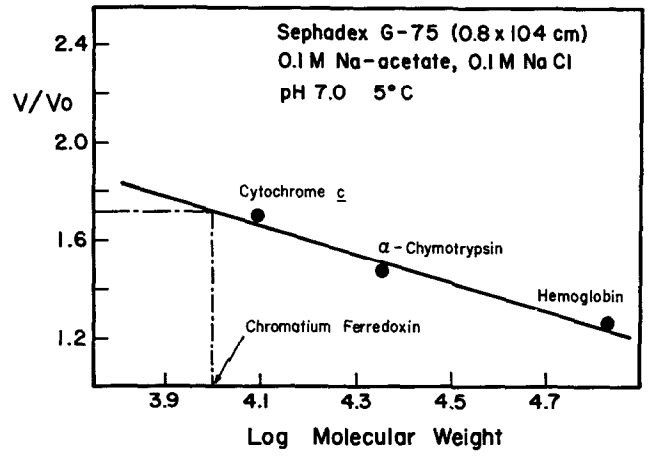

Fig. 2 Molecular weight determination of Chromatium ferredoxin by a dextran column. Blue dextran 2,000 was used to calculate the void volume (V_o). The ratio, V (elution volume for each sample)/V_o was plotted against the log molecular weight.

The molecular weight was determined by a gel filtration method and Chromatium ferredoxin was eluted a little later than cytochrome c. The molecular weight was calculated as 10,000 ± 1,000 (Fig. 2). This is slightly lower than that of spinach ferredoxin (Matsubara et al., 1967).

The amino acid composition is in Table 1. It was close to that obtained

TABLE 1

AMINO ACID COMPOSITION OF CHROMATIUM FERREDOXIN

	Time of Hydrolysis		Mean value[a]	Nearest Integer
	20 hr mole/mole	44 hr mole/mole	mole/mole	
Lysine	2.17	2.12	2.15	2
Histidine	1.95	1.88	1.92	2
Arginine	2.04	1.93	1.99	2
Aspartic Acid + Asparagine	8.63	8.62	8.63	9
Threonine	5.53	5.21	5.80	6
Serine	3.53	3.13	3.87	4
Glutamic Acid + Glutamine	16.41	16.03	16.22	16
Proline	4.90	4.91	4.91	5
Glycine	5.29	5.35	5.32	5
Alanine	3.75	3.85	3.80	4
Valine	6.01	6.42	6.22	6
Isoleucine	6.75	6.92	6.84	7
Leucine	3.24	3.31	3.28	3
Tyrosine	2.48	2.04	2.86	3
Phenylalanine	0.31	0.31	0.31	0
Cysteic Acid	8.81		8.81	9
Methionine Sulfone	1.03		1.03	1
Tryptophan			0.0	
Total Residues				84

[a] Threonine, serine and tyrosine values were obtained after extrapolation to zero hydrolysis time. Half-cystine and methionine values were calculated as cysteic acid and methionine sulfone, respectively. Tryptophan was measured as mentioned in the text.

The calculation of the number of residues was based on an assumption of lysine (2), arginine (2), aspartic acid (9), proline (5), glycine (5), alanine (4), leucine (3), and histidine (2).

by Dus† , although some discrepancies exist. Tryptophan was absent and one methionine residue was present. Although the analysis showed 0.31 mole phenylalanine per mole of protein, we assumed that no phenylalanine was present. As expected from the molecular weight determination, the total number of residues was 84, which corresponded to a molecular weight of approximately 9,250 excluding iron and labile sulfur. This result agrees well with that reported by Bearden et al. (1965). On this basis the mmolar extinction coefficient at 385 mμ was calculated to be about 31. The stoichiometry of the residues was not quite satisfactory and therefore this composition may be modified slightly in future experiments.

The compositions of various ferredoxins are shown in Table 2 which includes a ferredoxin from a unicellular green algae, Scenedesmus (Matsubara, in preparation). The ferredoxins in Table 2 may be placed in three groups; type i, from green plants and green algae, type ii, from photosynthetic bacteria, type iii, from nonphotosynthetic bacteria.

It is interesting that Chromatium ferredoxin shows some characteristics intermediate between those of ferredoxins of types i and iii. The total content of basic amino acids is close to that of the plant type ferredoxins although Chromatium ferredoxin is lower in lysine. The glutamic acid plus glutamine content is higher than that of aspartic acid plus asparagine. There is no tryptophan, and the content of half-cystine is high; in these respects it resembles the bacterial type. Its content of isoleucine is higher than that of leucine.

The fact that methionine was found in Chromatium and Scenedesmus ferredoxins was interesting. The ferredoxins of Swiss chard (Matsubara, unpublished), of taro (Rao and Mower, 1967) and of nonphotosynthetic bacteria lack methionine.

† Dr. K. Dus, Department of Chemistry, University of California at San Diego, California, kindly furnished analytical data obtained by him for Chromatium ferredoxin.

TABLE 2

COMPARISON OF AMINO ACID COMPOSITIONS OF FERREDOXINS

	Type i			Type ii	Type iii		
	Spinach (a)	Alfalfa (b)	Scene-desmus (c)	Chroma-tium (d)	Cl. past. (e)	Cl. buty. (e)	Micrococcus aerogenes (f)
Lysine	4	5	4	2	1	0	1
Histidine	1	2	1	2	0	0	0
Arginine	1	1	1	2	0	0	0
Aspartic Acid + Asparagine	13	10	12	9	8	9	8-9
Threonine	8	6	10	6	1	3	0
Serine	7	8	8	4	5	3	5
Glutamic Acid + Glutamine	13	17	10	16	4	5	4
Proline	4	3	4	5	3	3	5
Glycine	6	7-8	7	5	4	5	4-5
Alanine	9	10	10	4	8	7	8
Valine	7	9	5	6	6	6	4
Methionine	0	0	1	1	0	0	0
Isoleucine	4	4	3	7	5	4	6
Leucine	8	6	7	3	0	0	0
Tyrosine	4	4	4	3	1	0	2
Phenylalanine	2	2	3	0	1	2	0
Tryptophan	1	1	0	0	0	0	0
Half-cystine	5	6	6-7	9	8	8	8
Total	97	101-102	96-97	84	55	55	55-57
Iron	2	2	2	(4)	7	7	
Labile sulfur	2	2	2	(4)	7	7	

(a) Matsubara et al. (1967)
(b) Keresztes-Nagy and Margoliash (1966)
(c) Matsubara, in preparation
(d) Present communication
(e) Benson et al. (1966)
(f) Tsunoda et al. (1967)

The amino-terminal residue was identified as alanine, 8.19 mole/10^5 g, without any correction for the losses, 10-15%, during the process of identification, and therefore, the molecular weight was calculated to be about 12,210 for the oxidized protein. All ferredoxins examined so far have amino-terminal alanine residues.

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