Isolation and Characterization of Phytoferritin from Pea (*Pisum sativum*) and Lentil (*Lens esculenta*)

By ROBERT R. CRICHTON, YESID PONCE-ORTIZ, MICHEL H. J. KOCH,*
RAYMOND PARFAIT and HEINRICH B. STUHRMANN*
Unité de Biochimie, Université Catholique de Louvain, 1348 Louvain-la-Neuve, Belgium

(Received 29 July 1977)

Ferritin was isolated from the seeds of pea (*Pisum sativum*) and lentil (*Lens esculenta*). The homogeneity of the phytoferritins was established by polyacrylamide-gel electrophoresis. The subunit molecular weights were respectively 20 300 and 21 400 for the pea and lentil proteins. A neutron low-angle scattering study established the molecular weight of the oligomer as 480 000 for pea apoferritin and 510 000 for lentil apoferritin. Although the quaternary structure of 24 polypeptide chains is preserved, the phytoferritins have a larger cavity in the interior than mammalian ferritins and can thus potentially store 1.2–1.4 times as much iron. The amino acid composition of the phytoferritins show some similarities to those of mammalian apoferritins; tryptic 'fingerprinting' reveals that there are many differences in the amino acid sequence of plant and mammalian apoferritins.

Ferritin, together with haemosiderin, is the principal iron-storage protein in mammals. It is found principally in spleen liver and bone marrow (Granick, 1943), but in man appears to be present in all cell types, including mucosal cells, testis, kidney, heart and skeletal muscle, lung, pancreas, thyroid, reticulocytes, adrenal and placenta (Granick, 1946; Allfrey *et al.*, 1967; Arora *et al.*, 1970). More recently its presence in serum, originally established by Mazur & Shorr (1948, 1950), has become the basis of a routine method for measurement of body iron stores (reviewed by Jacobs & Worwood, 1975). Its distribution in mammals is extensive. Originally isolated from the horse (Laufberger, 1937), it has since been found in virtually all mammals examined (for a review see Crichton, 1973).

The distribution of ferritin in nature is not restricted to mammals. It has been isolated from tuna fish *Thunnus thynnus* and its presence confirmed in both eggs and early embryos of frog *Rana esculenta*, in snail *Helix pomatia* hepatopancreas and ovotestes, in octopus *Octopus*, in certain invertebrates notably worms *Lumbricus terrestris*, and in fungi and plants (reviewed by Crichton, 1973).

The identification of phytoferritin by electron microscopy in ribosomal preparations of pea embryonic axes prepared by the method of Ts'o *et al.* (1958) by Hyde *et al.* (1962) in California was followed by its isolation by these latter workers (Hyde *et al.*, 1963). It has subsequently been observed in various

tissues of a number of plants, mostly in cells not associated with active photosynthesis, such as apple fruit, pea and bean root, shoot meristem, conductive cells of *Atriplex*, *Acer pseudoplatanus* and willow *Salix fragilis*, excretional tissue of *Passiflora* and in epithem and hyathodes from *Taraxacum* and *Saxifraga*; its presence was also noted in leaf tissue, which had a lower photosynthetic capacity (reviewed by Seckbach, 1972a). Iron administration to iron-starved plants increases the storage of ferritin in the chloroplasts (Seckbach, 1968, 1969). Ferritin has been isolated from bean and pea by Hyde *et al.* (1963) and by Seckbach (1972b).

We have isolated ferritin from pea and lentil and describe here its isolation and characterization. The subunit structure has been determined by molecular-weight estimation, for the subunit by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate, and for the oligomer by low-angle neutron scattering. The iron contents of the phytoferritins have been determined, as have their amino acid compositions. Tryptic 'fingerprints' of the apophytoferritins have also been prepared. These characteristics are compared with those of mammalian ferritins.

Experimental

Materials

Dried peas (*Pisum sativum* L. var. Phéno) were obtained from Boerenbond, Leuven, Belgium, and dried lentils (*Lens esculenta* L. var. Anicia) from L. Clause, Brétigny, France. DEAE-cellulose was

* Present address: European Molecular Biology Laboratory, c/o Deutsche Elektronische Synchrotron, 2000 Hamburg 52, Germany.

from Serva, Heidelberg, Germany, and Sepharose 6B from Pharmacia, Uppsala, Sweden. Horse spleen apoferritin was from Boehringer, Mannheim, Germany, or was prepared by the method of Granick (1943). All other products were analytical grade.

Methods

Isolation of phytoferritins. The isolation of phytoferritins was carried out by a modification of the procedure described by Hyde *et al.* (1963). The dried legumes (usually 1 kg) were allowed to swell in water for 2 days at room temperature (20°C) and were then homogenized in water and filtered through three layers of gauze. Dialysis at 4°C against phosphate buffer led to precipitation of some of the starch present, which was removed by centrifugation at 27000g for 20 min. The supernatant was adjusted to 0.05 M-MgCl₂. This step was introduced by Hyde *et al.* (1963) to co-precipitate the phytoferritin and the ribosomes. However, we found it preferable to remove a large amount of starch by a low-speed centrifugation at 2000g for 5 min, and then to pellet the phytoferritin by ultracentrifugation at 100000g for 2 h. After resuspension of the pellet in water and homogenization, the cycle of low-speed and high-speed centrifugation was repeated, and finally the phytoferritin was passed through a column (2 cm × 50 cm) of DEAE-cellulose equilibrated in 0.01 M-sodium phosphate buffer, pH 7.3, and eluted with a linear gradient from 0.05 M- to 0.5 M-NaCl in the phosphate buffer. The phytoferritin, characterized by its yellow-brown colour, was concentrated by ultrafiltration (Amicon, Lexington, MA, U.S.A.) and either used directly or subjected to gel filtration on a column (4 cm × 120 cm) of Sepharose 6B in 200 mM-Tris/HCl buffer, pH 7.2.

Polyacrylamide-gel electrophoresis. The homogeneity of ferritin preparations was established by electrophoresis on 5% polyacrylamide gels in columns (1 cm × 10 cm) with 0.034 M-Tris/asparagine buffer, pH 7.3, as electrode buffer for 45 min at 2–3 mA per gel. Gels were stained in an Amido Black 10B solution (Bryce & Crichton, 1971) for 30 min. They were destained in acetic acid/methanol/water (3:2:35, by vol.). Gel electrophoresis in sodium dodecyl sulphate of protein solutions that had been denatured by incubation with sodium dodecyl sulphate was carried out by the method of Weber & Osborn (1969) as described in Bryce & Crichton (1971).

Amino acid analysis and peptide 'fingerprinting'. Amino acid analysis after hydrolysis for 16 h in 6 M-HCl at 110°C was carried out on a Locarte amino acid analyser (Locarte Co., London W12 9RT, U.K.). Tryptophan was determined by the method of Edelhoch (1967). Tryptic 'fingerprints' on thin layers of

micro-crystalline cellulose were as described in Huebers *et al.* (1976).

Protein and iron determination. Protein was determined by amino acid analysis of approx. 30 µg of ferritin or apoferritin after acid hydrolysis, assuming values of 10 and 11 residues of glycine/subunit for lentil and pea apoferritin respectively. Iron was determined at 520 nm as the αα'-bipyridyl complex after reduction of the ferritin.

Neutron-scattering experiments. Neutron-scattering measurements using the contrast-variation method (Stuhrmann & Kirste, 1967) were made at the high-flux-beam reactor of the Institut Max von Laue-Paul Langevin (Grenoble, France) with the low-angle scattering device D11 (Ibel, 1976).

For the preparation of the samples, part of a solution of the protein in H₂O was dialysed for 48 h against two changes of ²H₂O. Appropriate amounts of the solutions in H₂O and ²H₂O were mixed in quartz cells (150 µl, 1 mm thickness) to obtain nine contrasts (0, 20, 40, 45, 50, 55, 60, 70, 100% ²H₂O).

Corresponding H₂O/²H₂O mixtures were used for background measurements. The exact ²H₂O content of the samples was determined by transmission measurements. Protein and iron concentrations were measured after the neutron experiments as described above.

The values for the protein concentrations were 2.51 mg/ml for pea ferritin and 3.25 mg/ml for lentil ferritin. The iron concentration was 0.59 mg/ml in both cases.

The scattering curves were measured in three parts, with sample-detector distances of 10.5, 2.5 and 1.7 m and wavelengths of 0.65, 0.81 and 0.42 nm respectively; the range of momentum transfer ($\kappa = 4\pi \sin \theta / \lambda$, 2θ = scattering angle, λ = wavelength) extends from $5 \times 10^{-4} \text{ nm}^{-1}$ to $3 \times 10^{-2} \text{ nm}^{-1}$. A velocity selector with 8% full width at half maximum was used. Each sample was measured for approx. 15 min at 5°C. The partial scattering curves were merged by fitting the overlapping regions of momentum transfer.

The radii of gyration and the extrapolated zero-angle intensities were determined from a plot of the logarithm of the intensity [$I(\kappa)$] versus the square of the momentum transfer (κ) (Guinier plot).

Results

The yield of ferritin by the isolation procedure described in the Experimental section was of the order of 8 mg/kg of dry legumes for pea and 20 mg/kg for lentil. The two proteins were judged to be homogeneous on the basis of polyacrylamide-gel electrophoresis under denaturing and non-denaturing conditions. We did not observe higher oligomers than the ferritin monomer for the phytoferritins. Fig. 1

Fig. 1. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of ferritins
The samples are: (a) pea ferritin+lentil ferritin;
(b) pea ferritin+horse spleen ferritin; (c) lentil ferritin+horse spleen ferritin.

shows the results of polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate of mixtures of each of the phytoferritins with horse spleen apoferritin and of the two phytoferritins together. Both plant ferritins have a higher subunit molecular weight than horse ferritin and the lentil apoferritin has a higher molecular weight than pea apoferritin (Fig. 1). The subunit molecular weights of the apophytoferritins are given in Table 1 together with the standard deviation in the determinations. The iron content of the phytoferritins was also determined (Table 1).

In the contrast-variation method (Stuhrmann *et al.*, 1976) the H_2O/D_2O concentration ratio which determines the scattering density of the solvent is varied in order to modify the excess scattering density of the solute, $\rho(\vec{r})$. $\rho(\vec{r})$ is a continuous three-dimensional function, which is usually represented as a sum of two functions:

$$\rho(\vec{r}) = \bar{\rho} \cdot \rho_c(\vec{r}) + \rho_s(r) \tag{1}$$

$\rho_c(r)$ represents the shape of the solute and takes into account its H^2/H exchange properties, whereas $\rho_s(r)$ describes its internal structure.

The contrast ($\bar{\rho} = \rho_{\text{solute}} - \rho_{\text{solvent}}$) is the difference between the average scattering density of the solute (at $\bar{\rho} = 0$) and the scattering density of the solvent.

For a monodisperse solution a plot of the square root of the extrapolated zero-angle intensity [$\sqrt{I(0)}$] versus the scattering density of the solvent should thus result in a straight line which intercepts the abscissa when $\rho_{\text{solute}} = \rho_{\text{solvent}}$. Typical Guinier plots for lentil and pea ferritin are shown in Fig. 2. When $\sqrt{I(0)}$ is plotted against the scattering density of the solvent, pronounced deviations from linearity occur at low contrast, and most strikingly, $\sqrt{I(0)}$ never reaches zero (Fig. 3). This can be explained by the polydispersity of unfractionated ferritin samples resulting from the variable iron content of the molecules. Stuhrmann & Duée (1975) have shown that in such systems $\sqrt{I(0)}$ reaches a minimum when

Table 1. Physical properties of plant ferritins

The subunit molecular weights and iron content were determined as described in the text. S.E.M. values are given for subunit molecular-weight determinations; the numbers of determinations are given in parentheses. The iron contents are the means of four different preparations for each ferritin. Details of the methods used to determine oligomeric molecular weight and radius of gyration by low-angle neutron scattering, as well as the corrections used, are described in the text. Corresponding values for horse spleen ferritin are included for comparison [taken from Crichton (1973) and Stuhrmann *et al.* (1976)].

Property	Pea ferritin	Lentil ferritin	Horse spleen ferritin
Iron content (atoms/molecule)	2140	2130	2500
Subunit mol.wt.	20300 ± 200 (10)	21400 ± 400 (6)	18500
Oligomer mol.wt. (uncorrected)	637400	642700	—
Oligomer mol.wt. (corrected)	463700 ± 5000	498500 ± 5000	443000
Radius of gyration, R_g	5.27 nm	5.48 nm	4.90 nm*
Maximum radius, $r^\dagger$	6.80 nm	7.07 nm	5.17*
α	-0.00060	-0.00084	-0.00070*

* For a ferritin fraction containing 1980 iron atoms/molecule.
† Calculated for a homogeneous sphere.

Fig. 2. Guinier plots of neutron scattering, $I(\kappa)$, of lentil and pea ferritins
(a) Lentil ferritin in H_2O ; (b) lentil ferritin in 2H_2O ; (c) pea ferritin in H_2O . The broken line corresponds to the dimers.

Fig. 3. Square root of zero-angle scattering for lentil ferritin in different $H_2O/{}^2H_2O$ mixtures plotted against the scattering density of the solvent

the scattering density of the solvent is equal to the mean value of the scattering density distribution of the solute, and that its value is proportional to the root-mean-square deviation of this distribution. For lentil ferritin this minimum occurs in a solvent containing 49% 2H_2O ($\rho_{\text{solvent}} = 2.84 \times 10^{10} \text{ cm}^{-2}$). A typical value for pure protein would be 41% 2H_2O ($\rho_{\text{solvent}} = 2.285 \times 10^{10} \text{ cm}^{-2}$). This shows that the sample has a rather low iron content with very broad distribution, as indicated by the root mean square deviation $[0.4 (\pm 0.1) \times 10^{10} \text{ cm}^{-2}]$. The distribution of scattering densities is expected to be analogous to that observed for horse spleen ferritin, where sedimentation data (Crichton, 1973) indicate

the presence of a sharp peak corresponding to apoferritin and a broad distribution of molecules containing variable amounts of iron.

Zero-angle scattering is a direct measure of the weight-average molecular weight (Stuhrmann *et al*, 1976) and is described by eqn. (2):

$$M.W. = \frac{I(0)}{I_{H_2O}} \times \left\{ N \left[\exp \frac{(DN\sigma_{H_2O})}{M_{H_2O}} - 1 \right] \right\} \quad (2)$$

where $I(0)$ is extrapolated zero-angle scattering of the sample in water; I_{H_2O} is scattering intensity of water; σ_{H_2O} is total cross-section of water; N is Avogadro's number; v is specific volume of the solute calculated from the amino acid composition; c is concentration of the solute; ρ_p is scattering density of the solute measured at zero contrast; ρ_{H_2O} is scattering length density of H_2O ; ρ_c is loss factor due to $H/{}^2H$ exchange; D is thickness of the sample; M_{H_2O} is molecular weight of water; M.W. is molecular weight of the solute. This yields a mol.wt. of 642 741 for lentil ferritin. Assuming that all of the iron is present as $FeO \cdot OH$, we obtain a mol.wt. of 498 538 for the oligomer (Table 1).

Fig. 4. Square of the apparent radius of gyration, R^2 , of lentil ferritin in different $H_2O/{}^2H_2O$ mixtures plotted as a function of the reciprocal of the contrast

Error bars are included for scattering at low contrasts only.

Table 2. Amino acid composition of phytoferritins
The results are presented as residues of each amino acid/subunit based on the molecular weights for the subunit (Table 1). In all cases the results are means of at least 14 determinations on four different preparations. S.E.M. values are given for the pea and lentil apoferritins: the numbers of determinations are given in parentheses. The tryptophan values are the mean of seven determinations. Abbreviation: N.D., not determined.

Amino acid	Residues/subunit (mol.wt. 20300) pea (14)	Residues/subunit (mol.wt. 21400) lentil (16)
Cys	N.D.	N.D.
Asx	30.3 ± 1.11	20.9 ± 0.52
Thr	4.0 ± 0.42	5.0 ± 0.51
Ser	14.7 ± 0.64	9.7 ± 0.35
Glx	28.9 ± 0.91	27.6 ± 0.57
Pro	3.1 ± 0.43	6.0 ± 0.34
Gly	11.0 ± 0.55	9.6 ± 0.54
Ala	14.7 ± 0.31	15.4 ± 0.40
Val	12.8 ± 0.45	17.4 ± 0.60
Met	3.0 ± 0.26	3.9 ± 0.27
Ile	7.4 ± 0.33	6.9 ± 0.25
Leu	15.8 ± 0.43	17.4 ± 0.44
Tyr	6.0 ± 0.24	6.5 ± 0.37
Phe	8.4 ± 0.23	9.5 ± 0.18
His	5.0 ± 0.30	9.7 ± 0.41
Lys	8.9 ± 0.41	13.3 ± 0.36
Arg	6.7 ± 0.38	8.9 ± 0.30
Trp	1.6 ± 0.11	1.7 ± 0.06

The Guinier plot for pea ferritin in H₂O (Fig. 2) shows the presence of aggregates. It is shown below that these are mainly dimers. Making the same assumptions as for lentil ferritin a mol.wt. of 637418 is found when allowance is made for the presence of 6% dimers. This gives a corrected mol.wt. of 463 686 for the oligomer (Table 1).

Radii of gyration

In general the variation of the radius of gyration *R* with contrast is described by eqn. (3) (Stuhrmann & Kirste, 1967).

$$R^2 = R_c^2 + \frac{\alpha}{\rho} - \frac{\beta}{\rho^2} \tag{3}$$

This expression is not applicable to polydisperse systems. Only the values of *R_c*, the radius of gyration at infinite contrast, and α , the second moment of the internal structure, can be determined with accuracy. Those depend on the measurements at high contrast, where the variable iron content has no influence. A plot of *R*² versus the inverse of the contrast is shown in Fig. 4 for lentil ferritin. The value of *R_c* is 5.48 nm. For a spherical particle this leads

Fig. 5. Tryptic-peptide patterns of (a) lentil apoferritin and (b) pea apoferritin

The equivalent of 100 μg of digested protein was applied to a thin layer of cellulose and was subjected to electrophoresis at 20 V/cm for 60 min in pyridine/acetic acid/water (1:1:78, by vol.) and then to ascending chromatography in pyridine/acetic acid/butanol/water (10:3:15:12, by vol.).

to an upper limit of *r* (maximum radius) = $\sqrt{5/3} R_c = 7.07$ nm.
The slope of the curve is negative ($\alpha = -8.4 \times 10^{-4}$),

as expected for a particle with a dense core surrounded by a shell of low-density material. Corresponding values for pea ferritin are $R_c = 5.27$ nm, $r = 6.80$ nm, $\alpha = -6.0 \times 10^{-4}$ (Table 1). The Guinier plot of pea ferritin at very low angle shows the presence of aggregates, as mentioned above. The broken line in Fig. 2 corresponds to the radius of gyration of these particles ($R_{H_2O} = 8.54$ nm). Assuming that these particles are dimers one finds by application of the parallel-axis theorem that the separation between the centres of mass of the monomers in the dimers, $\Delta = 2 \sqrt{R_{dimer}^2 - R_{monomer}^2} = 137$ nm. Comparison with the diameter ($2r$) of pea ferritin suggests that the assumption is correct, hence justifying the calculations of the molecular weight.

The amino acid compositions of the two phyto-ferritins were determined, and the results are given in Table 2 together with the standard error of the mean. Cysteine was not determined. Fig. 5 shows the peptide maps of tryptic digests of the two apoferritins.

Discussion

The isolation of ferritin from dried legumes presents special problems, particularly because of the large amounts of starch present. However, the alternative of using etiolated seedlings leads to much lower yields of ferritins (Y. Ponce-Ortiz & R. R. Crichton, unpublished work). By a combination of low-speed centrifugation, $MgCl_2$ precipitation and DEAE-cellulose chromatography we were able to obtain homogeneous preparations of phyto-ferritins from the two legumes studied, which were free of starch. In the procedure originally described by Hyde *et al.* (1963) the $MgCl_2$ step was included to co-precipitate phyto-ferritin and ribosomes. We have found that after $MgCl_2$ treatment a large amount of starch could be eliminated by low-speed centrifugation (2000g) without appreciable loss of phyto-ferritin (as judged by the absence of coloration from the sediment). Since we have not observed contamination of our phyto-ferritin preparations with RNA, we assume that the ribosomes are either precipitated by the $MgCl_2$ or else are removed at the subsequent DEAE-cellulose step. We have tried to use the same procedure for isolation of phyto-ferritin from spinach leaves without success (R. R. Crichton, C. Monard & Y. Ponce-Ortiz, unpublished work). The yield obtained with dried lentils (20 mg/kg) suggests that this may be the most suitable source for obtaining plant ferritin.

Since we have used an ultracentrifugal method for the isolation of plant ferritin, we cannot draw any conclusions about the average iron content of these ferritins in the plant cells, since we are selecting those molecules that are sedimented at 100 000g in 2 h. We know that, for example, rat mucosal ferritin with an average iron content of 550 atoms/molecule is not

sedimented at 120 000g in 1 h (Huebers *et al.*, 1976). Seckbach (1972a) showed that iron loading increases the ferritin content of *Xanthium pensylvanicum* leaves. Clusters of electron-dense particles consisting of ferritin iron cores of diameter 50–60 nm were observed by electron microscopy in plastids and mature chloroplasts. This value of around 5.5 nm diameter for the iron core in phyto-ferritins (Seckbach, 1972a) can be compared with that calculated by Fischbach & Anderegg (1965) of 7.3 nm for full horse spleen ferritin (4300 atoms/molecule) and is consistent with an iron core containing 1840 atoms/molecule. In fact, for the horse spleen ferritin fraction in Table 1 (Stuhrmann *et al.*, 1976), the diameter of the core is 5.8 nm and the iron content 1980 atoms/molecule. Thus the average iron content found for the pea and lentil ferritins is consistent with the electron-microscopic data. However, our efforts to examine the distribution of iron in the phyto-ferritins were unsuccessful because of insolubility of the proteins in the density gradients (Y. Ponce-Ortiz & R. R. Crichton, unpublished work). In view of the fact that the phyto-ferritins have a larger interior volume than mammalian ferritins, it would be expected that full phyto-ferritins would contain more iron than their mammalian equivalents. We tried to load the phyto-ferritins with iron by using ferrous ammonium sulphate in 200 mM-imidazole buffer, pH 7.5, and were able to obtain pea ferritin containing 5054 atoms of iron/molecule. We do not know if this value represents the maximum possible content; it is consistent with the increase in interior volume.

The subunit molecular weights of the plant ferritins are higher than those found for most mammalian ferritins (18 000–19 000), although ferritins of subunit mol.wt. 21 000 have been reported in animal tissues such as horse liver (Crichton *et al.*, 1977). The quaternary structure of the plant ferritins is the same as that for mammalian ferritins, i.e. 24 subunits per molecule of apoferritin (Table 1). However, the increase in subunit and in oligomer molecular weight compared with mammalian ferritins results in a molecule that has a larger radius of gyration at infinite contrast, R_c , and a larger external and internal radius (Table 1). We would expect that both pea and lentil ferritin can store a greater quantity of iron than horse spleen ferritin can, and from calculation of the relative interior volumes we expect the maximum iron contents to be 5420 atoms/molecule and 6200 atoms/molecule respectively. The deviations from linearity in the Guinier zone observed for pea ferritin (Fig. 2) can be attributed to dimer formation, as shown by the application of the parallel-axis theorem (see the Results section). Self-aggregation is a well-documented phenomenon for mammalian ferritins (Crichton, 1973).

Table 3 presents a comparison of the amino acid compositions of pea and lentil ferritins with a number

Table 3. *Amino acid composition of mammalian and plant ferritins*

The values for human and horse ferritins are from Crichton (1973) and those for rat ferritins from Huebers *et al.* (1976). All results are given as residues of each amino acid/subunit. Abbreviation: N.D., not determined.

Amino acid	Human		Horse		Rat			Pea	Lentil
	Spleen	Liver	Spleen	Liver	Spleen	Liver	Mucosa		
Cys	1.7	1.5	2.9	2.6	N.D.	N.D.	N.D.	N.D.	N.D.
Asx	19.3	19.2	17.3	17.9	20.4	19.6	21.2	30.3	20.9
Thr	6.1	6.2	5.5	5.6	6.6	6.6	8.4	4.0	5.0
Ser	7.7	9.3	9.0	8.9	9.0	9.1	11.8	14.7	9.7
Glx	22.3	23.9	23.9	25.3	26.2	25.1	24.2	28.9	27.6
Pro	3.8	2.9	2.8	3.1	4.2	4.7	5.5	3.1	6.0
Gly	10.8	10.1	9.9	10.2	11.9	10.5	11.5	11.0	9.6
Ala	13.8	13.7	14.0	13.4	14.1	14.3	13.6	14.7	15.4
Val	6.0	6.3	6.9	6.9	7.2	6.9	7.9	12.8	17.4
Met	2.7	2.9	2.8	2.5	1.6	2.4	2.7	3.0	3.9
Ile	3.8	2.5	3.5	3.6	3.1	3.1	4.0	7.4	6.9
Leu	23.3	23.4	25.0	24.2	23.4	23.9	19.1	15.8	17.4
Tyr	4.4	6.0	5.0	4.1	3.1	4.0	5.1	6.0	6.5
Phe	7.0	6.9	7.3	7.7	6.6	6.7	5.8	8.4	9.5
Trp	2.2	2.2	2.1	2.1	N.D.	N.D.	N.D.	1.6	1.7
His	6.9	5.4	5.8	6.5	7.0	5.9	6.5	5.0	9.7
Lys	10.4	10.4	8.7	8.8	10.2	10.5	10.4	8.9	13.3
Arg	9.3	8.4	9.5	9.0	9.9	9.6	7.6	6.7	8.9

of other ferritins from mammalia. Although there are some similarities between the plant and animal proteins (for example for threonine, glycine, alanine, methionine, tyrosine, phenylalanine and tryptophan), there are large differences for most of the other amino acids. The percentage of non-polar amino acids remains constant at around 45%. The isoelectric point of pea ferritin is 6.4, and that of lentil ferritin 6.0 (H. Huebers, Y. Ponce-Ortiz & R. R. Crichton, unpublished work). The high values for asparagine+aspartate in pea ferritin should be considered in conjunction with the lower content of basic amino acids and its higher isoelectric point. Thus the asparagine content of pea ferritin must be considerably higher than in mammalian ferritins.

Comparison of the tryptic 'fingerprints' of the two plant ferritins reveals a few peptides that may be common to the two proteins. We have been unable to detect any similarities with horse spleen apoferritin, which suggests that there are large differences in sequence.

In conclusion, we have established a satisfactory purification procedure for phytoferritin from dried pea and lentil. The content in lentils is quite high; this reserve of iron is no doubt used for synthesis of haem and non-haem iron enzymes associated with photosynthesis, since phytoferritins have been detected in plastids from various plant tissues (Seckbach, 1972a). The phytoferritins are larger than mammalian ferritins and are hence capable of storing more iron. Preliminary studies reported here suggest

that although they have the same quaternary structure as mammalian ferritins, they have quite large differences in their primary structures.

We thank Mrs. Francine Brouwers for carrying out the amino acid analyses. The support of the European Molecular Biology Organisation (EMBO) through short-term fellowships to R. R. C. and R. P. is gratefully acknowledged.

References

- Allfrey, C. P., Lynch, E. C. & Whitley, C. E. (1967) *J. Lab. Clin. Med.* **70**, 419-427
- Arora, R. S., Lynch, E. C., Whitley, C. E. & Allfrey, C. P. (1970) *Tex. Rep. Biol. Med.* **28**, 189-193
- Bryce, C. F. A. & Crichton, R. R. (1971) *J. Biol. Chem.* **246**, 4198-4205
- Crichton, R. R. (1973) *Struct. Bonding Berlin* **17**, 67-134
- Crichton, R. R., Collet-Cassart, D., Ponce-Ortiz, Y., Wauters, M., Roman, F. & Pacques, E. (1977) in *Proteins of Iron Metabolism* (Brown, E. B., Aisen, P., Fielding, J. & Crichton, R. R., eds.), pp. 13-22, Grune and Stratton, New York
- Edelhoc, H. (1967) *Biochemistry* **6**, 1948-1954
- Fischbach, F. A. & Anderegg, J. W. (1965) *J. Mol. Biol.* **14**, 458-473
- Granick, S. (1943) *J. Biol. Chem.* **149**, 157-167
- Granick, S. (1946) *J. Biol. Chem.* **164**, 737-746
- Huebers, H., Huebers, E., Rummel, W. & Crichton, R. R. (1976) *Eur. J. Biochem.* **66**, 447-455

- Hyde, B. B., Hodge, A. J. & Birnstiel, M. L. (1962) *Electron Microscopy*, vol. 2, p. 1, Academic Press, New York
- Hyde, B. B., Hodge, A. J., Kahn, A. & Birnstiel, M. L. (1963) *J. Ultrastruct. Res.* **9**, 248–258
- Ibel, K. (1976) *J. Appl. Crystallogr.* **9**, 296–306
- Jacobs, A. & Worwood, M. (1975) *N. Engl. J. Med.* **292**, 951–956
- Laufberger, V. (1937) *Bull. Soc. Chim. Biol.* **19**, 1575–1583
- Mazur, A. & Shorr, E. (1948) *J. Biol. Chem.* **176**, 771–787
- Mazur, A. & Shorr, E. (1950) *J. Biol. Chem.* **182**, 607–627
- Seckbach, J. (1968) *J. Ultrastruct. Res.* **22**, 413–423
- Seckbach, J. (1969) *Plant Physiol.* **44**, 816–820
- Seckbach, J. (1972a) *J. Ultrastruct. Res.* **39**, 65–76
- Seckbach, J. (1972b) *Cytobiologie* **5**, 1–11
- Stuhrmann, H. B. & Duée, E. (1975) *J. Appl. Crystallogr.* **8**, 538–542
- Stuhrmann, H. B. & Kirste, R. G. (1967) *Z. Phys. Chem. Frankfurt am Main* **56**, 334–337
- Stuhrmann, H. B., Haas, J., Ibel, K., Koch, M. H. J. & Crichton, R. R. (1976) *J. Mol. Biol.* **100**, 399–413
- Ts'o, P. O. P., Bonner, J. & Vinograd, J. (1958) *Biochim. Biophys. Acta* **30**, 570–582
- Weber, K. L. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412