Biochem. J. (1972) **126**, 761–764
Printed in Great Britain

The Subunit Structure of Apoferritin and other Eicosamers

By ROBERT R. CRICHTON
Max-Planck-Institut für Molekulare Genetik, Berlin-Dahlem, West Germany

(*Received* 1 *December* 1971)

In a paper on the quaternary structure of proteins Cornish-Bowden & Koshland (1971) emphasized that it is not necessary to assume that all subunits in an oligomer are in the same environment, even though they may be chemically identical. As supporting evidence for this view they pointed out that 'the icosamer apoferritin is thought to have 20 subunits placed at the vertexes of a pentagonal dodecahedron, which is significant because it is impossible for asymmetrical units to occupy identical environments if they are so placed'. Hanson (1968) has also directed his attention to this problem, and concluded that apoferritin represents an oligomeric protein in which a single type of subunit appears to be capable of existing in two distinct conformations within the same oligomeric structure. He pointed out that this is one of a class of related structures with dihedral point-group symmetry. Other proteins quoted by Hanson (1968) as belonging to this class and having the appearance of pentagonal dodecahedrons from electron microscopy are dihydrolipoyl transacetylase from bovine kidney (Ishikawa *et al.*, 1966) and fibrinogen (Köppel, 1966). Whitehead (1965) cites evidence for a 20-subunit structure for glutamate dehydrogenase from chicken liver, and a dodecahedral model has been proposed for human low-density lipoprotein (Pollard *et al.*, 1969). It is the purpose of the present communication to discuss the evidence on which the 20-subunit model of apoferritin is based, to draw attention to the fact that more recent results obtained in this and other laboratories are at variance with this model, and to suggest that chemical and physicochemical data supporting the existence of 20-subunit structures are at present not very convincing.

If we consider first the basis for the 20-subunit structure of horse spleen apoferritin, it can be stated quite succinctly. Investigations by Harrison and her collaborators (Harrison & Hofmann, 1962; Hofmann & Harrison, 1962; Harrison *et al.*, 1962) led to the conclusion that the protein could be dissociated into subunits of molecular weight 25000–27000, that the content of tryptophan was 21 residues/oligomer of molecular weight 480000 and that the number of peptides released by digestion with trypsin was in good agreement with the content of lysine and arginine residues, based on a subunit molecular weight of 24000. Treatment with carboxypeptidase released 18–19 mol of arginine/mol, and 19.6 mol of *N*-acetyl group was found/mol of molecular weight 480000 (Mainwaring & Hofmann, 1969). X-ray-crystallographic analysis indicated that the molecule had pseudo-eicosahedral symmetry, but not exact eicosahedral or tetrahedral symmetry, and a model was proposed in which the 20 subunits were arranged at the vertices of a pentagonal dodecahedron with a small central space in each of the pentagonal faces (Harrison, 1963). A reinvestigation of the structure of the protein shell by electron microscopy with negative staining (Easterbrook, 1970) has provided results that seem to be compatible with this model.

The chemical and physicochemical evidence on which this model is based is now rather slight (Crichton, 1971*a*). Determination of the molecular weight of the subunit in two laboratories by four different methods leads in all cases to a value of 18500. This is also true for the apoferritins from horse liver, human liver and human spleen (R. R. Crichton, J. A. Miller, R. L. C. Cumming & C. F. A. Bryce, unpublished work). The molecular weight of the oligomer has been determined in five laboratories by several techniques. In all cases the value found was about 440000–460000. These data are presented in Table 1.

The value of 24 subunits/oligomer fits the physicochemical data very well, and leads to a model in which structural equivalence of the subunits would be possible, in agreement with our structural observations discussed below.

One of the problems of the 20-subunit model is that all of the subunits cannot occupy equivalent positions in the structure. Clearly, if there were two kinds of subunit, this would be more compatible with the dodecahedral model. For this reason we are engaged in the determination of the primary structure of horse spleen apoferritin, and up to the present can find no evidence for any heterogeneity. After cleavage of the protein with CNBr we can isolate four peptides, which together account for the complete amino acid composition of the protein and whose molecular weights together account for the molecular weight of the subunit (Crichton, 1970, 1971*b*; Crichton & Barbirolli, 1970; Bryce & Crichton, 1971*b*). During our investigations we have isolated several peptides, from tryptic digests, that are products of incomplete digestion, and in one case of non-tryptic digestion (R. R. Crichton & M. D. P. Boyle, unpublished work). This would explain why one finds more tryptic peptides than there are lysine+arginine residues in the molecule.

Table 1. *Molecular weights of eicosameric proteins*

Protein	Molecular weight	Method of determination	Reference
Apoferritin (horse spleen)			
Oligomer	443000	Sedimentation equilibrium	R. R. Crichton, R. Eason, A. Barclay & C. F. Bryce (unpublished work)
	430000	Light-scattering	Richter & Walker (1967)
	440000–465000	Sedimentation equilibrium	Björk & Fish (1971)
	465000*	Sedimentation diffusion	Rothen (1944)
	480000*	X-ray diffraction	Harrison (1959)
Subunit	18300–18800	Sodium dodecyl sulphate–gel electrophoresis, gel filtration and sedimentation equilibrium	Crichton & Bryce (1970); Bryce & Crichton (1971*a*,*b*)
	18000–19000	Sodium dodecyl sulphate–gel electrophoresis, gel filtration and sedimentation equilibrium	Björk & Fish (1971)
	25000–27000†	Sedimentation diffusion	Hofmann & Harrison (1962)
Dihydrolipoyl transacetylase (*E. coli*)			
Oligomer	1090000	Sedimentation equilibrium	Henney *et al.* (1967)
Subunit	36400	Sedimentation equilibrium	Willms *et al.* (1967); Henney *et al.* (1967)
Dihydrolipoyl transacetylase (pig heart)			
Oligomer	1980000	Sedimentation equilibrium	Hayakawa *et al.* (1969)
Subunit	90000	Lipoic acid content	Hayakawa *et al.* (1969)
Fibrinogen (bovine)			
Oligomer	330000	Sedimentation diffusion	Shulman (1953)
Subunit	47000	Sedimentation equilibrium	McKee *et al.* (1966)
	56000		
	63500		
Glutamate dehydrogenase (bovine liver)			
Oligomer	316000	Light-scattering	Eisenberg & Tomkins (1968)
Subunit	53500	Light-scattering	Eisenberg & Tomkins (1968)
Glutamate dehydrogenase (chicken liver)			
Oligomer	430000	Sedimentation velocity	Frieden (1962)
Subunit	326000	Sedimentation velocity	Anderson & Johnson (1969)
Low-density lipoprotein (human serum)			
Oligomer	2200000–2300000	Sedimentation equilibrium	Pollard *et al.* (1969)
	2300000–2500000	High-salt flotation method	Adams & Schumaker (1969)
	2800000–3000000	Light-scattering	Björklund & Katz (1956)
Subunit	27500	Sedimentation equilibrium	Pollard *et al.* (1969)

* A value of $\bar{v}$ of 0.747 was used instead of that based on the amino acid composition (0.731). This would decrease these two values to 440000 and 454000 respectively.

† A value of 18100–19000 is obtained if it is assumed that 1.4g of sodium dodecyl sulphate is bound/g of protein (Bryce & Crichton, 1971*a*).

We have redetermined the tryptophan content, and find 52 residues/mol of molecular weight 460000, in contrast with the previous value of 21 (Bryce & Crichton, 1971*a*). The accuracy of *N*-acetyl group and *C*-terminal determinations would not seem adequate to distinguish between 20 subunits and 24.

Cornish-Bowden & Koshland (1971) have suggested that isomorphism in the quaternary structure of proteins composed of identical subunits may occur, and hypothesize that the appearance of additional bands on isoelectric focusing of proteins that contain identical subunits could be an indication of such an agreement. The reported micro-heterogeneity of apoferritin on isoelectric focusing (Drysdale, 1970; Uroshizaki *et al.*, 1971) might appear to be an example of such a situation. However, there is good reason to doubt whether the appearance of multiple bands is necessarily an indication of heterogeneity (Hayes & Wellner, 1969), and it seems that with horse spleen apoferritin the apparent micro-heterogeneity may be an artifact (Lee & Richter, 1971; C. F. A. Bryce & R. R. Crichton, unpublished work).

Dihydrolipoyl transacetylase is the core of the pyruvate dehydrogenase complex. Whereas the transacetylase of *Escherichia coli* consists of 24 identical polypeptide chains of molecular weight 36400 and the electron-microscopic appearance indicates that the molecule contains eight morphological subunits each containing three polypeptide chains (Table 1), a different model has been proposed for the mammalian enzyme. Although the pyruvate dehydrogenase contains the same three enzymes as are found in the *E. coli* complex, the transacetylase appears to be different in negatively stained electron micrographs. The subunits appear to be arranged at the vertices of a pentagonal dodecahedron (Willms *et al.*, 1967). The physicochemical data on the pig heart enzyme are shown in Table 1. On the basis of the lipoate content one can compute that there are 22 lipoic acid molecules/oligomer. Information on the bovine kidney enzyme is not at present available.

The dodecahedral model proposed for bovine fibrinogen was also based on a negative-staining electron-microscopic study (Köppel, 1966). It has been established that the molecular weight of mammalian fibrinogens is 340000 (Table 1). Human fibrinogen appears to be a dimer composed of two pseudo-identical monomers that are joined by disulphide bonds (Blömback, 1969; Blömback *et al.*, 1968). Each monomer consists of three polypeptide chains, present in equal amounts and with molecular weights 47000, 56000 and 63500 (Table 1). It is clear from *N*-terminal analysis and molecular-weight determinations that in all species studied to date the fibrinogen molecule consists of three polypeptide chains and that the intact molecule contains a pair of these chains.

A molecular weight of 430000 was reported for chicken liver glutamate dehydrogenase, this apparently being compatible with a 20-subunit structure (Table 1). In more recent investigations a value of 326000 has been found, in agreement with data for the bovine enzyme (Eisenberg & Tomkins, 1968). The subunit molecular weight of the bovine enzyme is 56000. The active enzyme from both tissues probably consists of six identical subunits, as originally proposed by Eisenberg & Tomkins (1968).

Human serum low-density lipoprotein has a molecular weight of 2.3×10^6, although values obtained in various laboratories range from 2.3×10^6 to 3.0×10^6 (Table 1). A value of 27500 ± 2000 was found for the molecular weight of the apoprotein moiety (Pollard *et al.*, 1969). Assuming a molecular weight of 2.2×10^6 for the oligomer and a protein content of 22–24%, the number of subunits was calculated to be 18–22. Evidence for a 20-subunit structure was derived from negative-staining electron microscopy (Pollard *et al.*, 1969), and an isodensity map has since been constructed from the electron-microscopic data in which the best three-dimensional distribution of the isodensity data was that of a pentagonal dodecahedron with isodensity maxima at the vertices (Pollard & Devi, 1971). These maxima are thought to represent protein subunits. Once again it is clear that, in view of the difficulties involved in the determination of the molecular weight of the undissociated complex, it would be not be easy from the physicochemical data available to decide whether there were 20 subunits or some higher number such as 24.

In no case therefore is the evidence for a 20-subunit structure, proposed on the basis of electron-microscopic or X-ray-diffraction data, unequivocally confirmed by chemical and physicochemical investigations. For apoferritin, chicken liver glutamate dehydrogenase and fibrinogen such models would seem to be unlikely. For dihydrolipoyl transacetylase and human serum low-density lipoprotein the physicochemical data are not yet adequate. It seems therefore that we cannot at present dismiss the occurrence of 20-subunit structures or of chemically identical subunits existing in two distinct conformational states within the one oligomer, solely on the basis of available physicochemical data. However, we can conclude that the fivefold symmetry of the starfish may not have a parallel at the molecular level.

Much of the unpublished work cited in this communication was supported by the Deutsche Forschungsgemeinschaft.

Adams, G. H. & Schumaker, V. N. (1969) *Anal. Biochem.* **29**, 117

Anderson, P. J. & Johnson, P. (1969) *Biochim. Biophys. Acta* **181**, 45

Blömback, B. (1969) *Brit. J. Haematol.* **17**, 145

Blömback, B., Blömback, M., Henschen, A., Hessel, B., Iwagawa, S. & Woods, K. R. (1968) *Nature (London)* **218**, 130

Björk, I. & Fish, W. W. (1971) *Biochemistry* **10**, 2844

Björklund, R. & Katz, S. (1956) *J. Amer. Chem. Soc.* **78**, 2122

Bryce, C. F. A. & Crichton, R. R. (1971*a*) *J. Biol. Chem.* **246**, 4198
Bryce, C. F. A. & Crichton, R. R. (1971*b*) *J. Chromatogr.* **63**, 267
Cornish-Bowden, A. J. & Koshland, D. E. (1971) *J. Biol. Chem.* **246**, 3092
Crichton, R. R. (1970) *Biochem. J.* **117**, 34P
Crichton, R. R. (1971*a*) *N. Engl. J. Med.* **284**, 1413
Crichton, R. R. (1971*b*) *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 9
Crichton, R. R. & Barbirolli, V. (1970) *FEBS Lett.* **6**, 121
Crichton, R. R. & Bryce, C. F. A. (1970) *FEBS Lett.* **6**, 134
Drysdale, J. W, (1970) *Biochim. Biophys. Acta* **207**, 256
Easterbrook, K. B. (1970) *J. Ultrastruct. Res.* **33**, 442
Eisenberg, H. & Tomkins, G. M. (1968) *J. Mol. Biol.* **31**, 37
Frieden, C. (1962) *Biochim. Biophys. Acta* **62**, 421
Hanson, K. R. (1968) *J. Mol. Biol.* **38**, 133
Harrison, P. M. (1959) *J. Mol. Biol.* **1**, 69
Harrison, P. M. (1963) *J. Mol. Biol.* **6**, 404
Harrison, P. M. & Hofmann, T. (1962) *J. Mol. Biol.* **4**, 239
Harrison, P. M., Hofmann, T. & Mainwaring, W. I. P. (1962) *J. Mol. Biol.* **4**, 251
Hayakawa, T., Kanzaki, T., Kitamura, T., Fukoyoshi, Y., Sakurai, Y., Koike, K., Suematsu, T. & Koike, M. (1969) *J. Biol. Chem.* **244**, 3660
Hayes, M. B. & Wellner, D. (1969) *J. Biol. Chem.* **244**, 6636
Henney, H. R., Willms, C. R., Muramutsu, M., Mukherjee, B. B. & Reed, L. J. (1967) *J. Biol. Chem.* **242**, 898
Hofmann, T. & Harrison, P. M. (1962) *J. Mol. Biol.* **4**, 256
Ishikawa, E., Oliver, R. M. & Reed, L. J. (1966) *Proc. Nat. Acad. Sci. U.S.* **56**, 534
Köppel, G. (1966) *Nature (London)* **212**, 1608
Lee, J. C. K. & Richter, G. W. (1971) *Comp. Biochem. Physiol.* **39B**, 325
Mainwaring, W. I. P. & Hofmann, T. (1969) *Arch. Biochem. Biophys.* **125**, 975
McKee, P. A., Rogers, L. A., Marler, E. & Hill, R. L. (1966) *Arch. Biochem. Biophys.* **116**, 271
Pollard, H. & Devi, S. K. (1971) *Biochem. Biophys. Res. Commun.* **44**, 593
Pollard, H., Scanu, A. M. & Taylor, E. M. (1969) *Proc. Nat. Acad. Sci. U.S.* **64**, 304
Richter, G. W. & Walker, G. F. (1967) *Biochemistry* **6**, 2871
Rothen, A. (1944) *J. Biol. Chem.* **152**, 679
Shulman, S. (1953) *J. Amer. Chem. Soc.* **75**, 5846
Uroshizaki, I., Niitsu, Y., Ishitani, K., Matsuda, M. & Fukada, M. (1971) *Biochim. Biophys. Acta* **243**, 187
Whitehead, E. P. (1965) *J. Theor. Biol.* **8**, 276
Willms, C. R., Oliver, R. M., Henney, H. R., Mukherjee, B. B. & Reed, L. J. (1967) *J. Biol. Chem.* **242**, 889