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Iron and aluminium in relation to brain ferritin in normal individuals and Alzheimer's-disease and chronic renal-dialysis patients

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Ferritin has been isolated and its subunit composition, iron and aluminium content determined in the cerebral cortex and cerebellum of normal individuals and in the cerebral cortex of Alzheimer's-disease and renal-dialysis patients. An e.l.i.s.a. for ferritin has been developed and the ferritin, non-haem iron and aluminium content of the parietal cortex were determined in normal individuals and Alzheimer's-disease patients. It was found that ferritin from the cerebral cortex and cerebellum of normal individuals had a high H-subunit content, similar to that of heart ferritin. The subunit composition of ferritin isolated from the cerebral cortex was not significantly altered in Alzheimer's-disease or renal-dialysis patients. Ferritin from the cerebral cortex of normal individuals had only approx. 1500 atoms of iron per molecule and the iron content of ferritin was not significantly changed in Alzheimer's-disease or renal-dialysis patients. Ferritin isolated from the cerebral cortex of normal, Alzheimer's-disease and renal-dialysis patients had less than 9 atoms of aluminium per molecule. The failure to find increased concentrations of aluminium associated with ferritin in dialysis patients, who had markedly increased concentrations of aluminium in the cerebral cortex, shows that aluminium does not accumulate in ferritin *in vivo*. This has important implications for the toxicity of aluminium, since it implies that cells are unable to detoxify aluminium by the same mechanism as that available for iron. Comparison of the concentrations of ferritin, aluminium and iron in the parietal cortex from normal and Alzheimer's-disease patients showed that, whereas the concentration of aluminium was not increased, both ferritin and iron were significantly increased in Alzheimer's disease.

INTRODUCTION

Ferritin is the major cellular iron-storage protein and serves the role of a housekeeping protein, by providing bioavailable iron for the synthesis of iron-containing proteins such as cytochromes, and by detoxifying any excess iron which enters the cell. Apoferritin has a molecular mass of approx. 480 kDa and can store up to 4500 iron atoms per molecule as crystalline ferrihydrite ($5Fe_2O_3,9H_2O$) with some attached phosphate [1,2]. Ferritin is composed of 24 subunits of two distinct types, designated heavy (H) (molecular mass 21099 Da) and light (L) (molecular mass 19766 Da) [3]. H and L subunits are encoded by separate genes on chromosomes 11 and 19 respectively [4,5]. X-ray-crystallographic and electron-microscopic studies have shown the protein to be a hollow sphere of approx. 12 nm diameter surrounding an 8 nm core of crystalline hydrous iron(III) oxide-phosphate [1]. Apoferritin consists of 24 subunits arranged with 432 symmetry to produce six hydrophobic and eight hydrophilic channels which connect the exterior with the interior of the molecule. Ferritin possesses intrinsic ferroxidase activity, associated with the H subunit, which oxidizes soluble Fe(II) and provides Fe(III) for the nucleation of ferrihydrite in the protein interior [6]. Once a small core of ferrihydrite is formed inside the ferritin molecule, autoxidation of iron occurs on the surface of the crystalline core independently of the protein subunits [1,7].

Non-haem iron in the brain has a unique distribution pattern [8,9] with the highest concentrations being found in the extrapyramidal system, particularly the globus pallidus and substantia nigra zona reticulata, where the iron concentration is similar to that found in the liver. Histochemical and immunocytochemical studies [10,11] have shown that much of the stainable non-haem iron is in the form of ferritin which is localized mainly in microglia and astrocytes [12,13]. That brain non-haem iron is mainly present in ferritin may be of significance given the putative role of ferritin in the intracellular detoxification of iron. Iron in a free and reactive form is capable of catalysing free-radical-mediated lipid and protein peroxidation and the brain may be particularly vulnerable to free-radical damage owing to its relative lack of oxidant defence mechanisms, high oxygen consumption and high lipid content [14]. Indeed, iron and ferritin seem to play a role in the pathogenesis of Parkinson's disease where iron has been shown to accumulate in the substantia nigra, with a decrease rather than an increase in ferritin and a concomitant increase in lipid peroxidation [15–17].

Little is known of the composition of brain ferritin. One study has suggested that ferritin isolated from the entire human brain has a high H-subunit content and that in Alzheimer's disease there is an increased content of both iron and aluminium [18]. In the present study, ferritin has been isolated and characterized from normal human cerebral cortex and cerebellum in order to determine its subunit composition and iron and aluminium content. Ferritin has also been isolated and characterized from the cerebral cortex of both Alzheimer's patients and renal-

Abbreviations used: BBS, borate-buffered saline; TST, 50 mM-Tris buffer/100 mM-NaCl (pH 8.0)/0.5% (w/v) Tween 80.

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dialysis patients. The renal-dialysis patients had elevated concentrations of brain aluminium because of administration of aluminium-containing phosphate-binding compounds to control the hyperphosphataemia that occurs in such patients [19,20]. In addition, an e.l.i.s.a. for brain ferritin has been developed and the ferritin, non-haem iron and aluminium content of parietal cortex were determined in Alzheimer's-disease and age-matched normal patients.

MATERIALS AND METHODS

Patient details and brain dissection

Brains were obtained at *post mortem* from normal individuals with no known history of neurological disease, from patients with clinically diagnosed Alzheimer's disease and from patients who had received dialysis for chronic renal failure. Neuropathological assessment was carried out on all brains and showed that the normal and renal-dialysis patients had no significant Alzheimer-type pathological changes, whereas the Alzheimer's-disease patients all showed the presence of senile plaques and neurofibrillary tangles in the cerebral cortex. For ferritin isolation and characterization, seven normal individuals (four males, three females; mean age 69 ± 13 years, range 44–84 years; *post mortem* delay 41 ± 25 h), five Alzheimer's-disease patients (three males, two females; mean age 71 ± 8 years, range 62–85 years; *post mortem* delay 28 ± 12 h) and six renal-dialysis patients (two males, four females; mean age 58 ± 7 years, range 48–69 years; *post mortem* delay 35 ± 11 h) were studied. For the ferritin, iron and aluminium determinations in the parietal cortex, 11 normal individuals (four males, seven females; mean age 69.4 ± 12.6 years; range 48–89 years; *post mortem* delay 33 ± 22 h) and 11 Alzheimer's-disease patients (four males, seven females; mean age 81 ± 9 years, range 57–91 years; *post mortem* delay 25 ± 11 h) were studied. Removal of the brain followed standard autopsy room procedures and the brain was dissected fresh, using clean stainless-steel instruments, in a laminar flow cabinet to minimize airborne contamination. The cerebellum and brainstem were removed by a transverse incision at the level of the basis peduncularis/pontis. The left hemisphere of the cerebellum excluding the vermis was then removed by a sagittal incision. The forebrain was separated by a midline sagittal incision through the corpus callosum and midbrain. The left hemisphere was coronally sectioned at 1 cm intervals. Tissue slices were sealed in polythene and snap-frozen in melting liquid Arcton (Freon 12, ICI, Macclesfield, Cheshire, U.K.) before storage at −70 °C. The right hemisphere, cerebellum and brainstem were fixed by suspension in 4 % neutral formalin before routine neuropathological assessment.

Ferritin isolation and characterization

The initial steps in the purification of brain ferritin were similar to those described by Linder & Munro [21] for other tissues and made use of the relative resistance of ferritin to denaturation by heat and low pH. Brain tissue was thawed and grey matter separated from white matter in a laminar flow cabinet. Grey matter (60–200 g) was homogenized in a clean polyethylene beaker in 4 vol. of high-purity distilled water (Milli-Q, > 18MΩ.cm; Millipore) in a class 3 cabinet using an Ultraturrax homogenizer. The homogenate was then heated to 70–75 °C for 10 min before being rapidly cooled on ice. The homogenate was centrifuged at 10000 ***g*** for 30 min at 4 °C and the pH of the supernatant adjusted to 4.6 with 1 M-acetic acid and re-centrifuged at 10000 ***g*** for 30 min to remove material precipitated by the low pH. The supernatant was then adjusted to pH 7.2. At this point the supernatant was almost completely clear and one-ninth of the supernatant volume of 10-times-concentrated borate-buffered saline (10 × BBS) added. The final solution contained 140 mM-NaCl/KCl, 20 mM-borate and 0.02 % sodium azide at pH 7.2.

Further purification was carried out using a method similar to that described by Arosio *et al.* [22]. Ferritin was precipitated from the clear supernatant by the slow addition of 40 % (w/v) $(NH_4)_2SO_4$ with constant stirring. The precipitated ferritin was left overnight at 4 °C and then centrifuged at 10000 ***g*** for 30 min at 4 °C. The pelleted precipitate was redissolved in a minimum volume of BBS and dialysed against three changes of the same buffer. It was then applied to a Sepharose 6B column ($22\ cm^2 \times 90$ cm) and eluted with BBS. Ferritin-containing fractions were concentrated to a volume of approx. 2 ml using an Amicon 8MC ultrafiltration cell with a PM-10 membrane.

Preparative PAGE was performed by using a convex pore-size gradient obtained by varying the concentration of acrylamide in the gel as it was poured. The 1.5 mm-thick gels were typically 6–12.5 % (w/v) acrylamide (top to bottom)/2.5 % (w/v) bisacrylamide and electrophoresis was carried out with the discontinuous buffer system described by Laemmli [23] in a Raven slab-gel apparatus. Samples were loaded on to a stacking gel with a single well and up to 2 ml could be loaded in a single application, but it was possible to load more once the original amount had run into the gel. Electrophoresis was carried out at a constant voltage (150 V) overnight; after this period the ferritin would travel no further through the gel. Ferritin was clearly visible as the major fast running band corresponding to ferritin monomers, with a second diffuse band running more slowly, probably corresponding to molecular dimers and larger aggregates. Slices containing the ferritin bands were excised and homogenized in 3–5 vol. of BBS, using a Dounce-type tissue homogenizer with nylon rotary pestle. The homogenate was left for 4 h before gel fragments were removed by centrifugation at 15000 ***g*** for 15 min. The gel was re-extracted with 3 vol. of BBS and the supernatants were pooled and then washed with BBS and concentrated in an Amicon 8MC ultrafiltration unit with a PM10 membrane. The isolated ferritin was greater than 98 % pure as judged by SDS/PAGE.

The proportion of H and L subunits in the isolated ferritin samples was determined by SDS/PAGE (12.5 % acrylamide), the stained gels being scanned with a Quickscan R+D gel scanner (Helena Laboratories, Gateshead, Tyne and Wear, U.K.).

Total phosphate determination

Phosphate concentrations in ferritin samples were measured essentially as described by Ames [24]. Ferritin samples were reduced by adding 2 vol. of 6 mM-thioglycollic acid adjusted to pH 4.2 with sodium acetate, and incubated at 40 °C for 1–2 h. To 0.3 ml of the reduced sample was added 0.7 ml of a mixture of 1 part 10 % (w/v) ascorbic acid to 6 parts 0.42 % (w/v) ammonium molybdate.$4H_2O$ in 0.5 M-H_2SO_4, and the samples were incubated for a further 20 min at 40 °C. The absorbance was then determined at 820 nm and phosphate concentrations were determined from a standard curve of 5–40 nmol of phosphate made from a 1 mM stock solution of Na_2HPO_4.

In some samples 6 mM-thioglycollic acid apparently did not completely reduce the iron core since some colour remained even after 3 h of incubation. The samples could be completely reduced with 60 % acetic acid/750 mM-sodium sulphite. This did not affect the assay, as judged by the comparison of standard curves, and a single reduced sample could be used for iron and phosphate assays to provide Fe/phosphate ratios.

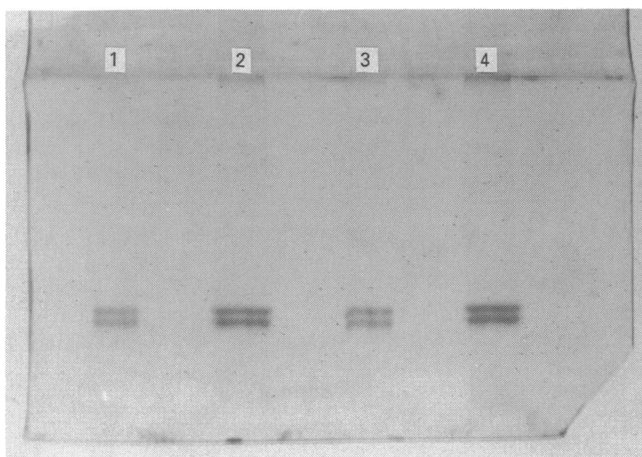

Fig. 1. SDS/PAGE (12.5%) of human brain ferritin after purification by preparative PAGE

1, Sample a, 2 μl; 2, sample a, 4 μl; 3, sample b, 2 μl; 4, sample b, 4 μl. The gel was stained with Coomassie Brilliant Blue R.

Non-haem iron determinations

Non-haem iron was determined in homogenates of grey matter from the parietal cortex (Brodmann area 40) in normal subjects and Alzheimer's-disease patients. The grey matter was homogenized with an Ultraturrax homogenizer in a class 3 cabinet for 20 s in 4 vol. of Milli-Q-grade water. Iron was determined by the method of Foy *et al.* [25] using the mixed ferrozine/neocuproine reagent of Carter [26].

Estimation of non-haem iron in ferritin samples was made by reducing the iron core and measuring the absorbance at 520 nm of the Fe(II) complex with the chelating agent 2,2′-bipyridyl (ϵ_{520} = 8580 $M^{-1} \cdot cm^{-1}$), as described previously by Drysdale & Munro [27]. To samples containing 1–6 μg of Fe, 0.3 ml of 60% (w/v) acetic acid, 0.3 ml of freshly prepared 750 mM-sodium sulphite and 0.3 ml of 0.5% (w/v) 2,2′-bipyridyl solution in 10% (v/v) ethanol were added. The samples were then placed in a boiling-water bath for 1 h. After cooling the absorbance at 520 nm was read against a reagent blank.

Ferritin e.l.i.s.a.

Antisera were raised against human brain ferritin in New Zealand White rabbits by subcutaneous injection of purified ferritin isolated from the cerebral cortex, emulsified with an equal volume of Freund's complete adjuvant. The rabbits were boosted with three further injections of ferritin in an emulsion with Fruend's incomplete adjuvant. IgG was purified using a CM Affigel Blue column and $(NH_4)_2SO_4$ precipitation and was biotinylated using *N*-biotinyl-ω-aminohexanoyl-NH-hydroxy-succinamide (Amersham) as described by Polak & Varndl [28].

The partial cortex homogenates (see non-haem iron determinations) were heated at 70°C for 10 min to destroy proteinase activity. A solubilizing solution (20 μl) containing 5% (w/v) Triton X-100 in 750 mM-NaCl/50 mM-sodium phosphate buffer, pH 7.2 was added to 80 μl of homogenate and agitated for 10 min at room temperature. The homogenate was then centrifuged for 10 min at high speed in an MSE Microcentaur and the supernatant assayed for ferritin. A microtitre plate was coated with 100 μl of anti-(brain ferritin) IgG/well at a concentration of 5 μg/ml in 50 mM-Na_2CO_3 buffer, pH 9.6. The plate was incubated overnight at 4 °C, washed three times with 50 mM-Tris buffer/100 mM-NaCl, pH 8.0, and 0.5% (w/v) Tween 80 (TST). Non-specific binding to the plate was decreased by incubation for 1 h at room temperature with 100 μl/well of a solution containing 1 mg of BSA/ml in TST. A standard curve of 5–5120 ng of ferritin/ml in TST + 1 mg of BSA/ml was prepared from a standard of pooled isolated human brain ferritin (Fig. 1). Ferritin samples were diluted 250- to 2000-fold in TST and 50 μl of standard or sample was added to the wells, and incubated at 40 °C for 2 h. The plate was washed three times with TST, drained and 50 μl of a biotinylated anti-(brain ferritin) IgG in TST added to each well. The plate was incubated for 1 h at 40 °C, and washed with TST. Then 50 μl of streptavidin–biotinylated horseradish peroxidase complex diluted 1000-fold in TST was added to the wells and incubated for 30 min at room temperature. The plate was washed three times in TST, three times in distilled water and dried. To each well was added 50 μl of substrate reagent containing 0.1 mg of 3,3′,5,5′-tetramethylbenzidine/ml, 0.015% H_2O_2 and 0.7 mM-citric acid in 0.1 M-sodium acetate; the colour was allowed to develop for up to 15 min and the reaction was stopped by the addition of 50 μl of 2 M-H_2SO_4. The absorbance of each well was measured at 450 nm using a Bio-Rad 2550 plate reader.

Aluminium determination using graphite furnace atomic absorption spectrometry

The concentration of aluminium in brain tissue samples was determined as described previously [19]. The concentration of aluminium in ferritin samples was measured against an aqueous standard curve prepared from an aluminium nitrate standard solution (BDH 'Spectrosol') using a Perkin–Elmer 3030 atomic absorption spectrophotometer with HGA 500 graphite furnace and AS40 autosampler. The lower detection limit (3 × S.D. of the reagent blank) was 27.6 nM (14.9 pg of Al in 20 μl). The concentration of aluminium measured in a standard solution of 3.7 μM-Al in the presence of 25 μg of horse spleen ferritin/ml as 96% of the control value. In the presence of 100 μM-Fe the Al concentration was 102% of the control value.

Protein determination

Protein assays of cortical grey matter and of isolated ferritin were carried out by the method of Lowry *et al.* [29]. Since the absorption coefficients of different proteins vary and the large amount of human brain ferritin necessary to make a working standard was not available, conversion factors were determined to allow a BSA standard to be used. The absolute protein concentration of brain ferritin samples was determined by amino acid analysis and the concentration of a portion of the same sample, at a range of dilutions, determined using the colorimetric assays with a BSA standard. In this way a conversion factor, x, was obtained, where x is the amount of ferritin which, in the colorimetric assay, gave the same reading as 1 μg of BSA. For human brain ferritin $x = 0.75$ μg for the Lowry assay.

Chemicals

Reagents used were of analytical grade or better unless otherwise stated and obtained from BDH/Merck (Poole, Dorset, U.K.). Fruend's complete and incomplete adjuvant, BSA and goat anti-rabbit IgG–horseradish peroxidase conjugate were obtained from Sigma (Poole, Dorset, U.K.) and streptavidin–biotin horseradish peroxidase was from Amersham (Amersham, Bucks., U.K.). Sepharose 6B and CM Affigel Blue were from Pharmacia (Milton Keynes, Beds., U.K.) and Bio-Gel P-100 was from Bio-Rad (Hemel Hempstead, Herts., U.K.).

RESULTS

Ferritin prepared from human cerebral cortex and cerebellum had a faster electrophoretic mobility when compared with human spleen ferritin in native polyacrylamide gels. Both monomer and

dimer bands were present, together with bands of aggregated ferritin molecules. Subunit analysis of ferritin isolated from native gels, using SDS/PAGE, showed that ferritin isolated from the cerebral and cerebellar cortices is relatively enriched in H subunits (Fig. 1). Ferritin isolated from the cerebral cortex and cerebellum of controls had an H-subunit content of approx. 65 % and 60 % respectively (Table 1). The H-subunit content of ferritin was not significantly changed in either Alzheimer's-disease patients or in renal-dialysis patients (Table 1).

Ferritin isolated from the cerebral cortex of controls contained approx. 1500 iron atoms/ferritin molecule, whereas ferritin isolated from normal cerebellum had a significantly higher iron content of approx. 1850 iron atoms/molecule (Table 1). No significant differences were found in the iron content of ferritin isolated from the cerebral cortex in Alzheimer's-disease or renal-dialysis patients compared with controls (Table 1).

The aluminium content of ferritin isolated from the cerebral cortex was low, with a mean value of 6 atoms of aluminium/ferritin molecule in the cerebral cortex from normal individuals. There was no significant difference between the aluminium content of cerebral cortex and cerebellum in normal subjects (Table 1). Similarly, there was no significant elevation in the aluminium content of ferritin in the cerebral cortex in Alzheimer's disease (Table 1). Ferritin isolated from renal-dialysis patients also did not have an elevated aluminium content (Table 1), despite the increased concentrations of aluminium in the cerebral cortex of these patients (mean 9.7±2.9 μg/g dry weight, range 6.3–14.1 μg/g dry weight) when compared with controls (mean 2.5 μg/g dry weight, range 1.3–5.5 μg/g dry weight [20]).

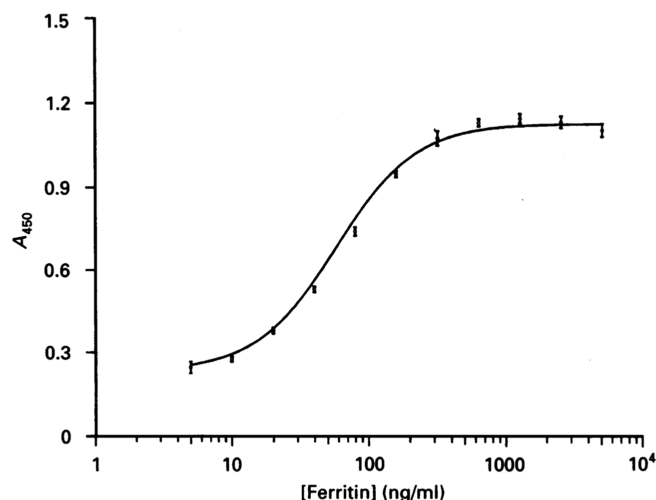

Fig. 2. Sandwich immunoassay of human brain ferritin

The immunoassay was carried out as described in the Materials and methods section. The ferritin standard was pooled from a number of different preparations. Each concentration of the standard was in triplicate. Error bars shows sample standard deviation.

Table 2. Ferritin, iron and aluminium concentrations in parietal cortex of normal and Alzheimer's-disease patients

Patients	Ferritin (μg/mg of protein)	Non-haem iron (μg/mg of protein)	Aluminium (ng/mg dry wt)
Normal (n = 11)	1.445±0.391	0.29±0.05	2.3±0.4‡
Alzheimer's disease (n = 11)	1.997±0.682*	0.42±0.13†	2.4±0.4‡

* Significantly different from control, $P < 0.05$ (Mann–Whitney U-test).

† Significantly different from control, $P < 0.01$ (Mann–Whitney U-test).

‡ $n = 7$.

Table 3. Iron to aluminium ratios in cortical grey matter and ferritin of normal and Alzheimer's-disease patients

The ratio for purified ferritin was calculated from the ferritin iron and aluminium content of normal and Alzheimer's-disease patients given in Table 1. The ratio for cortical grey matter was calculated from cortical Fe and Al concentrations given in Table 2. It was assumed that dry weight is equivalent to 15 % of wet weight and total protein is equivalent to 10 % of wet weight.

	Mean Fe/Al ratio	
	Purified ferritin	Cortical grey matter
Normal individuals	374±201	43+7
Alzheimer's-disease patients	270±213	63±22

There was no significant difference in the phosphate content of ferritin between the groups, with a mean value of 11 iron atoms to one phosphate irrespective of whether ferritin was isolated from cerebral cortex or cerebellum of controls or from renal-dialysis or Alzheimer's-disease patients (Table 1).

E.l.i.s.a. determination of ferritin in the grey matter of parietal cortex from normal and Alzheimer's-disease patients showed a significant increase in ferritin concentrations of 38.2 % in the Alzheimer's disease patients (Table 2). This increase in ferritin was paralleled by a significant elevation of the non-haem iron

Table 1. Composition of ferritin isolated from the cerebral cortex from normal subjects and Alzheimer's-disease and renal-dialysis patients

Numbers in parentheses indicate the number of cases in which isolation was carried out. Fe/protein, iron atoms per ferritin molecule; Al/protein, aluminium atoms per ferritin molecule; Fe/P_i, molar ratio of iron to inorganic phosphate in ferritin molecules.

	H subunit (%)	Fe/protein	Al/protein	Fe/P_i	Fe/Al
Normal, cortex (7)	64.6±5.5*	1507±226	6.2±4.0	11.1±3.7	243
cerebellum (4)	59.7±5.6	1847±170§	5.2±0.4	9.6±3.3	355
Alzheimer cortex (5)	56.7±5.8†	1626±353	8.9±4.6	9.3±1.8	183
Renal dialysis cortex (6)	62.7±4.7‡	1355±355	7.2±3.6	13.2±4.8	188

* Six samples.
† Three samples.
‡ Five samples.
§ $P < 0.05$, Student's t test.

content of 44.8 % in the parietal cortex of the Alzheimer's-disease patients (Table 2). The concentrations of aluminium in the parietal cortex of the control and Alzheimer's-disease patients were not significantly different (Table 2).

DISCUSSION

Ferritin isolated from the cerebral cortex and cerebellum of control patients had characteristics more in common with H-subunit-rich ferritins such as those in the heart and placenta than L-subunit-rich ferritins from liver and spleen [30,31]. The H-subunit content of ferritin from the cerebral cortex (64.6 %) and cerebellum (59.7 %) was similar to that of heart with an H-subunit content of 53.4 % [30]. The subunit composition of ferritin isolated from the cerebral cortex was not significantly different in Alzheimer's-disease and renal-dialysis patients. These results were in agreement with those of Fleming & Joshi [18] who found that ferritin isolated from the entire brain of two normal individuals and two cases of Alzheimer's disease had an H-subunit content of 70 %. The subunit composition of brain ferritin has implications for its function, since ferritins enriched in H subunits are more active in iron metabolism than L-rich ferritins which may have a long-term storage function [31]. Brain apoferritin like other H-subunit-rich ferritins may be expected to show a faster rate of uptake of iron than L-subunit-rich apoferritins [30]. Consequently brain ferritin may be more efficient than the L-rich ferritins of spleen or liver in detoxifying iron, since the rapid sequestration of iron in ferritin may limit iron-catalysed peroxidation reactions.

Ferritin isolated from the cerebral cortex of normal individuals has approx. 1500 atoms of iron per molecule, compared with 2000–3000 atoms of iron per molecule in liver and spleen ferritin [30]. This is consistent with the proposal that L-rich ferritins are associated more with a longer-term iron-storage function, whereas H-rich ferritins are more active in iron turnover. The absence of significant differences found here between the iron content of ferritin isolated from the cerebral cortex of normal, Alzheimer's-disease or renal-dialysis patients contrasts with the report by Fleming & Joshi [18] of a significant increase in the iron content of ferritin (isolated from the entire brain) from two patients with Alzheimer's disease compared with two normal patients. However, it should be noted that the iron contents of the isolated ferritins found by Fleming & Joshi [18] were two to three times lower than the values observed in the present study for both normal and Alzheimer's-disease patients.

Fleming & Joshi [18] also reported the presence of very high concentrations of phosphate in isolated brain ferritin which had an Fe/phosphate ratio of 2 compared with approx. 11 in the present study. Mammalian ferritins have an Fe/phosphate ratio of 8–20 [2]. Thus the Fe/phosphate ratio found in the present study is more consistent with ferritins isolated from other tissues where the phosphate appears to be loosely bound to the surface of the iron core [2,32]. Fleming & Joshi [18] used 5 mM-phosphate buffer in their isolation procedure which may have given rise to the high phosphate content of the isolated ferritin, since phosphate can be adsorbed on the iron core of ferritin.

Ferritin isolated from the cerebral cortex of normal, Alzheimer's-disease and chronic renal-dialysis patients had less than 9 atoms of aluminium/ferritin molecule. The lack of increase in aluminium associated with ferritin isolated from the cerebral cortex in Alzheimer's disease is in marked disagreement with the over fivefold increase reported by Fleming & Joshi [18]. The failure to find raised concentrations of aluminium in ferritin isolated from the cerebral cortex of renal-dialysis patients, who had markedly increased concentrations of aluminium in the cerebral cortex, clearly shows that aluminium does not accumulate in ferritin *in vivo*. It is of particular interest that there are marked differences between the Fe/Al molar ratios in cortical grey matter and isolated ferritin for both the normal and Alzheimer's-disease patients (Table 3). This suggests that either aluminium cannot be taken up into ferritin, because it lacks the redox properties of iron, or it is excluded from ferritin by some other means. The finding (D. Dedman, A. Treffry and P. M. Harrison, unpublished work) that horse spleen ferritin, *in vitro*, can incorporate at least 120 aluminium atoms/molecule with an Fe/Al ratio of approx. 25, suggests that aluminium is excluded from ferritin *in vivo*. The degree of accumulation of aluminium by ferritin is likely to reflect the composition of the pool of iron and aluminium available for uptake rather than the intrinsic properties of ferritin molecules. Thus the affinity of phosphoproteins and small carrier molecules for aluminium may diminish its availability to ferritin. The conclusion that, *in vivo*, ferritin does not accumulate aluminium even in situations where brain aluminium is significantly elevated, e.g. renal-dialysis patients, has important implications for the potential intracellular toxicity of aluminium, since it implies that cells are unable to detoxify aluminium by the same mechanism as that available for iron.

There was no significant difference between the aluminium concentration in the parietal cortex in Alzheimer's-disease patients and age-matched normal individuals. These results are in agreement with those of McDermott *et al.* [33] but are at variance with those of Crapper *et al.* [34]. An e.l.i.s.a. for brain ferritin developed here which used an antibody against ferritin from the cerebral cortex showed significantly increased ferritin concentrations in the parietal cortex of Alzheimer's-disease patients compared with age-matched normal subjects. The increase in ferritin, by approx. 38 %, was paralleled by a similar significant increase (approx. 45 %) in the non-haem iron content. It can be calculated that 85–88 % of the non-haem content of the parietal cortex is located in ferritin in both normal and Alzheimer's-disease patients. The increase in iron in the brains of Alzheimer's-disease patients accompanied by a concomitant increase in ferritin suggests that a regulatory mechanism for the translational control of ferritin synthesis is operational as in other tissues [35]. Ferritin appears to be predominantly located in microglial cells [12], and, in Alzheimer's disease, these microglial cells seem to be increased in number, are reactive as judged by HLA-DR expression [36] and are associated with senile plaques [37]. The increase in the iron and ferritin content in the cerebral cortex in Alzheimer's disease may therefore reflect a pathological response to a disease process rather than a primary cause of degeneration. Further information on the metabolism of brain iron and ferritin is required, however, before these issues can be resolved.

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REFERENCES

1. Ford, G. C., Harrison, P. M., Rice, D. W., Smith, J. M. A., Treffry, A., White, J. L. & Yariv, J. (1984) Philos. Trans. R. Soc. London Ser. B **304**, 551–565
2. Treffry A., Harrison, P. M., Cleton, M. I., de Bruijn, W. C. & Mann, S. (1987) J. Inorg. Biochem. **31**, 1–6
3. Boyd, D., Vecoli, C., Belcher, D. M., Byrd, S. K. & Drysdale, J. W. (1985) J. Biol. Chem. **260**, 11755–11761
4. Caskey, J. H., Jones, J. H., Miller, Y. E. & Seligman, P. A. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 482–486
5. Worwood, M., Brook, J. D., Cragg, S., Hellkuhl, J. & Jones, B. M. (1985) Hum. Genet. **69**, 371–374

6. Lawson, D. M., Artymiuk, P. J., Yewdall, S. J., Smith, J. M. A., Livingstone, J. C., Treffrey, A., Luzzago, A., Levi, S. & Arosio, P. M. (1991) Nature (London) **349**, 541–544
7. Xu, B. & Chasteen, N. D. (1991) J. Biol. Chem. **266**, 19965–19970
8. Spatz, H. (1922) Z. Ges. Neurol. Psychiat. **77**, 261–390
9. Hallgren, B. & Sourander, P. (1958) J. Neurochem. **3**, 41–51
10. Diezel, P. B. (1955) Iron in the Brain: A Chemical and Histochemical Examination, pp. 145–152, Academic Press, London
11. Hill, J. M. (1988) in Brain Iron: Neurochemical and Behavioural Aspects (Youdim, M. E. H., ed.), pp. 1–24, Taylor and Francis, London
12. Kaneko, Y., Kitamoto, T., Tateishi, J. & Yamaguchi, K. (1989) Acta Neuropathol. **79**, 129–136
13. Connor, J. R., Menzies, S. L., St. Martin, S. M. & Mufson, E. J. (1990) J. Neurosci. Res. **27**, 595–611
14. Halliwell, B. (1989) Acta Neurol. Scand. **126**, 23–33
15. Dexter, D. T., Wells, F. R., Agid, F., Agid, Y., Lees, A. J., Jenner, P. & Marsden, C. D. (1987) Lancet **ii**, 1219–1220
16. Dexter, D. T., Carayon, A., Vidailhet, M., Ruberg, M., Agid, F., Agid, Y., Lees, A. J., Wells, F. R., Jenner, P. & Marsden, C. D. (1990) J. Neurochem. **55**, 16–20
17. Dexter, D. T., Carayon, A., Javoy-Agid, F., Agid, Y., Wells, F. R., Daniel, S. E., Lees, A. J., Jenner, P. & Marsden, C. D. (1991) Brain **114**, 1953–1975
18. Fleming, J. & Joshi, J. G. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 7866–7870
19. Morris, C. M., Candy, J. M., Oakley, A. E., Taylor, G. A., Mountfort, S., Bishop, H., Ward, M. K., Bloxham, C. A. & Edwardson, J. A. (1989) J. Neurol. Sci. **94**, 295–306
20. Candy, J. M., McArthur, F. K., Oakley, A. E., Taylor, G. A., Chen, C. P. L.-H., Mountfort, S. A., Thompson, J. E., Chalker, P. R., Bishop, H. E., Beyreuther, K., Perry, G., Ward, M. K., Martyn, C. N. & Edwardson, J. A. (1992) J. Neurol. Sci., in the press
21. Linder, M. C. & Munro, H. N. (1972) Anal. Biochem. **48**, 266–278
22. Arosio, P., Adelman, T. G. & Drysdale, J. W. (1978) J. Biol. Chem. **253**, 4457–4458
23. Laemmli, U. K. (1970) Anal. Biochem. **48**, 266–278
24. Ames, B. N. (1966) Methods Enzymol. **8**, 115–118
25. Foy, A. L., Williams, H. L., Cortell, S. & Conrad, M. E. (1967) Anal. Biochem. **18**, 559–563
26. Carter, P. (1971) Anal. Biochem. **40**, 450–458
27. Drysdale, J. W. & Munro, H. N. (1965) Biochem. J. **95**, 857–858
28. Polak, J. M. & Varndl, I. M. (1984) Immunolabelling for Electron Microscopy, Elsevier, Amsterdam
29. Lowry, O., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
30. Wagstaff, M., Worwood, M. & Jacobs, A. (1982) Clin. Sci. **62**, 1–12
31. Theil, E. C. (1987) Annu. Rev. Biochem. **56**, 289–315
32. Treffry, A. & Harrison, P. M. (1978) Biochem. J. **171**, 313–320
33. McDermott, J. R., Smith, I. A., Iqbal, K. & Wisniewski, H. M. (1979) Neurology **29**, 809–814
34. Crapper, D. R., Krishnan, S. S. & Quittkat, S. (1976) Brain **99**, 67–80
35. Klausner, R. D. & Harford, J. B. (1989) Science **246**, 870–887
36. McGeer, P. L., Itagaki, S., Boyes, B. E. & McGeer, E. G. (1988) Neurology **38**, 1285–1291
37. Grundke-Iqbal, I., Fleming, J., Tung, Y.-C., Lassman, H., Iqbal, K. & Joshi, J. G. (1990) Neuropathology **81**, 105–110

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