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Organ-specific crystalline structures of ferritin cores in β-thalassemia/hemoglobin E

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Summary. The cores of ferritins isolated from different organs of human subjects with β-thalassemia/hemoglobin E (β-thal/HbE) disease have different size distributions and crystallinities depending on the source organ. These patients have not been treated by hypertransfusion regimen or iron chelation therapy. β-Thal/HbE spleens and livers yield ferritin cores which are less crystalline than those isolated from normal spleens and livers, reflecting the more rapid deposition of iron in the diseased state. Ferritins isolated from the hearts and pancreases of β-thal/HbE subjects were found to have larger, more crystalline cores than those from the β-thal/HbE livers and spleens, possibly as a consequence of the role of the heart and pancreas as long-term iron deposition sites in this iron overload pathology.

Key words: Ferritin – Thalassemia – Ferrihydrite – Crystallinity – Electron microscopy

Introduction

β-Thalassemia/hemoglobin E (β-thal/HbE) disease, a double heterozygote for the β-thalassemia gene and hemoglobin E gene, is common in South East Asia (Wasi 1981). The abnormality in β-thalassemia is a reduction in β-globin chain synthesis, whereas HbE is a structural mutation in the β-globin gene. The amino acid substitution is $\alpha_2\beta 26$ Glu→Lys (Hunt and Ingram 1961). The combination of these two genetic disorders results in chronic hemolytic anemia. The erythropoietic activity measured by ferrokinetics is markedly increased (up to 10 or more times normal) (Pootrakul et al. 1981a, 1988). Iron overload is a constant complication in severe thalassemia diseases. Although patients with β-thal/HbE in Thailand receive minimal or no blood transfusions, there is evidence that severe iron loading occurs (Bhamarabravati et al. 1967; Sonakul et al. 1978; Pootrakul et al. 1980, 1981b). This excess iron is the result of increased gastrointestinal absorption (Pootrakul et al. 1988) and is deposited in the form of ferritin and hemosiderin. Ferritin consists of a multisubunit protein shell surrounding a mineral core which contains iron while hemosiderin contains iron in the form of insoluble granules (St. Pierre et al. 1989; Ford et al. 1984).

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Materials and methods

Samples of spleen, liver, heart and pancreas obtained from β-thal/HbE patients (post-mortem) were supplied by the Thalassemia Center, Siriraj Hospital, Bangkok, Thailand. Normal spleen and liver obtained from non-thalassemic (normal) subjects were supplied by Queen Elizabeth II Hospital, Perth, Western Australia. Ferritin was isolated from each of these organs and then purified by methods published elsewhere (Tran et al. 1990a). The samples of heart and pancreas were separately pooled in order to obtain a reasonable yield of ferritin.

Ferritin core size measurements and crystallinity were determined by electron microscopy and selected area electron diffraction using a Jeol 2000FX transmission electron microscope operating at 200 keV.

Ferritin core size distributions were analysed using a one-way analysis of variance assuming a normal distribution. The probability that the difference in the means of any two core size distributions is due to random fluctuations in the sampling of the population of cores was calculated. *P* values of less than 0.01 were considered to indicate a significant difference between two mean values.

Results and discussion

Core size distributions for some of the ferritins are shown in Fig. 1. Comparisons among the ferritins isolated from β-thal/HbE organs show that the ferritins in the heart and pancreas have significantly larger core sizes (peaks of distributions are 7.0–7.5 nm) than those from the spleen and liver (peaks of distributions are 6.0–6.5 nm). Furthermore, the core sizes of the ferritin from β-thal/HbE liver are significantly smaller than those of the ferritin from the normal liver (peaks of distributions are 6.0–6.5 and 7.0–7.5 nm, respectively). Values of the mean core sizes are given in Table 1.

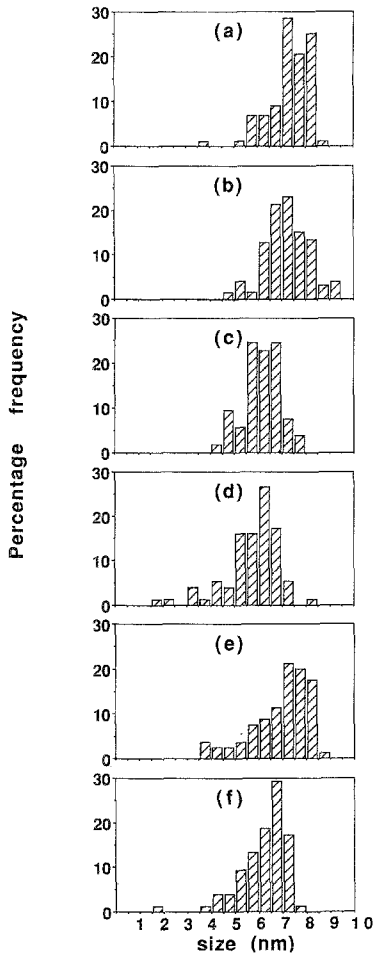

Fig. 1. Core size distributions of ferritins isolated from (a) β-thal/HbE heart, (b) β-thal/HbE pancreas, (c) β-thal/HbE spleen I, (d) β-thal/HbE liver III, (e) normal liver, (f) normal spleen. The distributions were measured from electron micrographs by measuring the largest dimension of each of a sample of 53–126 electron dense core images. The labels I and III (c and d, respectively) indicate different organ donors

Table 1. Means and standard errors on the means of the ferritin core size distributions

Source of ferritin	Number of core images measured	Mean (±SE) core size (nm)
β-Thal/HbE heart	88	7.37±0.09
β-Thal/HbE pancreas	126	7.18±0.08
β-Thal/HbE spleen I	53	6.19±0.10
β-Thal/HbE spleen II	103	6.08±0.09
β-Thal/HbE liver III	75	5.80±0.13
Normal liver	80	7.01±0.13
Normal spleen	75	6.25±0.11

The labels I, II and III indicate different organ donors

Table 2. *d*-spacings of ferrihydrite and ferritin cores from normal and β-thal/HbE organs measured from electron diffraction ring patterns

Sample	*d*-spacing (nm)					
Thal. heart ferritin	0.264	0.232	0.211	0.178	0.160[a]	0.153
Thal. pancreas ferritin	0.259	0.228	0.205	0.174	0.157	0.150
Thal. spleen I ferritin	0.263	0.229				0.152
Thal. spleen II ferritin	0.264	0.231[b]	0.207[b]			0.151
Thal. liver III ferritin	0.274	0.220			0.157	
Thal. liver IV ferritin	0.266	0.227[b]	0.205[b]		0.157	
Thal. liver V ferritin	0.259	0.228	0.202			0.150
Normal liver ferritin	0.258	0.228	0.201	0.175		0.150
Normal spleen ferritin	0.259	0.228	0.204	0.175	0.154	0.149
Ferrihydrite[c]	0.254	0.224	0.198	0.173	0.152	0.147
Ferrihydrite[d]	0.252	0.225	0.197	0.172		0.148

The labels I, II, III, IV and V indicate different organ donors
[a] Very diffuse line
[b] Very weak line
[c] From Towe and Bradley (1967)
[d] From Murad and Johnston (1987)

Electron diffraction patterns of the ferritins (Table 2) gave between three and six powder diffraction rings at positions which correspond to those of the mineral ferrihydrite (Ford et al. 1984; Murad and Johnston 1987; Towe and Bradley 1967). Ferrihydrite is known to exist with varying degrees of crystallinity giving between two (poorly crystalline) and six (well crystalline) diffraction lines (Murad and Johnston 1987). Of the β-thal/HbE ferritin cores, those from the heart and pancreas were shown to be significantly more crystalline (six lines) than those from the spleen and liver (three or four lines). The diffraction pattern data in Table 2 also show that the β-thal/HbE liver and spleen ferritins are less crystalline (three or four lines) than those from normal organs (five or six lines). In the case of the four-line diffraction patterns for the β-thal/HbE ferritins, the lines were generally very diffuse.

The data presented here on ferritin core size and crystallinity almost certainly reflect the kinetics of iron metabolism in the body and the differences between the dynamic equilibrium of iron in normal and β-thal/HbE subjects. The reticuloendothelial cells of the spleen and liver are the major sites involved in the reprocessing of the iron in red blood cells at the end of their lifetime. The lower degree of crystallinity of the ferritin cores from the livers and spleens of β-thal/HbE subjects compared to the normal subject suggests a more rapid rate of deposition of iron than in the latter case. This is consistent with the shorter mean lifetime of red blood cells in β-thal/HbE and the consequent approximate tenfold increase in erythroid iron turnover rates for β-thal/HbE (Pootrakul et al. 1988). The severity of iron overload varies between individual β-thal/HbE patients (Wasi 1981); this is possibly reflected in the variation of the crystallinity of the spleen and liver ferritins between three- and four-line electron diffraction patterns. Other biological systems that are known to deposit iron into ferritin rapidly, such as limpets and chitons (marine molluscs), also produce ferritin cores with a low degree of crystallinity (Mann et al. 1986; St. Pierre et al. 1986a, 1986b, 1989, 1990).

Ferritins isolated from the spleen of one β-thalassemic patient (Mann et al. 1986) and the liver and spleen of another (V. J. Wade, personal communica-

tion), both of whom had been treated with regular blood transfusions and iron chelation therapy, had cores with a higher degree of crystallinity (four, five or six ferrihydrite diffraction lines) than the β-thal/HbE liver and spleen ferritin cores described in the present study. This indicates that regular blood transfusions and iron chelation therapy may influence the crystal structure of the ferritin cores in the liver and spleen. Although regular blood transfusions introduce extra iron into the body in the form of hemoglobin of intact red blood cells, it has been shown that they also repress dietary iron uptake (Cavill et al. 1978) and slow erythropoiesis (Shuler et al. 1990; Pootrakul et al. 1981a). Both of these factors would tend to reduce the rate of iron turnover through the reticuloendothelial cells of the spleen and liver which in turn would lead to a slower rate of deposition and turnover of iron in the ferritin of these cells, even though the total body iron has been increased. This may explain the higher degree of crystallinity of the liver and spleen ferritin cores from the transfused patients. It should also be noted that the transfused patients received iron chelation therapy which tends to reduce total body iron.

While the heart and pancreas are not directly involved in the reprocessing of iron from red blood cells, heart and pancreas iron loadings are increased by about 2.7 and 47 times, respectively, in β-thal/HbE subjects (Shuler et al. 1990). The high degree of crystallinity and larger core size of the ferritins from the heart and pancreas compared to those from the liver and spleen of β-thal/HbE subjects indicates a slow rate of iron deposition over an extended period. This is consistent with the role of the heart and pancreas as sites for long-term iron deposition under conditions of iron-overload (Jacobs and Worwood 1981).

Isoelectric focussing and SDS/PAGE of each of the β-thal/HbE ferritins in this study gave very similar results (Tran et al. 1990b). This implies that the protein structures of the ferritins from each β-thal/HbE organ are also very similar. Thus it appears that the organ specificity of the ferritin core structures is likely to be a result of the iron kinetics in each organ rather than a result of any molecular characteristics of the protein, at least those detectable by various electrophoretic techniques (Tran et al. 1990a, 1990b).

The present study has focused on the protein ferritin. Presumably the core structure of hemosiderin will also be affected by the pathological conditions in the various organs of β-thal/HbE patients. It has already been shown that the core structure of hemosiderin from livers and spleens is specific to the type of iron-overload pathology (Mann et al. 1988; Dickson et al. 1988; Ward et al. 1988). Recognition of organ-specific crystalline structures of ferritin and hemosiderin cores in iron-overload pathologies provides another means of evaluation of iron-chelation therapies since the reactivity of this excess iron is dependent on core structure.

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