Ascorbic acid deficiency affects the metabolism of cytosolic ferritin but not lipid-associated ferritin in livers of guinea pigs

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Two physicochemically and metabolically separate pools of ferritin, namely cytosolic ferritin and lipid-associated ferritin, are present in the livers of guinea pigs. In this paper we establish that the iron content of cytosolic ferritin is dependent on and linearly related to ascorbic acid concentration, whereas changes in concentration of this vitamin do not affect the iron content of lipid-associated ferritin. In livers of ascorbic acid-deficient guinea pigs both synthesis and degradation of cytosolic ferritin are diminished equally. Consequently cytosolic ferritin is metabolized more slowly without changes in its pool size. In contrast with cytosolic ferritin, the metabolism of lipid-associated ferritin is unaffected by ascorbic acid deficiency. The differential effects of ascorbic acid deficiency on the physicochemical characteristics as well as on the metabolism of cytosolic ferritin and lipid-associated ferritin suggest that the two forms of ferritin have different functional roles.

INTRODUCTION

Ferritin has been defined as a soluble protein that serves as a source of iron storage in the body (Worwood, 1986). It has since been shown that a significant proportion of ferritin is present as non-soluble protein in the liver of various mammals (Cham *et al.*, 1986, 1988, 1989). This ferritin can be solubilized by lipid solvent systems (Cham *et al.*, 1986) or by non-ionic detergents (Cham *et al.*, 1988). The newly described ferritin pool has therefore been termed lipid-associated ferritin (LAF) (Cham *et al.*, 1986, 1988, 1989). The difference between cytosolic ferritin (CYF) and LAF is not only physicochemical (Cham *et al.*, 1986, 1988) but also metabolic (Cham *et al.*, 1989). At homeostasis the rate of turnover of CYF is much higher than that of LAF.

Ascorbic acid is involved in a large number of cellular metabolic events. Deficiency of this vitamin is known to alter the metabolism of serum ferritin in both animals (Roeser *et al.*, 1980; Roeser, 1983) and man (Cohen *et al.*, 1981) and of tissue ferritin in guinea pigs (Roeser *et al.*, 1983). In previous papers it was suggested that ascorbic acid has a physiological role in the reduction of intracellular iron and that it is the reduced form of iron that stimulates CYF synthesis in the liver (Roeser, 1983; Roeser *et al.*, 1983, 1986).

To date no information is available on the effect of ascorbic acid deficiency on the metabolism of LAF. The studies reported in the present paper were therefore designed to examine physicochemical characteristics and the metabolism of CYF and LAF in livers of scorbutic guinea pigs.

EXPERIMENTAL

Materials

$\text{Na}_2^{14}\text{CO}_3$ (specific radioactivity 50 mCi/mmol, as aqueous solution) was purchased from Amersham (Australia) Pty. Ltd. (Sydney, N.S.W., Australia). All other reagents were of the highest quality commercially available.

Animals

The diet and experimental conditions of the animals were as published previously (Cham *et al.*, 1989). Ascorbic acid deficiency

was induced, after 2-week-old weanlings had been maintained on the described diet for 7–8 weeks (body weights 412 ± 18 g; mean $\pm$ S.D.), by discontinuing the ascorbic acid supplements in their drinking water. Metabolic studies on the livers of animals were started after 12 days of ascorbic acid depletion.

The following experimental procedures have been previously described in detail (Cham *et al.*, 1989): (1) the conditions and measurement of the incorporation of ^{14}C from $\text{Na}_2^{14}\text{CO}_3$ into CYF and LAF; (2) the preparation of liver samples, and the separation and measurements of total liver ferritin, CYF and LAF concentrations; (3) the delipidation procedure for tissue samples and polyacrylamide-gel eluates; (4) the validation of the similar immunological reactivity of CYF and LAF in electro-immunoassays; (5) the calculation of rates of synthesis and catabolism of ferritin.

The iron concentration of ferritin was determined by atomic absorption spectrophotometry (Roeser *et al.*, 1983).

Concentrations of ascorbic acid in livers of the animals were measured by the method of Zannoni *et al.* (1974).

RESULTS

The decline in hepatic ascorbic acid was monitored in ascorbic acid-deprived guinea pigs. Tissue ascorbic acid concentrations at any time between 12 and 20 days of ascorbic acid depletion were similar. Hepatic concentrations of ascorbic acid were 23 ± 9 $\mu\text{g/g}$ wet wt. (mean $\pm$ S.D., $n = 16$). Concentrations of hepatic ascorbic acid for guinea pigs that had not been deprived of ascorbic acid were 375 ± 140 $\mu\text{g/g}$ wet wt. (mean $\pm$ S.D., $n = 15$). Centrifuged liver homogenates from the ascorbic acid-deficient animals were delipidated and assayed for ferritin. This yielded total liver ferritin, which was 791 ± 604 $\mu\text{g/g}$ wet wt. of liver (mean $\pm$ S.D., $n = 30$). CYF and LAF concentrations, determined as described, were 613 ± 521 and 178 ± 222 μg wet wt. of liver (means $\pm$ S.D.) respectively. Thus LAF represented $23 \pm 17\%$ (mean $\pm$ S.D.) of the total ferritin pool. Concentrations of CYF and LAF, when expressed as μg of ferritin in total liver per 100 g body wt., were 2215 ± 1822 and 912 ± 1089 (means $\pm$ S.D.) respectively. Ascorbic acid deficiency resulted in a marked fall in the iron/protein

Abbreviations used: LAF, lipid-associated ferritin; CYF, cytosolic ferritin.

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Fig. 1. Correlations of the iron/protein ratio of (a) hepatic CYF and of (b) hepatic LAF and ascorbic acid concentration

The iron/protein ratio expressed as a percentage is plotted against the concentration of ascorbic acid. The line drawn is based on least-squares linear-regression analysis. Correlation coefficients determined by linear-regression analyses of the plotted data were 0.7042 ($P = 0.003$) for CYF and 0.1064 ($P = 0.717$, not significant) for LAF.

Table 1. Known physicochemical and metabolic differences between CYF and LAF

CYF	LAF
Physicochemical	
Not associated with lipid	Associated with lipid (can be dissociated with organic solvents and non-ionic detergents)
Heat-stable	Heat-unstable
Iron content affected by ascorbic acid deficiency:	Iron content unaffected by ascorbic acid deficiency:
Control* animals, $8.80 \pm 2.15^\dagger$	Control* animals, $9.34 \pm 4.58^\dagger$
Scorbutic animals, $5.80 \pm 1.82^\dagger$	Scorbutic animals, $10.48 \pm 9.36^\dagger$
Metabolic	
Affected by ascorbic acid deficiency:	Unaffected by ascorbic acid deficiency:
Control animals, $k_s 0.51 \pm 0.07^\ddagger$	Control animals, $k_s 0.15 \pm 0.11^\ddagger$
Scorbutic animals, $k_s 0.20 \pm 0.09^\ddagger$	Scorbutic animals, $k_s 0.20 \pm 0.11^\ddagger$
Control animals, $k_c 0.48 \pm 0.10^\ddagger$	Control animals, $k_c 0.13 \pm 0.09^\ddagger$
Scorbutic animals, $k_c 0.31 \pm 0.09^\ddagger$	Scorbutic animals, $k_c 0.07 \pm 0.06^\ddagger$

* Control data obtained in concurrent experiments with those for data for ascorbic acid-deficient animals (Cham *et al.*, 1989).

† Iron/protein ratio (w/w), expressed as percentage (mean $\pm$ s.d.).

‡ Rate constants for synthesis (k_s) and catabolism (k_c) per day (mean $\pm$ s.e.m.).

(w/w) ratio of hepatic CYF. The percentage iron/protein ratio of hepatic CYF for guinea pigs that had not been deprived of ascorbic acid was 8.80 ± 2.15 (mean $\pm$ s.d., $n = 15$). In ascorbic acid-deficient guinea pigs the percentage iron/protein ratio of CYF was 5.80 ± 1.82 (mean $\pm$ s.d., $n = 10$). The differences in iron/protein ratios of CYF were highly significant ($P < 0.001$). The percentage iron/protein ratio of hepatic LAF for normal guinea pigs was 9.34 ± 4.58 (mean $\pm$ s.d., $n = 15$). The percentage iron/protein ratio of LAF for ascorbic acid-deficient animals

had a large variation (10.48 ± 9.36 ; mean $\pm$ s.d., $n = 10$) but was not significantly different from control values. A significant correlation was found between the percentage iron/protein ratio of CYF and ascorbic acid concentration. Linear-regression analysis gave an r value of 0.7042 with a P value of 0.003 (Fig. 1a). In contrast, there was no correlation between the percentage iron/protein ratio of LAF and ascorbic acid concentration ($r = 0.106$; $P = 0.717$) (Fig. 1b).

The decline with time in the specific radioactivities of CYF and LAF represented the rates of dilution of pre-labelled ferritin by newly formed unlabelled protein, and thereby provided a quantitative index of ferritin synthesis. The rate constant for synthesis (k_s) in scorbutic livers was 0.20 ± 0.09 day $^{-1}$ for the CYF pool and 0.20 ± 0.11 day $^{-1}$ for the LAF pool (means $\pm$ s.e.m.).

The decline with time of total liver ferritin radioactivity represented catabolism of pre-labelled ferritin. The rate constant for catabolism (k_c) in scorbutic livers was 0.31 ± 0.09 day $^{-1}$ for the CYF pool and 0.07 ± 0.06 day $^{-1}$ for the LAF pool (means $\pm$ s.e.m.). For each pool of liver ferritin the rate constant for synthesis did not differ significantly from that of catabolism, suggesting that a steady state prevailed throughout the period of the study. Comparisons of the rate constants for the liver CYF and LAF pools in ascorbic acid-deficient animals with those of ascorbic acid-replete animals (controls) are shown in Table 1.

DISCUSSION

The present study has examined the effects of ascorbic acid deficiency on the metabolism of two pools of guinea-pig liver ferritin: heat-stable ferritin and lipid-associated ferritin (LAF). The former has been called cytosolic ferritin (CYF), although it is clear that not all heat-stable ferritin is in solution in cytosol (Sargent & Munro, 1975). It was previously demonstrated that CYF and LAF have markedly different rates of turnover, with CYF being synthesized and degraded twice as rapidly as LAF, under basal conditions (Cham *et al.*, 1989). The present study has indicated that ascorbic acid deficiency lowers the rate of turnover of CYF, but does not affect the metabolism of LAF (Table 1). It may be noted that ascorbic acid deficiency in guinea pigs resulted in CYF metabolic rates lowered to values that were similar to the metabolic rates of LAF. Although the pool sizes of CYF and

LAF were not significantly affected by ascorbic acid deficiency and thus steady state was assumed, it cannot be ruled out that homeostasis during the experimental period had not prevailed, resulting in the observed wide variation in the catabolic rate of LAF.

The current observation further underlines the distinct metabolic behaviours of the two forms of tissue ferritin. The mechanisms by which ascorbic acid deficiency altered CYF metabolism was not determined. However, current evidence suggests that the major (and possibly only) physiological determinant of liver ferritin concentration and turnover is the intracellular iron concentrations (Theil, 1987), and it seems likely that the known effects of ascorbic acid deficiency on iron metabolism (Roeser, 1983) have mediated the observed changes. This is supported by the decrease in ferritin iron in CYF as a result of ascorbic acid depletion, while the iron content of LAF was unaffected (Table 1).

These results suggest that LAF represents a relatively inert portion of total cellular ferritin. It has a low rate of turnover and appears to be relatively unaffected by intracellular events affecting iron metabolism. In control animals the iron content of LAF is similar to that of CYF. Furthermore, once the lipid moiety of isolated LAF is removed by solvents, the residual ferritin regains its heat-stability (B. E. Cham, H. P. Roeser & A. C. Nikles, unpublished work). This may indicate that LAF represents ferritin recruited from cytosol and then sequestered to lipid membranes, where it acts as a metabolic sink for iron, thus decreasing the risk of iron-catalysed peroxidative damage to lipids (Thomas & Aust, 1986). Interestingly, it has been pointed out that this same association of ferritin and lipid may, under pathological conditions, serve to enhance peroxidative damage, rather than

contain it (Reif *et al.*, 1988). The low rate of turnover of LAF may then merely reflect a low rate of exchange of LAF with the more rapidly turning over CYF pool.

We thank Mrs. K. Ridgway for technical assistance, Mr. B. Maher of the Prentice Computer Centre for assistance with the data analyses and the National Health and Medical Research Council of Australia for financial support.

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Received 23 November 1989/5 February 1990; accepted 19 February 1990