Iron speciation in hypotransferrinemic mouse serum.


A mouse model of human disease was recently described by Bernstein [1]. Hypotransferrinemic mice have 50-fold decreased serum transferrin levels, hypochronomic, microcytic (iron deficient) anaemia but develop liver iron overload [1,2]. These mice parallel for the rare human disease atransferrinaemia [3,4] and may be a useful model for human iron overload disorders. Great interest is currently focussed on the non transferrin-bound iron reported to be present in serum iron patients with iron overload [5-8], as the nature and reactivity of the non transferrin-bound iron species may be of significance in determining tissue uptake of iron as well as tissue damage. Hypotransferrinemic mice provide a useful model for the study of iron species produced in the serum during iron overload, without the complicating presence of transferrin.

Serum from homozygous hypotransferrinemic (data are given for a mixed group of males and females aged 6-7weeks) was found to contain apparently very low levels of serum iron (mean 1.5+0.5μM (SEM, n=5), range <0.5-4μM). This iron fraction includes all species reactive with the Fe(III) chelator ferrozine at pH 4.5 in the presence of hydroxylamine. Model compounds such as transferrin-Fe, FeNTA2, Fe/urate (1:2) or Fe/albumin (prepared by mixing 50μM FeCl3 with 100μg/ml bovine serum albumin (pH7)) are all detectable by this assay, but ferritin iron or FeEDTA (1:2) are relatively unreactive.

Study of hypotransferrinemic mouse serum with a bleomycin-dependent assay [9], sensitive to iron ions and low molecular mass complexes revealed 28±9μM (n=3) iron (wild-type controls showed 3.3±3.3μM, n=7), suggesting the presence of non transferrin-bound iron species. Determination of non-haem iron by boiling serum with 12.5% trichloroacetic acid/ 2% Naapprophosphate (TCA/PPI) [10] the iron being detected with ferrozine/ ascorbate [10] demonstrated large concentrations of iron in hypotransferrinemic serum. The quantity of iron detectable was found to increase progressively with repeated extraction of the serum (11.7±2.3μM, n=7, with a single extraction, 39.3±9.4μM, n=9 with 3 extractions, p<0.01). Even higher quantities of iron were detectable by atomic absorption spectrophotometry (65±16.5μM, n=9, p<0.01). Serum non-haem and total iron were positively correlated with liver non-haem Fe (Figure 1).

Two possible explanations for these observations are the presence of haem iron or ferritin iron in the serum. Haemoglobin iron was detectable at approx. 10μM by spectrophotometry, based on the alpha absorption band near 540nm, but test experiments showed that less than 20% of haemoglobin-Fe is released by boiling 3 times in TCA/PPI.

Serum ferritin was determined by fluorogenic ELISA [11] using anti-mouse liver ferritin with values of 64±128μg/l (n=14) in hypotransferrinemic mice (wild-types 44±6μg/l, n=14). Even if highly loaded with iron, this ferritin could not explain the observed levels of iron in the hypotransferrinemic serum. Ferritin was found to 80-90% extractable by a single boiling with TCA/PPI, as was the iron in overloaded livers from transferrinemic mice. This suggests that known tissue iron storage proteins and haem cannot account for most of the iron present in hypotransferrinemic serum.

Serum was fractionated by Sephadex G200 chromatography (elution with 10mM HEPES/ 0.15M NaCl, pH 7.0) and fractions were assayed for iron by atomic absorption spectrophotometry. Three distinct species were observed with apparent molecular weights of 200kd, 30-50kd and 1-5kd. The non transferrin-bound Fe therefore consists of several distinct species, possibly with differing toxicity and availability for tissue uptake. Further investigation of the nature of the iron species in hypotransferrinemic mouse serum should provide new insights into non transferrin-bound iron in serum from iron overloaded mammals.

Figure 1 Liver non-haem iron and serum iron in hypotransferrinemic mice.

Liver iron was determined by extraction with TCA/PPI (Simpson and Peters, 1990). Serum iron was determined by extraction [9] and by atomic absorption spectrophotometry (10). Liver iron was significantly correlated with both measures of serum iron (p<0.05).