Ferroxidase activity of rat ceruloplasmin

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PREVIOUS STUDIES in hypoceruloplasminemic swine indicated that ceruloplasmin is essential for the flow of iron from reticuloendothelial cells to plasma transferrin (17). Cell-to-plasma flow of iron became sufficiently impaired in such animals as to cause hypoferrremia when the ceruloplasmin concentration decreased to less than 1/3rd of the normal value. The administration of small amounts of ceruloplasmin (0.5-10% of that normally present) was followed by a prompt increase in plasma iron.

Based on the studies in vitro demonstrating that ceruloplasmin (ferroxidase) catalyzes the oxidation of iron (14) and on the observations that ferric but not ferrous iron is bound by apotransferrin (4), it was proposed that ceruloplasmin functions in iron metabolism by oxidizing iron, thereby enhancing the rate of transferrin formation.

This concept of the role of ceruloplasmin in iron metabolism was difficult to reconcile with observations in the rat. Rat ceruloplasmin, when studied at pH 6.7 in phosphate buffer in the presence of ascorbate, possessed very little ferroxidase activity (17). Therefore, the present study was undertaken to define the ferroxidase activity of rat plasma and purified rat ceruloplasmin under various assay conditions, to compare the ferroxidase activity of rat ceruloplasmin to the ferroxidase activity of porcine and human ceruloplasmin, and to ascertain if ceruloplasmin is required in the rat for the flow of iron from reticuloendothelial cells to transferrin.

MATERIALS AND METHODS

All blood specimens were collected in specially cleaned glassware (3) with heparin as an anticoagulant. Human apotransferrin was obtained from Certified Blood Service, Inc., Woodbury, N. Y. and dialyzed against 0.15 M sodium chloride before use (8). Porcine, rat, and human ceruloplasmin were prepared by a method reported elsewhere (13). The final preparations were dialyzed against 0.15 M sodium chloride before use. Plasma iron and copper were determined by atomic absorption spectroscopy after deproteinization by the method of Trinder (18). p-Phenylenediamine (pPD) oxidase activity was assayed by the method of Rubin (16), modified by the use of up to 1 ml of plasma when ceruloplasmin levels were low.

Ferroxidase activity was measured by the method of Johnson et al. (7). The reaction was carried out in sodium phosphate buffer, final concentration 0.0133 M, or in acetate buffer, final concentration 0.2 M, at the pH specified. The 1.0-ml reaction mixture consisted of 0.2 ml of appropriately diluted plasma (1:5-1:50), apotransferrin (30 μM), ferrous ammonium sulfate (30 μM), and buffer. Ascorbic acid, when used, was added to the iron solution to make a final concentration in the reaction mixture of 300 μM. The final concentration of sodium azide, when added, was 100 μM. After the addition of the iron or iron-ascorbate solution, the rate of increase in absorbance at 460 mμm was measured at room temperature in a Cary recording spectrophotometer, model 15. Determined values were corrected by subtracting the nonenzymatic rate of transferrin formation, which was measured in the appropriate buffer containing all the above agents except plasma. The nonenzymatic rate was approximately one-sixth the enzymatic rate.

Ceruloplasmin ferroxidase activity was calculated by subtracting the ferroxidase activity measured in the presence of azide from the ferroxidase activity measured in the absence of azide. Since only about 78% of the ferroxidase activity of ceruloplasmin is inhibited by 100 μM azide (9, 14, 19), this calculation gives an underestimate of the ceruloplasmin ferroxidase activity measured in plasma.

Copper deficiency was produced in weanling female rats by feeding a condensed milk diet low in copper. A similar diet has been used to produce copper deficiency in swine and is described elsewhere (10). All animals were given 5 mg of iron in the form of iron dextran (Imferon) intramuscularly daily for the first 6 days of the experiment.

RESULTS

Comparison of ferroxidase activity of rat plasma with that of porcine and human plasma. As judged by the plasma copper concentration and the pPD oxidase activity, the ceruloplasmin concentration of rat plasma was intermediate between pig and man (Table 1). However, the ceruloplasmin ferroxidase activity of rat plasma, when measured in phosphate buffer at pH 6.7 in the presence of ascorbate, was less than that of man and pig.
The PPD oxidase : copper ratio was similar in the plasma of all three species (Table 1). The ceruloplasmin ferroxidase : copper ratio and the ceruloplasmin ferroxidase : PPD oxidase ratio were greater in pig plasma than in the plasma from human subjects and lowest in the rat, indicating the relative ferroxidase activities per unit of ceruloplasmin when measured at pH 6.7 in phosphate buffer in the presence of ascorbate.

Ferroxidase activity of purified ceruloplasmins. The ferroxidase activity of purified ceruloplasmin from all three species was studied as a function of pH in phosphate and in acetate buffer (6). The effect of added ascorbate was also assessed in these buffers.

Comparison of the ferroxidase activity of the three ceruloplasmins when measured under optimal assay conditions is given in Table 2.

The ferroxidase activity of porcine ceruloplasmin was greatest at pH 6.8 in acetate buffer in the absence of ascorbate. Maximal ferroxidase activity of human ceruloplasmin was observed at pH 6.8 in phosphate buffer in the presence of 300 μM ascorbate. The ferroxidase activity of rat ceruloplasmin was greatest at pH 6.0–6.2 in acetate buffer without ascorbate.

Rat ceruloplasmin had more PPD oxidase activity per microgram of copper than either human or porcine ceruloplasmin. However, the ferroxidase activity of rat ceruloplasmin was about 1/3rd that of human ceruloplasmin and 1/10th that of porcine ceruloplasmin.

Role of ceruloplasmin in iron metabolism in copper-deficient rats. If ceruloplasmin is required for the optimal movement of iron from reticuloendothelial cells to transferrin in the rat, as it is in the pig (17), it follows that 1) hypoferremia should develop during the course of copper deficiency in the rat, 2) hypoceruloplasminemia should precede the development of hypoferremia, and 3) the administration of ceruloplasmin should be followed by a prompt increase in plasma iron. Accordingly, copper deficiency was induced in rats and these events were monitored.

The PPD oxidase activity and the concentration of iron and copper in the plasma of rats during the development of copper deficiency are shown in Fig. 1. The plasma iron remained above 200 μg/100 ml for the first 36 days on the deficient diet and then decreased to 133 μg/100 ml over the next 14 days. The decline in ceruloplasmin concentration, as measured by the PPD oxidase activity and the plasma copper concentration, preceded the development of hypoferremia. On the 22nd day of the copper-deficient diet, at which time the mean plasma iron value was 227 μg/100 ml, the mean plasma copper was 9% of the control value (11 μg/124 μg). The PPD oxidase activity was 1.2% of the control value (.0042/.360). On the 36th day of the experiment, at which time the plasma iron was 235 μg/100 ml, the plasma copper was 7% of the control value (9 μg/124 μg) and the PPD oxidase activity was 0.7% of the control value (.0027/.360). Thus, as in the pig (17), the flow of iron was not impaired until after the PPD oxidase activity decreased to less than 1% of the normal value.

Purified rat ceruloplasmin was infused rapidly intravenously into a group of copper-deficient rats. Purified pig ceruloplasmin was infused into a second group of animals. The amount of ceruloplasmin PPD oxidase activity infused was calculated to represent 20% of the amount in the circulation of the normal rat. Physiologic saline was infused into a third group of animals as a control, and copper in the form of copper sulfate was infused intravenously into a fourth group of rats. The amount of copper infused was calculated to be the same as the amount of copper contained in the ceruloplasmin which was infused into the first two groups. The animals in all four groups were sacrificed by exsanguination 3 h after the completion of the infusion, and plasma was obtained for the measurement of iron, copper,
DISCUSSION

As previously reported (17), the ceruloplasmin ferroxidase activity of rat plasma is negligible as compared with human and porcine plasma when measured at pH 6.7 in phosphate buffer in the presence of 300 μM ascorbate. The ferroxidase activity of rat ceruloplasmin is maximal at pH 6.0-6.2 in acetate buffer in the absence of ascorbate. Under these conditions, the ferroxidase activity is about 1/3rd that of human ceruloplasmin and 1/10th that of porcine ceruloplasmin.

In spite of the relatively low iron-oxidizing activity of rat ceruloplasmin, hypoceruloplasminemic rats developed hipoferremia, and the administration of ceruloplasmin was followed by a prompt increase in plasma iron. As in the pig (17), hypoferremia did not develop until after the ceruloplasmin concentration had been reduced to less than 1% of the normal concentration. Thus, ceruloplasmin is essential for the flow of iron from reticuloendothelial cells to transferrin in the rat as well as in the pig.

The manner in which ceruloplasmin functions in the

and pH oxidation activity. The plasma iron was appreciably greater in the two groups receiving ceruloplasmin than in saline- and copper-injected control groups (Table 3), indicating that the ceruloplasmin mobilized iron into the plasma.

REFERENCES


