Ferrioxamine Iron in Rabbit Reticulocyte  
Heme Synthesis

By M. D. GERLANC, E. J. ZAPOLSKJ, M. Rubin AND J. V. PRINCIOTTO

DESFERRIOXAMINE, the sideramine of the Actinomyces, is a potent chelator of ferric iron. It is believed that all aerobic organisms produce sideramines which function as specific iron donors (coenzymes) for the iron incorporating enzyme of the heme synthesis. In light of the role of ferrioxamines in heme synthesis in microorganisms, the present study was undertaken to investigate the ability of ferrioxamine to provide iron for heme incorporation by rabbit reticulocytes.

Ferrioxamine and three of its derivatives used in this study were supplied as the iron chelates.* The iron was removed and the free ligands prepared. Iron chelate was reformed in Ringer's solution by the addition of ferric chloride and $^{59}\text{FeCl}_3$, and the pH was adjusted to 7.4. The solutions so prepared were 15 μM with respect to iron and 30, 75 and 150 μM with respect to ligand, yielding ligand : iron ratios of 2 : 1, 5 : 1 and 10 : 1. Prior to use, fresh rabbit plasma was incubated with tracer $^{59}\text{FeCl}_3$.

Rabbit reticulocytes were obtained and used as we described in an earlier publication. Total radioiron incorporation by these reticulocyte suspensions, as well as hemolysate and stromal radioiron were determined in a well-type scintillation counter. In addition, radioiron in the hemolysate fraction was investigated after electrophoretic separation upon cellulose acetate strips which were air dried and scanned for radioactivity with a 4π radiochromogram scanner. After three washes with ice cold water and counting, stroma was further washed with 0.5 M EDTA (pH 7.4) and recounted.

The data obtained from this study are presented in Fig. 1. Total iron uptake from ferrioxamine B was equivalent to that demonstrated by plasma-bound iron but significantly less radioiron was present in the cell hemolysate fraction. Two hours after incubation of the reticulocytes with ferrioxamine, the stroma contained 82–90 per cent of the totally accumulated iron, whereas stromal...
For each compound, percentage of iron incorporated by reticulocytes presented at 2:1, 5:1 and 10:1 ligand:iron ratios (15 μM Fe²⁺). Shaded area of each bar represents stromal accumulation; solid area, hemolysate accumulation.

Accumulation from plasma-bound iron was 23 per cent of the total. Each of the ferrioxamine derivatives uniformly demonstrated a very high stromal accumulation, which was unaffected by increasing ligand concentrations. Attempts to remove this iron by washing the stroma with 0.5 M EDTA only removed 20–25 per cent of the iron in these membranes.

Radioscan of cellulose acetate strips after electrophoresis of hemolysate samples from each of the compounds studied indicated that the radioiron migrated with the hemoglobin moiety and was incorporated into heme. No activity was observed at the origin of these radioscans, the locus of ferrioxamine and its derivatives under the conditions of the separation. The ferrioxamine derivatives were able to increase hemolysate heme-iron levels two-fold compared to ferrioxamine B, but were yet only one third as effective as transferrin in this respect.

The present evidence that ferrioxamine iron is poorly available for heme synthesis in vitro for rabbit reticulocytes supports inferences from clinical studies. Desferrioxamine is used in the therapy of hemachromatosis and acute iron poisoning. In these applications it does not modify red cell iron incorporation from ⁵⁹Fe transferrin, nor does the mobilized iron alter hematologic indices.

Modification of the molecular structure by acylation of the terminal amine reduces ionic charge and increases its lipophilic character. Changes of this kind have been shown to increase membrane permeability of synthetic amino acid iron chelates and shift their in vivo excretion from the renal to the biliary route. Analogous results have been reported for N-benzoylation of ferrioxamine. The increase in reticulocyte membrane deposition consequent to the
lipophyllic derivatization of ferrioxamine (Fig. 1) must occur by direct transport of the chelate-bound iron to the cell membrane. The membrane-bound iron was neither eluted by EDTA wash as in the case of ferric ion attachment, nor was its deposition significantly decreased with an increase in the ligand:iron ratio from 2:1 to 10:1. The absence of ferrioxamine-bound iron in the hemolysate indicates that the intact iron chelate did not traverse the reticulocyte cell membrane. This suggests that the limited hemolysate iron observed after incubation with ferrioxamine increased somewhat with the ferrioxamine derivatives, may occur by a direct though inefficient transfer of the metal via membrane bound transferrin. This finding is in accord with in vivo studies which show that red cell incorporation of ferrioxamine iron is only one-third as efficient as iron derived from transferrin.

**SUMMARY**

Ferrioxamine B, a naturally occurring sideramine, serves as an iron transport agent and iron donor for heme synthesis in microorganisms. It is unable to effectively replace transferrin in iron delivery for rabbit reticulocyte hemo-globin formation.

**REFERENCES**
