CITRATE-MEDIATED EXCHANGE OF Fe^{3+} AMONG TRANSFERRIN MOLECULES

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Under physiologic conditions of pH and CO_2 tension, the apparent equilibrium constant for the overall reaction

$$Fe^{3+} + \text{Transferrin} \rightleftarrows \text{Fe Transferrin}$$

is approximately 5×10^{23} M^{-1} (Aasa, Malmström, Saltman and Vänngård, 1963). Even assuming that the forward kinetic constant is that expected of a diffusion-controlled reaction in which there may be forces between interacting particles - about 10^{10} M^{-1} sec^{-1} (Eigen and Hammes, 1963; Friedman, 1966) - the constant calculated for the back reaction cannot exceed 10^{-13} sec^{-1}. This would require that the time for release of an iron atom from transferrin would approach 10^4 years.

Accordingly, the mechanism for release of iron from transferrin in vivo cannot proceed by a simple diffusion-limited pathway.

Saltman (1965) has called attention to the probable role of small molecular weight chelating agents in the physiologic transport of iron. Ternary complexes of transferrin, Fe^{3+} and a variety of small molecular weight chelating agents have been demonstrated (Aisen, Aasa, Malmström and Vänngård, 1967; Bates, Billups and Saltman, 1967). The ability of one such agent, citrate, to act as a mediator promoting a rapid exchange of Fe^{3+} between transferrin molecules under physiological conditions is reported in this communication.

Materials and methods - Human transferrin was isolated and purified by methods previously described (Aisen, Leibman and Reich, 1966). Asialotransferrin was prepared by incubation of 375 mg of transferrin and 1000 units of V. cholerae neuraminadase (Sigma) in a total volume of 25 ml for 48 hours at 37°, followed by chromatographic purification on a DEAE Sephadex column. This procedure resulted in isolation of asialotransferrin free of neuraminadase. The resulting preparation contained less than .02 residues of neuraminic acid per molecule of transferrin, by the methods of Jamieson (1966). The optical absorption spectra and iron-binding capacity of the asialotransferrin were identical to those of untreated protein. Asialotransferrin has also been shown to be the same as transferrin in its ability to serve as an Fe^{3+} source for heme synthesis in the reticulocyte (Kornfield, 1968).

Mixtures of the native and enzyme-treated proteins, each at the same concentration (near 1%) but only one of which was labelled with ^{59}Fe, were incubated in the presence and absence of 10^{-3} M citrate for 24 hours at 37°. Following incubation, the asialo-and native forms of transferrin were separated from each other by chromatography on DEAE-Sephadex. The radio-activity of the column eluates was determined in a scintillation counter equipped with pulse-height analyzer. Figure 1 illustrates the efficiency of the separation procedure as well as the absence of exchange at physiological pH when no complexing agent for Fe^{3+} is present.

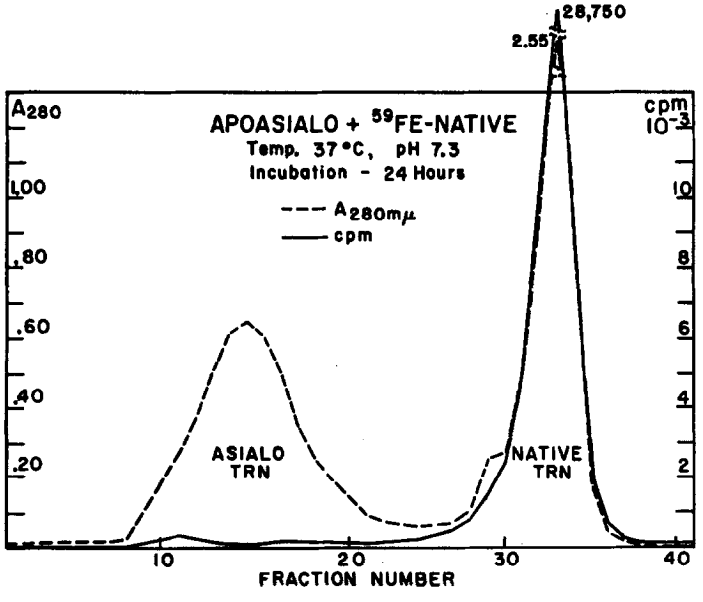

Fig. 1 Apoasialotransferrin and ^{59}Fe-labelled transferrin, pH 7.3, incubated 24 hours at 37° C and separated by column chromatography in DEAE Sephadex.

Results and discussion - At pH 7.4, in the absence of a complexing agent for Fe^{3+} as mediator, there is no detectable exchange of iron between transferrin molecules (Fig. 1). This is true whether the non-labelled protein is iron-saturated or iron-free. However, in the presence of 10^{-3} M citrate, a concentration comparable to that present in circulating blood, there is relatively rapid and complete exchange of iron between native and enzyme-treated transferrin (Fig. 2). This exchange occurs when the initially labelled protein is untreated or in the asialoform, and when the non-labelled protein is iron-saturated or iron-free. When the concentration of citrate is as low as 10^{-5} M, virtually complete exchange is evident after 5 days of incubation at 25° C.

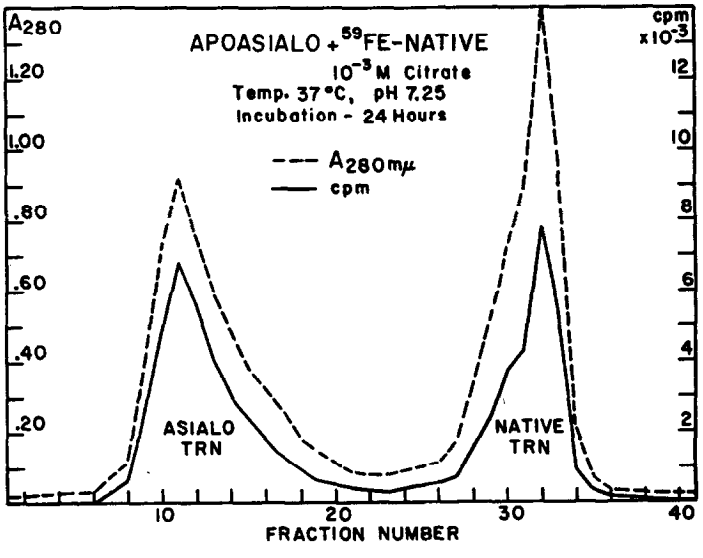

Fig. 2 Same as Fig. 1, except that 10^{-3} M citrate included in incubation mixture.

When a 10^{4} M solution of transferrin, saturated with Fe^{3+}, is incubated with 10^{-3} M citrate under the conditions described above, less than 5% of the absorbancy at 470 mμ is lost. It is clear, therefore, that at any given time the fraction of total Fe^{3+} bound to citrate in the exchange experiments is negligible, so that citrate is functioning as a true mediating agent for Fe^{3+} transfer between transferrin molecules.

At pH 6.0, significant exchange is observed even in the absence of a complexing agent for Fe^{3+} (Fig. 3). Based on

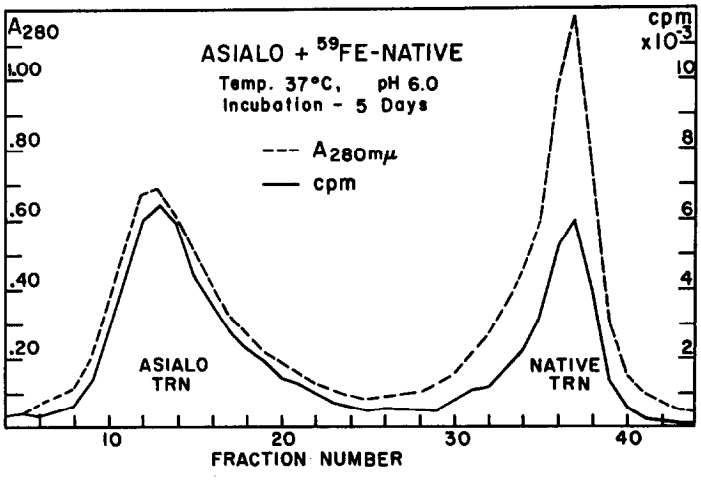

Fig. 3 Asialotransferrin and ^{59}Fe-labelled transferrin incubated at pH 6.0 for 5 days at 25° C, and separated by chromatography on DEAE Sephadex.

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studies done at higher pH, the apparent equilibrium constant for the association of an Fe^{3+} ion with a transferrin molecule is about 5×10^{15} at this pH (Aasa et al., 1963). Since little or no exchange would be effected with a binding constant of this

magnitude, the observed exchange may well be explained by a conformational change in the binding sites at low pH (Aasa and Aisen, 1968), so that the binding constants measured at higher pH do not adequately account for the equilibria at low pH (Aisen and Leibman, 1968).

The present results also provide an additional simple explanation for observations reported by Morgan, Marsaglia, Giblett and Finch (1967). These investigators noted in vivo exchange of Fe^{3+} between asialotransferrin and native transferrin, and attributed this exchange to reflux of Fe^{3+} bound to reticulocytes. It may be, however, that part or all of the exchange observed occurred in the plasma by a citrate- (or other Fe^{3+} - complexing agent) mediated transfer similar to that described in this report.

A likely mechanism for exchange involves formation of a ternary complex of Fe^{3+}, transferrin and citrate (Bates, Billup and Saltman, 1967), possibly by displacement of bicarbonate by citrate, and subsequent release of a molecule of the Fe-citrate complex which is known to function as an effective donor of Fe^{3+} to the binding sites of transferrin (Bates, Billup and Saltman, 1967).

It is not clear from the present experiments whether the uptake of Fe^{3+} from transferrin by reticulocytes engaged in the biosynthesis of heme actually proceeds by a chelate-mediated pathway. The trypsin-removable receptor at the reticulocyte surface may be involved in the transfer of iron as well as in the

binding of transferrin to the reticulocyte membrane (Jandl, Inman, Simmons and Allen, 1959). Furthermore, EDTA does not appreciably impede specific iron uptake by reticulocytes (Jandl et al., 1959). Nevertheless, the possible involvement of chelating agents in the transfer of iron to and from transferrin corroborated by the present observations.

REFERENCES

Aasa, R., and Aisen, P., J. Biol. Chem. 243, 2399 (1968).

Aasa, R., Malmström, B. G., Saltman, P., and Vänngård, T., Biochem. et Biophys. Acta, 75, 203 (1963).

Aisen, P., Aasa, R., Malmström, B.G., and Vänngård, T., Biol. Chem., 242, 2484 (1967).

Aisen, P., and Leibman, A., Biochem. Biophys. Res. Comm. 30, 407 (1968).

Aisen, P., Leibman, A., and Reich, H.A., J. Biol. Chem. 241, 1666 (1966).

Bates, G. W., Billups, C., and Saltman, P., J. Biol. Chem. 242, 2810 (1967).

Eigen, M., and Hammes, G.G., Adv. Enzymol., 25, 1 (1963).

Friedman, H. L., J. Phys. Chem. 70, 3931 (1966).

Jamieson, G.A., Biochem. Biophys. Acta, 121, 326 (1966).

Jandl, J., Inman, J. K., Simmons, R. L., and Allen, D. W., J. Clin. Invest., 38, 161 (1959).

Kornfield S., Biochemistry 7, 945 (1968).

Morgan, E. H., Marsaglia, G., Giblett, E. R., and Finch, C.A., J. Lab. and Clin. Med., 69, 370 (1967).

Saltman, P., J. Chem. Ed., 42, 682 (1965).