Transferrin: Intermolecular Iron Exchange

Current concepts of internal iron exchange visualize transferrin (Tr) as the only iron carrier, provided with two functionally identical metal-binding sites. Moreover, the high association constant of Tr to iron is thought to prevent any movement of iron between Tr molecules[1]. Regulation of iron transport is little understood, however, and comprehension of the mechanisms involved must appreciate possible functional heterogenity of the transport compartment. Recently, evidence was produced that Tr molecules carrying 2 iron atoms are superior in iron-donating activity to reticulocytes compared to the mono-iron species[2]. Based on this observation, a system was developed to demonstrate iron exchange among Tr molecules.

Equal amounts of $^{59}FeCl_3$ (200 µg Fe^{+++}/ml; 10 µCi/µg; Institut für Reaktorforschung, CH-5303-Würenlingen) were added to either 1.0 ml (I) or 20.0 ml (II) of pooled sideropenic rat serum raising the Tr saturation level of the former aliquot to 90%. By small additions of 1 *N* NaOH, the pH was kept continuously at 7.4. After 30 min of incubation at 37 °C, 19 ml of the original serum were admixtured to the 1.0 ml sample. Immediately thereafter, 1.0 ml aliquots of both preparations were frozen. At this time serum I contained a small proportion of di-^{59}Fe-Tr and a large excess of apotransferrin, while in serum II the mono-^{59}Fe-Tr species largely predominated[3]. Subsequently these samples were incubated at 37 °C and pH 7.4 for time periods between 96 h and a few min and then simultaneously exposed to equal volumes of washed packed reticulocytes obtained from bled rats. Then 6 min after reticulocyte admixture, cellular radioactivity was determined.

At the beginning of the experiment serum I was more than twice as potent in iron-donating capacity as serum II (Figure). Through the decreasing activity of serum I, the initial difference was halved after 12 h, and diminished progressively as preincubation periods increased. This finding is best explained by a redistribution of iron atoms among Tr-molecules. At the functional level, serum I was mainly affected because di-^{59}Fe-Tr molecules gradually disappeared due to transformation into the mono-^{59}Fe-type. The possibility that cellular uptake of radioiron unbound to Tr caused the difference between the two preparations was excluded by measuring the proportion of whole cell radioactivity appearing in heme[4]. After a 6 min exposure of reticulocytes to freshly prepared serum, it amounted to 78 and 85% for preparation I and II respectively, while 20.9 and 11.7% of original serum activity had been taken up by cells. It is well documented that significant quantities of Tr-free iron do not reach the heme moiety[5].

At 2 °C, and in serum dialyzed against bicarbonate buffered saline, iron exchange did not occur. It was restored by addition of 12×10^{-4} *M* citrate, pointing to a role of low molecular weight compounds as mediators of iron exchange. Citrate, in fact, removes iron from transferrin in greater amounts than expected from the stability constant[6], and the transfer of ferric iron from citrate to apotransferrin in an isomolar solution is a slow process terminated after 20 h only[7]. Further, citrate mediated exchange of iron among Tr molecules has been postulated on the basis of experiments using Tr and asialo-Tr separated by column chromatography[8]. Finally, ultracentrifugation studies revealed the presence of a small low molecular weight fraction in normal serum binding ferric iron most likely identical with citrate[9].

Evidence is accumulating that the two Tr iron binding sites are structurally different[10], and functional asymmetry has been unequivocally demonstrated in vitro[2], but contraversial findings exist in vivo[11,12]. This discrepancy might in part be explained by continuous intraplasmatic iron redistribution, possibly taking place at a faster rate in vivo than in the experiments reported here.

Zusammenfassung. Die Eisenabgabefähigkeit doppelt beladener Transferrinmoleküle an Retikulozyten, ist jener einfach beladener Moleküle überlegen, und vermindert sich rasch wenn Serum unter physiologischen Bedingungen inkubiert wird. Als Erklärung wird die Neuverteilung von Eisenatomen unter den Transferrinbindungsstellen postuliert[14].

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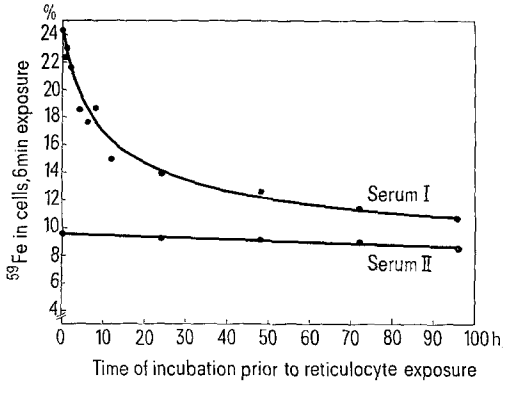

Changes in radioiron donating capacity to reticulocytes of 2 serums identical in ^{56}Fe (cold) concentration, total iron binding capacity (35 and 816 µg/100 ml respectively[13]), and ^{59}Fe content (37 µg/100 ml) over various time periods of preincubation at 37 °C under 95% air + 5% CO_2 (pH 7.4). At O-time serum I contained mainly di-^{59}Fe-Tr in presence of an excess of apotransferrin, while in serum II the mono-^{59}Fe-Tr species predominated.

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