the converse is true, possibly because of the aromatic character of enterobactin and its propensity to adhere to proteins such as albumin.

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The Two-Sited Nature of Transferrin: Spectroscopic, Thermodynamic and Physiologic Studies

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The transferrins comprise a class of two-sited metal-binding proteins widely distributed in cells and fluids of vertebrates. Each of the major transferrin varieties—serum transferrin, ovotransferrin (conalbumin) and lactoferrin (lactotransferrin)—consists of a single polypeptide chain with extensive internal homology indicating evolutionary origin from a single-sited precursor protein. In human transferrin and hen ovotransferrin, at least, evolutionary development has altered the two sites so that they are chemically and spectroscopically distinguishable, but whether the differences between the sites are physiologically important is not yet clear.

The metal-binding activities of all transferrins are dependent on the concomitant binding of structurally-suitable anions. In human serum transferrin, anion- and metal-binding activities alike vary between the two sites. For example, the sites may be distinguished by the thightness with which they bind ion, by their accessibility to different chemical forms of iron, by their vulnerability to acid, by the rates at which they exchange anions with the medium, by their capacity to accommodate different anions when Cu(II) is the bound metal ion, and by their spectroscopic features. When single-sited transferrin fragments are prepared by selective proteolytic digestion, the tightness of ion-binding by these fragments is virtually identical to that of corresponding sites in the holoprotein. Evidently, therefore, site-site interactions are not responsible for the observed differences between the sites in native transferrin.

Because each of the sites of transferrin resides in its own independent domain, and empty-sited domains are more susceptible to denaturation than occupied domains, each of the four possible molecular species of transferrin (apoprotein, monoferric-A and monoferric-B proteins, and differic protein) can be separated and displayed by electrophoresis in a 6M urea-gel system introduced by Makey and Seal. This procedure has been exploited to study the physiological iron-exchanging properties of the sites, *in vitro* and *in vivo*. In studies conducted in our laboratory, the two sites of diferric transferrin differ considerably in their ability to donate iron to isolated hemoglobin-synthesizing reticulocytes in an artificial culture medium. In *in vivo* studies in the rabbit, however, the two iron atoms are removed from diferric transferrin in pairwise fashion, so that the sites now appear equivalent in their iron-donating properties. Furthermore, iron, from stores and sites where it is absorbed or recovered from senescent red blood cells, is returned to empty sites of circulating transferrin, predominantly one atom at a time. Since studies of iron distribution among the sites of circulating transferrin in normal human subjects indicate that the sites are unequally occupied, with the more weakly binding site of the N-terminal half of the molecule favored, it seemed that the sites might differ not in their iron-donating abilities, but in their iron-accepting properties. Accordingly, the distribution of absorbed radioactive iron between the two sites of circulating transferrin was studied in the rabbit. In four of five animals the B-site was strongly favored by the newly absorbed iron. This may account, then, for the unequal distribution of iron between the sites of circulating transferrin. If the chemical differences between the two sites of transferrin are mapped into differences in function, therefore, it is probably on the 'supply side' of the iron economy.

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