Annotation

THE ROLE OF TRANSFERRIN IN IRON TRANSPORT

The Need for a Physiological Iron Carrier

At the pH and oxygen tension of most physiological fluids, including blood, the kinetically-favoured form of iron is Fe(III). The solubility of ferric hydroxide, however, is such that the equilibrium concentration of Fe(III) in blood could not exceed \(10^{-16}\) M. Since the turnover of iron in man approximates 30 mg per day, or 0.5 millimoles, the need for a specific carrier to maintain iron in soluble form and accommodate this traffic is clear. Transferrin, the iron-binding protein of blood pungingly named by the Swedish investigators Holmberg & Laurell (1947), meets this need.

Perhaps an even more critical function than transport is carried out by transferrin. As shown by Jandl et al. (1959), the protein specifically 'recognizes' the haemoglobin-synthesizing reticulocyte and, possibly, other iron-requiring cells. This recognition function assures delivery of iron only where there is a specific requirement for the metal. A dramatic indication of the importance of transferrin in regulating the metabolism of iron is shown by the effects observed when transferrin is absent from blood plasma. Several patients with congenital transferrinaemia have been studied in detail (Goya et al, 1972). The defect is one of failure to synthesize transferrin, since the survival time of infused labelled transferrin is normal. These patients suffer from the paradoxical coexistence of an anaemia resembling that of iron deficiency and a generalized iron-overload, presumably the consequence of repeated transfusions, but possibly also a failure in the regulation of iron metabolism due to the absence of transferrin. The anaemia is hypochromic and associated with a low concentration of serum iron, but does not respond to administration of iron. In one subject studied, intravenous administration of transferrin resulted in a significant improvement in haematologic parameters. Without transferrin, it appears that neither the delivery of iron to haemoglobin-synthesizing sites, nor its mobilization from stores, is successfully controlled.

Physiological Properties of Transferrin

Transferrin is readily isolated in highly purified form from whole blood plasma or from Cohn Fraction IV-7. The transferrin molecule has a molecular weight in the range 76 000-80 000 (Greene & Feeney, 1968; Mann et al, 1970) and consists of a single polypeptide chain on which are disposed two remarkably similar, if not identical, metal-binding sites (Aisen, 1973). These sites will accommodate a variety of transition metal ions but iron, as Fe(III), is by far the most tightly bound and will displace other metal ions. The binding of iron confers a characteristic salmon-pink colour to the transferrin molecule. For each Fe(III) bound a suitable anion must also be bound. The 'co-operativity' between metal- and anion-binding is complete: neither is bound in the absence of the other (Aisen et al, 1967; Price & Gibson, 1972a). When it is available, bicarbonate is the anion preferentially bound. If care is taken to exclude bicarbonate from the reaction mixtures, ternary complexes of Fe(III), transferrin and cations may be prepared. These are best recognized by their distinctive electron paramagnetic resonance spectra, since differences among their optical spectra may be small.
Upon the addition of bicarbonate they will revert to the Fe(III)-transferrin-bicarbonate complex at rates which depend on the particular anion in the ternary complex.

About 6% of transferrin consists of carbohydrate residues, arranged in two identical branched chains terminating in sialic acid (Jamieson et al, 1971). The carbohydrate seems to have no function in iron-binding or transfer of iron to the reticulocyte (Kornfeld, 1968). Unlike what is observed with other serum glycoproteins, enzymatic cleavage of the sialic acid residues does not affect the average survival time of transferrin in the circulation (Morell et al, 1971).

At the pH and bicarbonate concentration of blood, the apparent stability constant for the binding of Fe(III) to either site of transferrin is about $10^{33}$ M$^{-1}$. Such an extraordinary large binding constant is required if transferrin is to resist losing its iron to the competing reaction of ferric hydroxide formation. It implies, though, that a specific physiological mechanism must exist for removing iron from transferrin, since the protein is conserved and recycled in its interaction with reticulocytes and other tissues, and spontaneous dissociation of iron is excluded by the large binding constant (Aisen & Leibman, 1968).

Are the Binding Sites Identical in Structure and Function?

It is only natural that considerable attention should have been directed to this question in a two-sited protein with measurable biological activity. The first studies of the Fe(III)-binding properties of transferrin, by equilibrium dialysis and free electrophoresis, indicated that the binding sites were equivalent and non-interacting (Aasa et al, 1963). In 1967, however, Fletcher & Huehns reported that the sites did not appear to be equally effective in their ability to donate iron to the reticulocyte. These investigators further suggested that one of the sites might function preferentially in iron donation to the reticulocyte, and the other in iron transport across the placenta (Fletcher & Huehns, 1968). Recently, a preliminary report by Awai, Chipman & Brown appears to confirm the Fletcher-Huehns hypothesis in all essential respects (Awai et al, 1972). From the physicochemical viewpoint, differences between the sites are clearly demonstrable by electron paramagnetic resonance spectroscopy in Fe(III)-transferrin in the presence of 0.4 M perchlorate (Price & Gibson, 1972b), in Cr(III)-transferrin (Aisen et al, 1969), and, it has been argued, even when Fe(III) is bound under physiological conditions (Aasa, 1972). Whether these observations provide a structural basis for the Fletcher-Huehns effect remains to be determined. If there is a structural difference between the sites, it is surprising that the binding constants appear to be so similar. It is also remarkable that in conalbumin (ovotransferrin), where a structural difference between the sites appears well established (Aisen et al, 1973), a functional heterogeneity is not demonstrable (Williams & Woodworth, 1973). Since crystals of transferrin are readily prepared, and the techniques of protein X-ray crystallography are continually being improved, it seems likely that the structure of the binding sites will become available in the foreseeable future.

The Role of Transferrin in the Absorption of Iron

Unfortunately, the data bearing on this fundamental question are conflicting. Research by Wheby and his co-workers, using rats (Wheby & Jones, 1963) and human volunteers (Wheby & Umpierre, 1964), failed to indicate any role for the level of transferrin saturation in regulating the absorption of iron from the gut. However, one critical effect was observed:
when circulating transferrin was nearly saturated with iron, most of the iron absorbed was deposited in the liver. If unsaturated transferrin was available to bind absorbed iron, this did not occur. The function of transferrin, from these studies, would appear to be limited to the transport of absorbed iron to cells requiring it for the biosynthesis of haemoglobin and other iron-proteins. This view appears consistent with the defects observed in atransferrinaemia.

An additional role for transferrin, which seems to be at variance with the foregoing, is suggested by other, in vitro studies (Levine et al, 1972). Release of orally administered iron from proximal intestinal epithelial cells to an incubation medium was substantially increased when transferrin was present. Epithelial cells from iron-deficient animals released no more iron than did normal cells. The nonbiologic iron chelator desferrioxamine had no effect in these experiments. In contrast to what is observed with reticulocytes, the affinity of $^{125}$I-tagged transferrin for isolated intestinal epithelial cells was greater when the protein was almost devoid of iron than when it was saturated. From this work, it appears that transferrin may also serve to regulate the transfer of ingested iron from the labile intestinal epithelial pool to the plasma. Clearly, further studies are needed to establish whether this is indeed the case.

The Anion-Binding Site of Transferrin and the Transferrin–Reticulocyte Interaction

Because of its ready availability, the reticulocyte has been widely used as a model for the interaction of transferrin with the erythron. It is conceivable that marrow cells behave differently than reticulocytes, and this possibility should be considered in what follows.

Morgan (1964) has suggested that the sequence by which transferrin relinquishes its iron to the reticulocyte for the biosynthesis of haemoglobin entails four steps:

1. Physical adsorption of transferrin to receptor sites on the surface of the reticulocyte.
2. Formation of a firmer union between transferrin and the reticulocyte. This step, which is time and temperature dependent, may involve actual penetration of the protein into the cell (Morgan & Appleton, 1969), but the detailed fate of the transferrin molecule during its sojourn with the reticulocyte is for the most part unknown.
3. Transfer of iron from protein to the haemoglobin synthesizing apparatus of the cell.
4. Release of protein to the circulation. This step probably depends, at least in part, on the fact that transferrin devoid of iron binds much more weakly to the reticulocyte than does the metal-bearing protein. Interestingly, the opposite seems to obtain in the intestinal mucosal cell, as indicated above (Levine et al, 1972).

Because of the interdependence of anion and iron binding by transferrin, research in several laboratories has converged on the role of the anion-binding site of transferrin during its interaction with the reticulocyte (Aisen & Leibman, 1973; Egyed, 1973; Williams & Woodworth, 1973). Comparisons were made of the behaviour of Fe-transferrin–bicarbonate and Fe-transferrin–oxalate. The oxalate complex was chosen because it is relatively easy to prepare and comparatively stable, reverting to the bicarbonate form slowly over the course of many hours even when bicarbonate is available to displace the oxalate, as it must be in a physiological experiment. In one experiment, the ability of rabbit reticulocytes to take up $^{59}$Fe from the two transferrin complexes was measured. After an incubation of 1 hr, 5.0 $\mu$g of Fe per ml of reticulocytes was taken up from the oxalate complex. Much of the uptake
observed with the oxalate preparation may have been due to the formation of a small amount of Fe-transferrin–bicarbonate, so that the true uptake from the oxalate complex is even less than that measured. Once taken up by the reticulocyte, the iron from either form of the protein is readily incorporated into haem.

When reticulocytes are incubated with an equimolar mixture of Fe-transferrin–bicarbonate and Fe-transferrin–oxalate, the uptake of iron from the bicarbonate form of the protein is half of what is observed in the absence of the oxalate complex. A control experiment showed that this effect could not have been due to inhibition of reticulocyte function by free oxalate. It seems reasonable to suppose, therefore, that the oxalate and bicarbonate forms of transferrin compete on an equal basis for the reticulocyte receptors. The oxalate complex, when occupying a receptor site, prevents an Fe-transferrin–bicarbonate molecule from taking its place there, but does not itself function as an iron donor. Further evidence for this hypothesis was provided by measuring the binding of 125I-labelled proteins to reticulocytes (Aisen & Leibman, 1973). No appreciable difference was detected in the binding behaviour of Fe-transferrin–oxalate and Fe-transferrin–bicarbonate. The relative inability of the reticulocyte to utilize iron from the oxalate complex, then, is probably due to an inability to transfer iron from protein already bound to the reticulocyte. The suggestion was therefore offered that the iron-releasing mechanism of the reticulocyte involves an attack on the anion-binding site of the protein. When bicarbonate occupies the site, it is removed by the reticulocyte, following which the iron–protein bond is readily broken. When the site is occupied by oxalate, the reticulocyte is unable to remove this anion, and the iron–protein bond remains stable. Conceivably, the obverse of this mechanism operates to facilitate the binding of iron from storage sites by transferrin. It may be, then, that the mechanism by which transferrin operates in the regulation of iron metabolism depends on the ‘co-operativity’ between its metal- and anion-binding sites.

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