# TRANSFERRINS IN THE METABOLISM OF IRON

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UDC 577.1.112.854

The main physicochemical characteristics of the transferrins, their physiological role, and questions of reticulocyte-transferrin interaction and the biosynthesis of transferrin are discussed.

Transferrins occupy a special position in the processes of the metabolism of a microelement of vital importance for the organism — iron. They belong to the so-called minor proteins of the blood serum, their level in the blood being about 0.3%, but the role of these proteins in the organism is incomparably higher than their quantitative amount. The main function of the transferrins consists in the transport of iron into the reticulocytes where the biosynthesis of hemoglobin takes place. This does not exhaust the value of the transferrins for the organism. They apparently form a kind of buffer for iron. How important this function of the transferrins is can be seen from the case of a transferrinemia patients who completely or partially lack this protein in the blood. In atransferrinemia, iron is deposited in large amounts in the tissues of the organism, which leads to poisoning by it [1].

Transferrins form a class of single-chain glycoproteins capable of reversibly binding ferric iron ions and also some other metal ions, for which there are two specific binding sites in the protein molecule [2, 3]. Transferrins are extremely widespread in the physiological fluids and cells of vertebrates [2]. The best-studied transferrin is that of the blood serum, sometimes called serotransferrin or siderophyllin. Lactotransferrin or lactoferrin has been detected in milk, the lachrymal secretions, and in leucocytes [4, 5]. Ovotransferrin or conalbumin is found in large amounts in birds' eggs [2].

Transferrins form one of the most polymorphic systems of blood serum protein. In man, no fewer than 17 alleles present in an autosomic two-allele system controlled by a single locus have been found [6].

Interest in the study of the structural features of transferrin, the mechanisms of its biosynthesis, and its physiological function as a carrier of ferric ions is due to the fact that a deficiency of this protein in the blood or a disturbance of the mechanism of its biosynthesis causes disturbances in the hemopoietic system and this, in its turn, leads to the development of various types of iron-deficiency anemias [7]. In hereditary hemochromatosis in man a fundamental, although possibly not the primary, link in the pathogenesis of disturbances to the iron metabolism is a disturbance of the processes of the binding of iron to transferrin [8]. There are hereditary atransferrinanemias with a pronounced quantitative inadequacy of the synthesis of this protein [7-9]. The participation of so-called lactotransferrins in antimicrobial and antiviral immunity [4] is also arousing interest in this protein. Furthermore, with some tumoral diseases [10], chronic hepatitis and sclerosis of the liver [11], meningitides of viral and bacterial origin [12], and disseminated sclerosis [13] the determination of the level of transferrins in the blood is of undoubted diagnostic value.

In this review we have attempted to generalize modern information relating to the physiological role of the transferrins, their properties and their structural features, and questions of their biosynthesis and of the transferrin—reticulocyte interaction.

## PHYSIOLOGICAL ROLE OF TRANSFERRIN

The fact that iron is necessary for the maintenance of the normal vital activity of the organism was convincingly demonstrated as long as three centuries ago. Physiologists differ somewhat in their evaluations of the amount of iron in the human organism. It is considered that the organism of an adult man normally contains approximately 3 to 4 g of this metal,

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Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 139-151, March-April, 1984. Original article submitted November 29, 1982.

0009-3130/84/2002-0129$08.50  

about 70% of it (2.1-2.8 g of iron) being present as the iron of heme, which is a component of hemoglobin — the protein fulfilling the function of transporting oxygen into the cells and tissues of the organism. The remainder of the iron is present mainly in hemosiderin and ferritin, and a smaller amount in such proteins as the cytochromes, the transferrins, and myoglobin [14, 15]. It is well known that some tissues possess a high affinity for iron; for example, the consumption of this metal by so-called cells of the erythroid series, in which the biosynthesis of hemoglobin takes place, or the placenta, through which iron is transported from the mother to the developing fetus, is extremely high [16].

An inadequate supply of iron to the organism disturbs the formation of hemoglobin, which leads to anemia. The demand for iron in the adult man is 15-25 mg per day. In certain pathologies connected with a congenital deficiency of the iron-carrying protein [1], iron deposits in the tissues of the organism in such amounts that poisoning by it is possible. All this requires the presence of a system which would regulate and rapidly supply this metal to the sites of its demand. Serum transfer is a component part of such a system.

Serum transferrin, a protein of the β-globulin fraction of blood serum, is capable of reversibly binding two trivalent (ferric) iron ions and participates in the transport of this metal from the site of its absorption in the cells of the epidermis of the small intestine to the sites of its consumption (i.e., to the cells of the erythroid series); it transports it to the cells of the liver, where this metal is stored, and it also traps the iron liberated on the decomposition of hemoglobin [17-19].

Transferrin determines the amount of iron in the blood by binding free iron ions circulating in the blood, the excess of which could cause poisoning of the organism [17]. The properties of transferrin as an effective buffer for iron ions are determined by its capability for adapting itself to the changing concentrations of iron, since the transferrin circulating in the blood is only 30% saturated with iron ions [16, 17]. The amount of transferrin fluctuates between 200 and 400 mg per 100 ml of serum [20].

The term "transferrin" unites a class of proteins which covers all the serum transferrins and may also be taken to include the ovotransferrins and lactotransferrins. The physiological role of ovotransferrin is still obscure, although as early as 1946 Alderton put forward a hypothesis of the participation of the ovotransferrins in antimicrobial immunity during the development of the embryo [21]. No specific role of ovotransferrin in the transport of iron has been shown.

Lactotransferrin was first isolated from human milk [22] but it is also found in the respiratory, digestive, urinary, and lachrymal tracts [4]. So far as concerns its physiological function, this, as in the case of ovotransferrin, is still not completely clear. Lactotransferrin may participate in the transport of iron from the blood into the milk of the feeding mother, but this has not been accurately established. There are hypotheses on the participation of lactotransferrin in antimicrobial immunity [4].

Hypotheses on the participation of proteins of the transferrins of the protein type in antimicrobial immunity have obtained good confirmation by the results of investigations due to Bezkorovainy [23]. He has shown that transferrins (serotransferrins, ovotransferrins, lactotransferrins), which are present in practically all biological fluids, can suppress the development of aerobic microorganisms by creating intense competition for the iron necessary for vital activity of the microorganisms. Conversely, the saturation of the transferrins with iron deprives them of bacteriostatic action [23]. Bezkorovainy has also given interesting information according to which the milk of cows suffering from mastitis contains ten times more lactoferrin than in the norm, which shows the functioning of this protein in protective mechanisms of the cattle organism.

## Physicochemical Properties of the Transferrins

The main physicochemical properties of the transferrins have been studied fairly well [24]. Each transferrin molecule consists of a single polypeptide chain with a molecular mass averaging 76,000-79,000 daltons. For human serum transferrin the figures are more accurate, and this polypeptide chain has a molecular weight of 77,000 daltons [25] and bears two identical carbohydrate chains each with a molecular mass of about 2400 daltons [26, 27]. Knowing the molecular masses of the transferrins it is possible to calculate the average number of amino acid residues in the molecules of these proteins — about 640 amino acid residues.

All known transferrins are monosubunit proteins. For human and rabbit serotransferrins, and also for ovotransferrins, this has been shown in an investigation of the products of their

reduction and carboxymethylation in the ultracentrifuge [28]. The transferrin molecule has two specific iron-binding sites, each of which can ensure the transport of the iron necessary for the biosynthesis of hemoglobin in reticulocytes [29]. Transferrins are also capable of binding a whole series of bivalent and trivalent metal irons such as, for example, $Cu^{2+}$, $Zn^{2+}$, $Vo^{2+}$, $Cr^{3+}$, $Mn^{3+}$, $Co^{3+}$, $Ga^{3+}$, $Ho^{3+}$, $Er^{3+}$, $Tb^{3+}$, $Nd^{3+}$, $Pr^{3+}$ [30-34] and $Gd^{3+}$ [35]. However, a surprising fact is that, in spite of this capacity of the transferrins for accepting a large number of different metal ions, they do not bind bivalent iron ions, or in the best case, bind them very feebly, as can be seen from the results of differential ultraviolet spectroscopy with an isotopic label and those of kinetic studies [36-38].

Transferrin saturated with ferric iron ions possesses a characteristic absorption spectrum in the visible region with a maximum of 465-470 nm. A probable exception is formed by fish transferrins which have an additional absorption maximum that is characteristic for heme-containing proteins, at 412 nm [39]. However, the absorption maxima in transferrin saturated with iron depends on which anion is bound to the protein. Thus, the $Fe^{3+}$—transferrin—anion complex has an absorption maximum at 465 in the case of carbonate, oxylate, malonate, and nitrilotriacetate ions, 488 nm for the glycinate ion, and, for example, 446 nm for the salicylate anion. The $Fe^{+}$—transferrin complex has a characteristic pink color that is stable in the pH range of 7.5-10.0. At lower pH values the characteristic color of the complex disappears, since it undergoes dissociation. The color of the complex also disappears in the presence of iron-binding agents such as ethylenediaminetetraacetate (EDTA). At a pH below 3.0, the complex of the protein with iron dissociates even in the absence of iron-binding agents.

At the present time it has been accurately established that the presence of an anion is necessary for iron to bind with transferrin [40-42]. Under physiological conditions, this necessary component is a carbonate or bicarbonate anion [43]. In the basence of carbonate, anions such as oxalate, thioglycolate, malonate, nitrilotriacetate, and others may also bind to the so-called "anionic center" in the protein [40, 41, 44]. Under physiological conditions in the blood unsaturated transferrin always circulates in the form of a complex with carbonate [43]. Other inorganic anions bind to transferrin extremely feebly [41].

The isoelectric points of serum transferrin and ovotransferrin are in the pH range below 7.0 [45], but in lactotransferrin the isoelectric point has shifted to the pH range above 7.0 [46], which is apparently due to its capacity for binding acidic macromolecules [47]. The isoelectric points of transferrin depend on the metal bound to the protein, since the binding of each metal ion increases the total negative charge of the protein in accordance with the equation

$$Fe^{3+} + H_3\text{—}\ \text{transferrin}\ + HCO_3^- \rightleftarrows Fe\text{—}\ \text{transferrin}\ \text{—}\ HCO_3^- + 3H^+$$

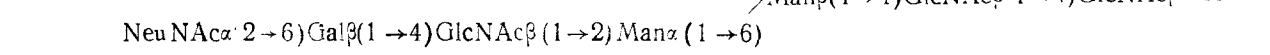
It is still not completely clear whether the protein binds a carbonate or a bicarbonate anion. But in any case the binding of the iron increases the total negative charge of the protein and, consequently, its anionic mobility. The modern method of isoelectric focusing enables monoferri-, differi-, and apotransferrins to be obtained. For example, the isoelectric points of human serotransferrin in the monoferri-, diferri-, and apo-forms have been determined by isoelectric focusing as 5.0, 5.3, and 5.6, respectively [29].

The study of avian serum transferrin and of the ovotransferrin of egg protein [48] shows that these two proteins have the same amino acid composition. In the immunochemical respect, they are also identical, although synthesized at different sites [49]. These results agree well with those of Palmiter et al. [50] who have shown that these proteins are the products of the expression of the same structural gene. The amino acid compositions of human lactotransferrin and of rabbit lactotransferrin differ appreciable from those of the corresponding serum protein [25, 51]. In contrast to avian transferrin and ovotransferrin, there is no cross-immunoreactivity between the transferrin from human serum and human lactotransferrin [52].

Carbohydrate Content

All known transferrins are glycoproteins. For example, human serum transferrin contains approximately 7% of carbohydrates attached to the protein molecule in the form of two identical chains with molecular masses of about 2500 daltons that are symmetrically branched [53]. The carbohydrate chains are terminated by sialic residues and are attached to the proteins by asparaginyl bonds [54]. The terminal sialic acid residues can be eliminated by treatment with neuroaminidase [55].

The sequence of each chain has been established by chemical and enzymatic methods [27] and has been confirmed by NMR spectroscopy [56] and mass spectroscopy [57].




This "two-antenna" structure differs from that proposed previously by Jamiesson [58]. The carbohydrate chains are attached to residues 413 and 610 in the protein by β-N-glycosidic bonds, the carbohydrate component being in the C-terminal section of the protein molecule [59]. The number of heteropolysaccharide chains in the transferrin molecule varies in different species [60].

A qualitative similarity of the carbohydrate prosthetic groups of human, bovine, and porcine transferrins and of duck and hen ovotransferrins, which consist of mannose, galactose, N-glucosamine, and sialic acid residue, has been reported. Some quantitative differences exist in the amounts of these components [60]. Fucose has also been found in the carbohydrate component of porcine transferrin [60].

The presence in the carbohydrate component of sialic acid residues is characteristic for the transferrins, just as for other serum glycoproteins. It is just the presence of different numbers of sialic acid residues that explains the electrophoretic polymorphism of the transferrins.

The carbohydrate prosthetic groups of transferrins and, in particular, the sialic acid residues play a fundamental role in the sorption of the transferrins on the reticulocyte membrane. This conclusion can be deduced from experiments on the desialation of the transferrin molecule, as a result of which it is no longer sorbed on reticulocytes [61].

Lactotransferrin possesses a similar "two-antenna" structure the composition of which is basically similar to that of serum transferrin. Unlike the latter, lactotransferrin also contains several fucose residues attached by α-1,6- or α-1,3- bonds to a N-acetylglucosamine residue [62].

On considering transferrins in connection with the well-known hypothesis of the carbohydrate components of secretory proteins — the serum glycoproteins are secretory for the cells and before passing out into the bloodstream they are subjected to glycosylation — it may be assumed that the carbohydrate components are necessary for the structural rearrangement of the cell membrane and the subsequent passage of the molecule from it into the bloodstream. However, results obtained by Stuck et al. indicate differently. Such an antibiotic as tunicamycin blocks by more than 75% the glycosylation of newly synthesized transferrin in rat liver cells, but this leads to a less than 15% inhibition of the secretion of transferrin [63].

## Hypothesis of a Domain Structure of Transferrin

The hypothesis of a domain structure of transferrin presupposes the presence in the native molecule of this protein of two structural domains (sections) each of which has a metal-binding center that is independent of the other. In the case of bovine transferrin [64], avian transferrin (ovotransferrin) [65, 66], and rat transferrin [67], the structural domains can be obtained by limited proteolysis without appreciable changes in the spectroscopic properties of the iron-binding sites. It has been established that the ion-binding sites present, respectively, in the two domains of the native protein molecule are autonomous, and the denaturation of one of these sites does not affect the capacity of the other four binding iron.

In recent years, there have been a number of publications devoted to the primary structures of serotransferrin, lactotransferrin, and ovotransferrin [59, 68-70]. MacGillivray and Brew [69] have published investigations on the primary structure of cyanogen bromide fractions obtained from human serotransferrin. They have described two sections of the primary structure of serotransferrin consisting of 87 and 57 residues. When suitably superposed upon one another, about 40% of the amino acid residues in the corresponding positions are identical. The complete amino acid sequence of human serotransferrin has now been studied [59]. It has been shown that the polypeptide chain of the transferrin contains two homologous domains comprising approximately equal numbers of amino acid residues. The degree of homology between the structural domains is fairly high (about 40% of the amino acid residues are identical).

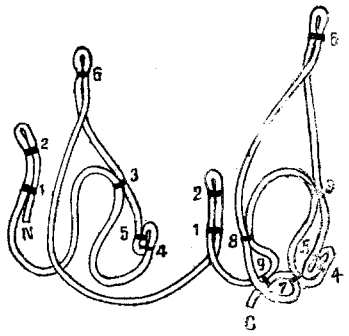


Fig. 1. Model of the ovotransferrin molecule according to Williams et al., showing the homology of the disulfide bonds [70].

Williams et al. [70] have studied the primary structure of ovotransferrin. The amino acid sequence of peptide fragments of ovotransferrin obtained by the sequencing method that is traditional for protein chemistry correlate fairly well with the complete amino acid sequence of ovotransferrin deduced from the nucleotide sequence of the cDNA transcribed from the messenger RNA for ovotransferrin obtained by Jeltsch and Chambon [71]. The two structural domains of ovotransferrin exhibit considerable similarity in their amino acid sequences and contain about 27% of identical residues. Of the 15 disulfide bonds present in the molecule, six are homologous and have identical positions in the two domains (Fig. 1).

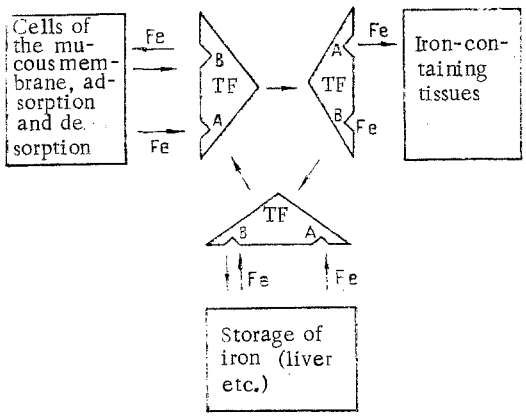
The structural domains, both in the case of human serotransferrin and in that of ovotransferrin, are connected with one another by a short-chain peptide having approximately the same number of amino acid residues about 40% of which are identical and they each bear one metal-ion-binding site. The structural domains of human serotransferrin and of ovoferrin also have a number of interesting differences. Thus, the C-terminal domains contain a larger number of disulfide bonds than the N-terminal sequences (11 as compared with 8 for serotransferrin, and 9 as compared with 6 for ovotransferrin). In the native molecule of human serotransferrin, only the C-terminal domain is glycosylated [59].

Recently, internal structural homology has also been shown for human lactotransferrin [72]. A similarity has been found in the amino acid sequences between human serotransferrin and lactotransferrin as well [72]. Such a phenomenon of the internal homology of proteins of the transferrin type suggests that in the course of phylogenesis a doubling of a structural gene for transferrin precursors took place. Apparently, in the early stages of evolution a primary ancestor protein containing one iron-binding site and having a mass of approximately half that of the present-day proteins circulated in the animal plasma. Such a conclusion is also permitted by the results of a comparative study of the monoferri fragments obtained on the proteolysis of ovotransferrin [73] and of the serotransferrins of different animals [67, 74, 75]. Such monoferri fragments have practically identical amino acid compositions and molecular masses, although they differ in their immunochemical and spectral characteristics [67, 70, 74]. The monoferri fragments retain the capacity for transferring iron ions into the reticulocytes, although the inclusion of iron in heme on using the native transferrin is approximately twice as great as on the addition of a mixture of monoferri fragments [76].

It would be tempting to assume that the monoferri fragments obtained on the proteolysis of native transferrin were the ancestors of present-day transferrins. However, such a hypothesis has been refuted by experiments due to Williams et al. [77], which showed that the half-fragments of the ovotransferrin molecule are excreted through the mouse kidney. At the same time, whole ovotransferrin molecules do not pass through the kidneys and are retained in the circulating blood. In view of this, it is assumed that the evolutionary precursor of transferrin had greater dimensions than the N- and C-terminal monoferri fragments of transferrin.

## The Binding of Iron by Transferrin

More than 10 years ago, Fletcher and Huehns [78] showed that transferrin with 50% saturation was a less effective donor of iron to reticulocytes than transferrin with 100% saturation. Furthermore, they reported that the two iron-binding sites differed in their capacity




Scheme 1. The Fletcher–Huehns hypothesis on the role of transferrin in the transport of iron and the regulation of the iron metabolism [100].

for liberating iorn. One site, denoted by A, was more effective than the other, denoted by B. Their results were confirmed by those of other scientists [79-83]. On the basis of these facts, Fletcher and Huehns put forward the hypothesis that transferrin is not simply a passive iron carrier but is a regulator of its absorption and distribution in the organism [84]. According to this hypothesis, iron is bound in the two centers of the transferrin molecule but is liberated selectively according to the accepting tissue. Thus, the A and B sites in the protein molecule can bind free iron from the cells of the mucous membrane of the small intestine, but the A site supplies its iron to the reticulocytes of the placenta, while the B site is oriented functionally towards the liver, where iron is laid up and stored until use, or to the cells of the mucous membrane for the desorption of iron. Graphically, this process is illustrated by the scheme.

The development of the methods of protein chemistry, which has permitted individual structural domains of transferrin each containing one ferric ion to be obtained and effectively separated, has shown that the transferrin molecule contains acid-stable and acid-labile iron-binding sections [75]. Under weak acid conditions, transferrin loses iron mainly from the center present in the N-terminal half of the molecule [85]. The results of the work of Azari's group [76] show that the iron-binding site present in the N-terminal half of transferrin is 44% more effective in its capacity for supplying iron ions to the erythrocytes. However, the N- and C-terminal monoferri fragments are bound to erythrocytes almost identically [76].

We must also mention papers that give results of an opposite nature [86-88]. According to these publications, the two iron-binding sites in transferrin molecules are functionally equivalent and do not differ in their capacity for liberating iron to different tissue receptors. Results in favor of the hypothesis that the two sites in transferrin do not differ in their capacity for transporting iron to reticulocytes in *in vitro* experiments have been obtained by the use of homologous systems including human transferrin and human reticulocytes [86, 87] or rabbit transferrin and reticulocytes [86]. When using heterologous systems consisting, for example, of the human transferrin and rabbit reticulocytes, a different efficiency of the centers in the transfer of the metal in the reticulocytes is observed [86, 89].

The Fletcher-Huehns phenomenon apparently gives a satisfactory explanation of the results of work by Okada et al. [90] and also by others [80, 91, 92]. The use of the method of isoelectric focusing permits the isolation of individual iso forms of transferrin differing from one another by their numbers of sialic acid residues. It is known that the transferrin pool, particularly in rats, is represented by transferrins that are fast-migrating (F) and slow-migrating (S) on electrophoresis. Okada et al. [90] have shown that different iron isotopes from the S- and F-transferrins are distributed differently in different accepting tissues. Thus, in rat reticulocytes, bone marrow hemes, and spleen iron from the S-transferrins predominates, while a larger amount of iron from the F-transferrins accumulates in the blood plasma and the liver ferritins. Furthermore, it has been reported that rat reticulocytes extract and absorb iron from the S-transferrins at a greater rate than from the F-transferrins. However, in a similar investigation by other authors [8] no functional differences between the isotransferrins were detected.

The method of isoelectric focusing permits not only iso forms of transferrin but also transferrins "loaded" with different amounts of iron ions to be obtained. Harris and Aisen [29] have obtained human differi and monoferri transferrins in this way. The technique of isoelectric focusing has enabled the fact to be established that the protein molecule acquires one negative charge for each ferric ion bound, which can be represented as

$$Fe^{3+} + apoTF^{2-} + HCO_3^- \rightarrow [Fe-TF-HCO_3]^{-2} + 3H^+$$

These investigations show that diferri transferrin (2Fe-TF) from human blood serum is a more effective donor of iron ions (calculated to one iron atom) for rabbit reticulocytes than monoferri transferrin. However, differi and monoferri transferrins supply their iron ions to reticulocytes at the same rate. Experiments must be mentioned from which it can be seen that iodine-125-labeled diferri transferrin shows a higher affinity for the reticulocyte membrane than monoferri transferrin [29]. In apotransferrin, affinity for the membrane is weak [93].

The differences between apotransferrin and diferri and monoferri transferrins in the degree of their binding with the reticulocyte membranes also explain to some degree by their dissimilar behavior on ion-exchange resins [94]. It is likely that the binding of iron by the protein causes some conformational changes in the protein molecule and this, in its turn, changes the total charge of the transferrin. Lane has suggested that the change in the total charge may play a definite role in the regulation of the binding of iron with the receptor in the reticulocyte membrane [94]. A number of workers have reported that the conformation of the protein changes after the binding of iron; in particular, it becomes more spherical and increases slightly in dimensions [95-97].

Binding of Anions

Certain anions must be present for ferric iron ions to be bound with transferrin. They may be bicarbonate, oxalate, malonate, EDTA, glycinate, thioglycolate, or nitrilotriacetate ions [98]. The necessity for an anion can be demonstrated fairly easily. In the absence of, for example, the bicarbonate ion the characteristic pink coloration of the complex of iron with transferrin does not develop. It has been shown that the specific binding of ferric ions with apotransferrin takes place only after the formation of a specific complex of the bicarbonate or carbonate anion with the protein [99].

In blood unsaturated with iron, under physiological conditions, transferrin always circulates in the form of a complex with an anion [99]. Bates and Schlabach have made broad studies of the interaction of $Fe^{3+}$, transferrin, and anions under conditions excluding the presence of the bicarbonate anion. In the experiments they used more than 25 anions [100]. The results of the experiments show that in the absence of anions the binding of the metal by the protein either does not take place or it is bound nonspecifically.

Aisen et al. [98] have shown that the specific binding of metal ions with transferrin can take place even in the absence of bicarbonate anion. In the absence of the bicarbonate anion the development of the characteristic coloration of the iron–transferrin complex was not observed but according to Aisen et al., the electron paramagnetic resonance spectroscopy method gives a signal that is characteristic for the complex. Apparently, the bond of the metal ion with the protein in the absence of carbonate is more unstable than in its presence. Thus, when the iron–transferrin complex is allowed to stand in the absence of bicarbonate at the physiological pH hydrolysis of the iron takes place with the formation of insoluble iron hydroxide.

On investigating the relationship between structure and the capacity of various anions for being bound in the anionic site of the protein, Schlabach and Bates come to the conclusion that this anionic center has the following dimensions: 3 Å in depth, 6 Å in width, and from 4 to 6 Å in length [100]. This center is asymmetric and is located close to the surface of the protein globule [100].

The spectral properties of the iron–transferrin complex change when the bicarbonate ion is replaced by other anions, which once more shows a fairly strong interaction between the binding sites for the metal and the anion. A figure given by Bates and Schlabach shows the absorption spectra in the visible region of complexes of 2Fe–transferrin with a number of anions. As can be seen from the figure, the absorption maxima of the complex differ fairly greatly according to the anion bound. As mentioned previously, the mechanism of the liberation of iron by the protein in tissues according to their demand for it is one of the basic

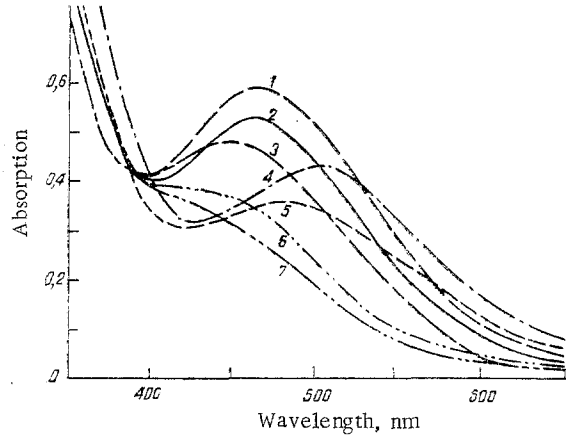



Fig. 2. Absorption spectrum in the visible region of $Fe^{3+}$—transferrin—anion complexes. Anions: 1) nitrilotriacetate; 2) carbonate; 3) salicylates; 4) thioglycolate; 5) glycinate; 6) glyoxylate; 7) glycolate.

features of the chemistry of the transferrins. A number of workers consider that the anion plays a key role here. The replacement of carbonate by oxalate in the anion site lowers the rate of transfer of the iron by the transferrin into reticulocytes by approximately 65% [101, 102]. Similar results have been obtained in the case of ovotransferrin [103]. The malonate ion is more effective than the oxalate ion but less than the carbonate anion [104].

Transferrin—Reticulocyte Interaction

Every day about 30 mg of iron is included in a hemoglobin of an adult man, the synthesis of the hemoglobin taking place in the cells of the erythroid series. Transferrin is an integral part of the system for the transport and supply of iron ions to these cells. The transferrin molecule probably fulfills the function of an iron carrier into the reticulocytes repeatedly in view of the fact that the "lifetime" of the protein is reckoned as several days and the actual process of transferring iron into reticulocytes from transferrins takes place fairly rapidly. The supply of iron to the reticulocytes depends on the time of incubation, the temperature, and a sufficient supply of energy, and an important role here is played by the native nature of the protein molecule and of the reticulocytes [105-107]. A convincing proof that it is in fact transferrin that is the donor of iron to the reticulocytes is provided by atransferrinemia patients in whom transferrin is not synthesized at all, which is apparently due to a mutation of the corresponding gene. Such patients form an example of a biological paradox when the organism, on the one hand, suffers from iron-deficiency anemia, and, on the other hand, is overloaded with iron [1].

For transferrin to be able to fulfill its function as an iron carrier to the reticulocytes, there must be special receptors on the surface of the latter. The existence of specific receptors in the reticulocyte membrane for transferrin was first shown by Jandl [108], who observed that the capacity of reticulocytes for accepting iron from transferrin fell after their treatment with trypsin. Treatment with neuraminidase also lowered the accepting capacity of the reticulocytes. The possibility of the solubilization of proteins from the reticulocyte membranes under the action of various detergents has stimulated the search for the membrane transferrin receptor. The technique of isolating the corresponding receptors in the reticulocyte membrane for transferrin has been well described [109]. Information given in the literature characterizes the membrane transferring receptor as a protein with a complex subunit structure having a molecular mass of 350,000 daltons (since the molecular mass of the receptor—transferrin complex is estimated at 430,000 daltons from a determination of the molecular mass of the complex by the gel filtration method, simple deduction of the known mass of transferrin gives the desired molecular mass for the receptor) [110].

Aisen et al. [109] have suggested a model of the transferrin receptor from rabbit reticulocytes as a protein structure consisting of two polypeptide chains with molecular masses of 95,000 and 165,000 daltons, respectively. Other workers have deduced lower values of the molecular mass of the receptor—transferrin complex, determining it as 225,000 daltons [111].

Witt and Woodworth [112] have estimated the molecular mass of the receptor for ovotransferrin as 35,000 daltons. Schneider et al. [113] have determined the transferrin receptor from human reticulocytes as a subunit glycopeptide structure consisting of two polypeptide chains each with a molecular mass of 90,000 daltons. In an investigation of the problem of reticulocyte–transferrin interaction, substantial interest is presented by the question of how iron passes from transferrin into the reticulocyte for the needs of hemoglobin synthesis. At the present time, two hypotheses explaining the reticulocyte–transferrin interaction exist. According to the first hypothesis, the interaction of a transferrin molecule with a reticulocyte is delimited by the surface of the latter. For example, Jandl and Katz [114] have shown that after a disturbance of the integrity of the reticulocyte as the result of hemolysis, about 90% of iodine-131-labeled transferrin remained bound to the cell residues of the membranes of the destroyed reticulocytes. Similar results can be found in the reports of other workers [115]. According to the other hypothesis, the transfer of iron into reticulocytes can take place as the result of the micropinocytosis of the transferrin by the cells. In favor of this hypothesis are experiments due to Sullivan and Grasso [116] in which rat reticulocytes and normoblasts were incubated with transferrin that was conjugated with ferritin or with antibodies to transferrin labeled with ferritin. On analysis under the electron microscope the binding of the conjugates with the cells and their appearance in micropinocytic vesicles was revealed.

The number of receptors on the surface of a reticulocyte has been estimated differently by different workers: from 50,000 [117] to 105,000 [118]. The rate of absorption of iron by reticulocytes does not depend on the degree of iron-saturation of the transferrin with which they are incubated providing that the total concentration of the iron–transferrin complex is constant. The receptors in the reticulocyte membrane have a pronounced selectivity for iron-saturated transferrin. The experimental results show that the affinity of the receptors for transferrin not saturated with iron (apotransferrin) is lower than for that which is saturated [114].

## The Biosynthesis of Transferrin

Transferrin molecules are synthesized mainly in the liver but they can also be synthesized in other organs: the spleen, kidneys, lungs, and bone marrow [119]. In birds, ovotransferrin is one of the main products of protein synthesis in the oviduct. Palmiter et al. [120] have shown that in birds the blood serum transferrin and ovotransferrin have identical primary structures and are the products of the expression of a single gene. They are synthesized in the form of precursors, and in the process of maturation a 19-membered peptide having a hydrophobic sequence is split out from them. However, the mechanisms for the regulation of the synthesis of serum transferrin and of apotransferrin in birds are different. Thus, estrogens, which are powerful inductors of the synthesis of ovotransferrin in the oviduct, stimulate synthesis of transferrin in the liver only insignificantly [120, 122]. Recent investigations [123] have shown that the biosynthesis of, in particular, rat transferrin is localized in membrane-bound polyribosomes, and this agrees well with the general concept that in fact predicts the biosynthesis of export proteins by the cell just in membrane-bound polyribosomes. Recent investigations on the dynamics of the appearance and accumulation of newly synthesized transferrin in the subcellular fractions of the rat liver and its excretion into the bloodstream have shown that the synthesis of mature transferrin has a complex and multistage nature of a process of post-synthetic maturation [124]. It has been shown that the intracellular transport of the newly-formed transferrin is accompanied by a change in its molecular mass (88,000 for the transferrin precursors in fractions of the endoplasmic reticulum, 84,000 for immunoreactive transferrin from the Golgi apparatus, and 77,000 for mature transferrin circulating in the blood). Such changes in molecular mass show the existence of a complex sequence of reactions in the maturation of transferrin in the liver, apparently including stages of proteolysis and glycosylation accompanied by the migration of this protein through the membrane systems of the hepatocytes and its secretion into the blood stream. The processing of the serum proteins apparently has a universal nature. Complex processes of post-synthetic maturation, including the proteolysis of the excess amino acid sequences in the precursors and glycosylation of the finished protein, have also been reported for the copper-transporting protein of the blood ceruloplasmin [125] and for albumin [126].

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# DERIVATIVES OF UNSATURATED AROMATIC ALCOHOLS IN PROPOLIS AND STYRAX BENZOIN

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UDC 547.915

The benzoate of trans-coniferyl alcohol and the benzoate of trans-p-coumaryl alcohol have been obtained from propolis and the styrax benzoin, this being the first time that the latter has been described.

The basis of propolis consists of the resinous secretions of certain species of woody plants, including the axillary buds of the birch, poplar, and pine [1, 2]. One of the main components of propolis collected from pine buds is, as we have shown previously [2], a substance with a molecular weight of 284 and the empirical formula $C_{17}H_{16}O_4$. It is readily detected by thin layer chromatography on silica gel in the form of a cherry-red spot when the plates are sprayed with concentrated $H_2SO_4$ and is convenient for the purposes of standardization.

The isolation of this compound in the pure form is associated with considerable difficulties in view of its instability in the presence of atmospheric oxygen. The best results in the purification of the substance were achieved with preliminary separation of the extract on a column of alumina (Brockman activity grade V) followed by separation of the enriched fraction on a column of silica gel and its final purification by preparative thin-layer chromatography (PTLC). To prevent subsequent oxidation, a large part of the fraction isolated was acetylated by the action of $Ac_2O$ in pyridine. The substance isolated in this way (I) and its acetate were investigated by the methods of physicochemical analysis.

M. M. Shemyakin Institute of Bioorganic Chemistry, Academy of Sciences of the USSR, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 152-160, March-April, 1984. Original article submitted February 22, 1983.

 0009-3130/84/2002-0140$08.50 