Interaction of Anions with Iron · Transferrin · Chelate Complexes*

TERRY B. ROGERS,‡, § ROBERT E. FEENEY,§ and CLAUDE F. MEARES‡

From the ‡Department of Food Science and Technology, and the §Department of Chemistry, University of California, Davis, California 95616

Preliminary evidence suggested that phosphate or borate destabilize iron · ovotransferrin · nitrilotriacetate complexes in the absence of added bicarbonate. The iron ovotransferrin · EDTA complex was prepared in the absence of bicarbonate, and a number of anions, including phosphate, sulfate, and citrate, were found to perturb the visible absorbance (λₘₐₓ = 490 nm) of this complex. Other anions, such as chloride, nitrate, and perchlorate, had little or no effect on the spectrum. Also, when bicarbonate was added to a solution of the iron · transferrin · EDTA complex, the visible absorbance had decreased to λₘₐₓ = 470 nm, evidently the iron · transferrin · CO₃ complex. These observations are best explained by a paradigm which includes anion binding to the apoprotein. It is clear that there is an intimate relationship between anions and the binding of iron chelates by transferrin.

The transferrins comprise a homologous class of vertebrate iron transport proteins which simultaneously bind 2 atoms of iron and two anions. The physiological anion in the iron · transferrin · anion complex is one of the ionic forms of the hydrated CO₃ molecule, but a number of other anions can substitute in the ternary complex if bicarbonate is excluded from solution (1).

In the absence of an appropriate anion, there is no stable interaction between Fe²⁺ and transferrin so that hydrolysis, polymerization, and nonspecific binding of iron is most likely to occur (2-4). There is little information available concerning transferrin · anion interactions in the absence of a metal, and it has been generally assumed that no significant binding to the protein does occur (5).

A number of chelating agents have been shown to be effective anion substitutes in the transferrin · iron complex including citrate, nitrilotriacetic acid, and EDTA. The notion of specific anion binding to transferrin is implicated in the mechanism of iron incorporation into transferrin from iron chelates. In general, the postulated mechanism involves the formation of an iron · transferrin · chelator ternary complex followed by displacement of the chelator by carbonate in the solution forming the physiological iron · transferrin · carbonate complex (6-11). It is clear from these studies that the rates of exchange of Fe²⁺ between chelates and apotransferrin in part depend upon the stability of the iron chelate as well as upon the concentration of bicarbonate in the solution (10, 11).

The present paper describes studies on interactions between anions and iron · transferrin · EDTA and iron · ovotransferrin · EDTA complexes. The results are consistent with a model for transferrin · anion interaction that includes specific binding of anions to the apoprotein.

EXPETIMENTAL PROCEDURES

Materials — Human serum transferrin was purchased from Behring Diagnostics (lot 1672). Chicken ovotransferrin was isolated by a procedure previously reported (12). A final step of gel filtration on a Sephadex G-100 column was included. Bio-Gel P-100 and Chelex 100 were obtained from Bio-Rad Laboratories. Sephadex G-100 and G-25 were purchased from Pharmacia Fine Chemicals. Disodium EDTA and nitrilotriacetic acid were purchased from J. T. Baker Chemical Co. HEPES was available from Sigma Chemical Co. Carrier free [²⁵FeCl₂] in HCl was purchased from New England Nuclear Co. and [¹⁴C]EDTA was obtained from ICN Life Sciences. All other compounds used were purchased as reagent grade. The water used in all of the experiments was doubly deionized and Pyrex distilled.

Methods — All of the buffers that were used in experiments were treated to ensure a minimum of contamination due to iron or other

* This research was supported in part by Research Grant HL15619 from the National Heart and Lung Institute of the United States Public Health Service. A portion of this work was presented at the 1974 Meeting of the American Chemical Society, Chicago, Illinois, August 29 to September 2, 1977. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ This material was taken from the thesis submitted in partial fulfillment of the requirements for the Ph.D. degree in Agricultural Chemistry, University of California, 1977.

§ The abbreviation used is: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Received for publication, June 13, 1977

8106
heavy metals. Usually, the buffers were extracted with 0.1% dithi-  
zon in CCl₄. The buffers were boiled briefly to remove traces of  
CCl₄ prior to their use. All of the buffers containing bicarbonate  
were treated with Chelex 100 according to the procedure of Willard  
et al. (13).

Solutions of Fe²⁺-EDTA and Fe³⁺-nitrilotriacetic acid were pre-  
pared as follows. The chelating agent or its sodium salt was dissolved  
in water. A standard iron solution was prepared by dissolving iron  
wire, which had been sanded and washed in 2 m nitric acid prior to  
weighing, in concentrated HCl. The iron solution, generally around  
0.2 mM, was added to the solution of nitrilotriacetic acid or EDTA.  
The nitrilotriacetic acid to iron ratio was 2:1 and the EDTA:iron  
solution was prepared as follows. The chelating agent or its sodium salt was dissolved  
in water. A standard iron solution was prepared by dissolving iron  
wire, which had been sanded and washed in 2 m nitric acid prior to  
weighing, in concentrated HCl. The iron solution, generally around  
0.2 mM, was added to the solution of nitrilotriacetic acid or EDTA.  

The nitrilotriacetic acid to iron ratio was 2:1 and the EDTA:iron  
solution was prepared as follows. The chelating agent or its sodium salt was dissolved  
in water. A standard iron solution was prepared by dissolving iron  
wire, which had been sanded and washed in 2 m nitric acid prior to  
weighing, in concentrated HCl. The iron solution, generally around  
0.2 mM, was added to the solution of nitrilotriacetic acid or EDTA.  

Anion Binding to Iron Transferrins

Addition of Iron-Nitrilotriacetic Acid to Ovotransferrin—  
Preparatory spectral observations were made when 2 eq of  
iron-nitrilotriacetic acid were added to 0.14 mM ovotransferrin  
in 50 mM phosphate or borate at pH 7.8. An initial absorbance  
at 470 nm was observed, usually around 0.50, which decreased  
rapidly with time. After 10 min, less than 15% of the original  
absorbance at 470 nm remained. When the same experiment  
was repeated using a 50 mM phosphate, 50 mM bicarbonate,  

In several experiments designed to measure iron and anion  
displacements, radiolabeled iron-transferrin-EDTA complexes were  
prepared, as above, using either ⁴⁰Fe-EDTA or iron-¹¹⁴C-EDTA  
solutions. After complex formation, bicarbonate was added to these  
solutions to a final concentration of 100 mM. The solutions were  
then passed through a Sephadex G-25 column equilibrated with 100 mM  
acetate, 10 mM EDTA at pH 4.5. After dialysis against  
water, the protein solutions were dialyzed versus 100 mM NaClO₄ to  
remove the chelating agent (31, and finally versus water before  
the pH adjustment. After preparation, the iron-EDTA solutions  
were analyzed by thin layer chromatography to verify that all of  
the added iron was bound to the chelating agent. An acidic aqueous  
acetone solvent (35 ml of acetone, 0.5 ml of 0.2 N HCl made to 50 ml  
with H₂O) was used. Any unchelated iron remained at the origin  
and could be visualized on fluorescent silica gel plates. Only those  
solutions in which all of the iron was bound as the EDTA chelate  
were used in experiments.

Iron-free ovotransferrin and iron-free human serum transferrin  
were prepared by first dialyzing the protein against several changes  
of 100 mM acetate, 10 mM EDTA at pH 4.5. After dialysis against  
water, the protein solutions were dialyzed versus 100 mM NaClO₄ to  
remove the chelating agent (3), and finally versus water before  
lyophilization. The diferric transferrin-carbonate complexes were  
prepared by adding 2.5 eq of iron-nitrilotriacetic acid to the protein  
dissolved in 100 mM NH₄HCO₃. The solution was then passed  
through a Sephadex G-25 column equilibrated with 100 mM  
NH₄HCO₃, the protein eluate was collected, and absorbances at 280  
and 470 nm were obtained. Only those fractions greater than 95%  
saturated with iron (Å absorptions Å 24.0) were pooled and lyophilized.  
The following extinction coefficients (Å²/Å) were used: apo-ovotrans-  
ferrin, 11.2 at 280 nm; diferric ovotransferrin, 14.0 at 280 nm, 0.62  
at 470 nm; apo-human serum transferrin 11.3 at 280 nm; diferric  
serum transferrin, 14.3 at 280 nm and 0.57 at 470 nm (12).

Complexes between iron EDTA and ovotransferrin and human  
serum transferrin were prepared under CO₂-free conditions. A solu-  
tion of 59Fe·EDTA complexes were prepared. When the  
iron-EDTA and either apo-ovotransferrin or apotransferrin  
solutions were mixed in the absence of bicarbonate, the  
formation of a complex was indicated by the appearance of a  
colored species in solution. The λmax was 490 nm and 515 nm  
for ovotransferrin and serum transferrin, respectively, as  
compared to a visible λmax of 465 nm for both of the iron protein-carbonate complexes. In a series of parallel  
experiments, various molar excesses of iron-EDTA were added to the ovotransferrin in the absence of bicarbonate in order to determine the levels of iron-EDTA required to  
saturate the protein. Fig. 1 shows the relationship between  
absorbance at 490 nm and concentration of iron-EDTA when  
the concentration of ovotransferrin was held constant at 0.19  
mM. When the ratio of iron-EDTA to binding site was 1:1,  
about 15% of the sites are filled. Ovotransferrin is approximately  
80% saturated when the molar ratio of iron-EDTA to binding  
site is 7.5:1. Estimation of the dissociation constant  
could be made since Kd = [iron-EDTA] at the half-saturation  
point. The Kd value is close to 1.5 mM, which correlates well  
with the binding constant of 1.3 mM calculated for iron-EDTA  
and human transferrin (9).

Addition of Other Anions to Iron-Ovotransferrin-EDTA  
Complexes—A number of anions were added to iron-ovotrans-  
ferrin-EDTA complexes and changes in the visible spectrum  
were followed at 490 nm. The protein, 0.19 mM, was 90%  
saturated with iron-EDTA, 2.94 mM. The results are shown  

RESULTS

Addition of Iron-Nitrilotriacetic Acid to Ovotransferrin—  
Preparatory spectral observations were made when 2 eq of  
iron-nitrilotriacetic acid were added to 0.14 mM ovotransferrin  
in 50 mM phosphate or borate at pH 7.8. An initial absorbance  
at 470 nm was observed, usually around 0.50, which decreased  
rapidly with time. After 10 min, less than 15% of the original  
absorbance at 470 nm remained. When the same experiment  
was repeated using a 50 mM phosphate, 50 mM bicarbonate,  

PH 7.8, buffer, a similar initial absorbance at 470 nm was  
observed which was stable for at least 24 h. Also, if the  
lyophilized diferric ovotransferrin·CO₂ complexes were  
dissolved in phosphate or borate buffer to 0.14 mM, an initial  
absorbance of 0.55 was observed and did not decrease over  
time. These results suggest that the formation of an initial  
iron-ovotransferrin-nitrilotriacetic acid complex, λmax = 470  
nm, is unstable in phosphate or borate buffer unless sufficient  
bicarbonate is present. As expected, iron-ovotransferrin·CO₂  
complexes are stable at relatively high concentrations in  
these buffers at pH 7.8.

Iron·Transferrin·EDTA Complex Formation—In order to  
investigate further the spectral changes due to interaction of  
phosphate and other anions with transferrin complexes,  
iron transferrin EDTA complexes were prepared. When the  
iron-EDTA and either apo-ovotransferrin or apotransferrin  
solutions were mixed in the absence of bicarbonate, the  
formation of a complex was indicated by the appearance of a  
colored species in solution. The λmax was 490 nm and 515 nm  
for ovotransferrin and serum transferrin, respectively, as  
compared to a visible λmax of 465 nm for both of the iron  
protein-carbonate complexes. In a series of parallel  
experiments, various molar excesses of iron-EDTA were added to the ovotransferrin in the absence of bicarbonate in order to determine the levels of iron-EDTA required to  
saturate the protein. Fig. 1 shows the relationship between  
absorbance at 490 nm and concentration of iron-EDTA when  
the concentration of ovotransferrin was held constant at 0.19  
mM. When the ratio of iron-EDTA to binding site was 1:1,  
about 15% of the sites are filled. Ovotransferrin is approximately  
80% saturated when the molar ratio of iron-EDTA to binding  
site is 7.5:1. Estimation of the dissociation constant  
could be made since Kd = [iron-EDTA] at the half-saturation  
point. The Kd value is close to 1.5 mM, which correlates well  
with the binding constant of 1.3 mM calculated for iron-EDTA  
and human transferrin (9).

Addition of Other Anions to Iron-Ovotransferrin·EDTA  
Complexes—A number of anions were added to iron-ovotrans-  
ferrin·EDTA complexes and changes in the visible spectrum  
were followed at 490 nm. The protein, 0.19 mM, was 90%  
saturated with iron·EDTA, 2.94 mM. The results are shown  

FIG. 1. The amount of excess iron·EDTA required to saturate  
apo-ovotransferrin. The protein concentration was maintained at  
0.19 mM and the concentration of iron·EDTA was varied from 0.19  
to 5.88 mM. The values for the absorbance at 490 nm were deter-  
mined relative to a blank containing an equivalent amount of iron·  
EDTA.
in Fig. 2. It is clear that the visible spectrum could be perturbed by a number of anions added to a concentration of 100 mM, including phosphate, sulfate, citrate, and carbonate. Glycine, nitrate, perchlorate, and chloride had little effect on the complex. There was no observed effect when chloride was added to 300 mM, so the variation in ionic strength does not appear to be an important factor. When 100 mM nitritotriacetic acid was added, there was a rapid increase in the absorbance at 470 nm, probably due to the formation of an iron-ovotransferrin-nitritotriacetic acid complex.

In order to look for interactions between anions and iron-EDTA that might perturb the equilibrium of the protein-iron-EDTA system, spectra were taken of a 3.0 mM iron-EDTA solution with various anions added. Significant changes in the iron-EDTA absorption spectrum were seen only when nitritotriacetic acid was added to the chelate solution. These data indicate that no significant interaction occurs between the other anions and iron-EDTA free in solution, as expected. It appears that the shift in equilibrium observed spectrally when anions are added to the iron-transferrin-EDTA solutions is due to protein-anion interactions rather than anion-iron-EDTA complexation.

Addition of Bicarbonate to Iron-Transferrin-EDTA Complex-The decrease in the visible absorbance when bicarbonate was added was unexpected. Reports in the literature suggest that carbonate ought to displace EDTA directly and to form a new ternary complex with a $\lambda_{\text{max}}$ at 470 nm (9, 11). The spectral changes observed when bicarbonate was added to ovotransferrin complexes were therefore studied in more detail.

Ovotransferrin was 80% saturated with iron-EDTA by forming the complex in the presence of a 1.25 molar excess of iron-EDTA to the number of binding sites present as before. A small aliquot of NH$_4$HCO$_3$ (usually 50 $\mu$L) was added to 3.0 ml of the iron-ovotransferrin-EDTA solution so that the final concentration of bicarbonate was 50 mM. Visible difference spectra were taken at various intervals with 2.94 mM iron-EDTA in the reference cell. These spectra are shown in Fig. 3. Within 5 min after the addition of bicarbonate, the absorbance at 490 nm due to the iron-ovotransferrin-EDTA complex decreased from 0.51 to a value of 0.18. After this time, the formation of a new species with a $\lambda_{\text{max}}$ of 465 nm was observed, typical of the iron-ovotransferrin-EDTA complexes. Similar results were obtained when iron-human serum transferrin-EDTA complexes were exposed to 50 mM HCO$_3^-$.

Several experiments were done in 75 mM Hepes and there was no change in the observed pH during the bicarbonate addition. Thus, a shift in pH was not an important factor in perturbing the complex in these experiments.

If the conversion of the ternary iron-ovotransferrin-EDTA complex ($\lambda_{\text{max}}$ = 490 nm) to the ternary iron-ovotransferrin-CO$_3^-$ complex ($\lambda_{\text{max}}$ = 465 nm) does not involve significant quantities of any intermediate species, one or more isosbestic points should be observed in Fig. 3. The distinct lack of such an isosbestic point is consistent with formation of significant quantities of species other than reactant and product during the conversion of the iron-ovotransferrin-EDTA complex to the iron-ovotransferrin-CO$_3^-$ complex.

In order to investigate the mechanism of this conversion, $^{57}$Fe-ovotransferrin-EDTA and $^{59}$Fe-serum transferrin-EDTA complexes were prepared. After bicarbonate was added to a final concentration of 100 mM, the amount of iron bound to the protein was monitored by passing aliquots through a gel filtration column and measuring the amount of radioactivity associated with the protein. The absorbance at 470 nm was monitored simultaneously and the results are shown in Fig. 4. The percentage of iron saturation of ovotransferrin was calculated from the percentage of the total radioactivity in the gel filtration eluate associated with the protein. Since there is a 7.5 molar excess of iron-EDTA initially, 100% saturated protein would have 17.5 or 13.3% of the total radioactivity, the rest eluting with the chelate peak. Before the addition of bicarbonate, the protein was approximately 80% saturated with iron as the iron-EDTA-protein complex, as determined by the initial absorbance at 490 nm. One minute after the bicarbonate was added, the first aliquot removed and subjected to gel filtration showed that the protein was only approximately 12% saturated with $^{59}$Fe. During the next 21 h, there is a parallel increase in absorbance at 470 nm.
and the amount of radioactivity associated with the protein as shown in Fig. 4. Similar results were obtained for the experiments using human serum transferrin. Apparently, the added bicarbonate is able to displace iron from the iron-protein EDTA complex, either via a direct displacement or more likely by shifting the equilibrium of the system.

Similar experiments were done using iron-ovotransferrin-[^1]CIEDTA complexes in order to determine the fate of the bound EDTA after the bicarbonate addition. The results are shown in Fig. 5. Before the addition of the bicarbonate, the protein was 70% saturated with EDTA as determined spectroscopically. However, 2.0 min after the addition of bicarbonate, there was less than 1% saturation of the binding sites with EDTA and this labeled molecule showed no further stable interaction with the protein as indicated in Fig. 5.

These labeling experiments are consistent with the notion that carbonate binds to the protein in some manner and perturbs the iron-protein-EDTA complex. Almost certainly, iron-EDTA dissociates from the complex in a concerted manner during the first minute or two of the reaction.

**DISCUSSION**

It was observed that phosphate and borate ions interfered with the exchange of iron from a nitrilotriacetic acid chelate to the binding sites of ovotransferrin. When iron-nitrilotriacetic acid was added to apo-ovotransferrin in 50 mM phosphate, it was concluded that very little of the added iron was incorporated into the binding sites of the protein, since the absorbance of the solution at 470 nm was low after 5 min. The initial burst in absorbance at 470 nm observed when iron-nitrilotriacetic acid was added appears to be the result of the formation of a iron-ovotransferrin-nitrilotriacetic acid complex. If the experiment is repeated in 50 mM phosphate, 50 mM bicarbonate, the absorbance at 470 nm increases rapidly as before and then remains constant for 24 h, indicating the formation of a stable iron-protein complex.

These results suggest that in the absence of sufficient bicarbonate, the iron-protein-nitrilotriacetic acid complex initially formed is destabilized by phosphate or borate. In the presence of 50 mM bicarbonate, iron can be incorporated from nitrilotriacetic acid by the protein to form the iron-transferrin-CO\(_3\) complex through a mechanism as reported in the literature (6, 7). A ternary complex of iron-transferrin-CO\(_3\) is formed which is stable to phosphate and borate. Consistent with this interpretation is the observation that the absorbance at 470 nm does not decrease when ditherr transferrin (prepared as the carbonate complex) is dissolved in 50 mM phosphate buffer.

Studies were pursued in more detail with the iron-transferrin EDTA and iron-ovotransferrin EDTA complexes which have the advantage over the nitrilotriacetic acid complexes in that these complexes have a maximum absorbance at longer wavelengths (515 nm and 490 nm, respectively) than the carbonate complexes (\(\lambda_{	ext{max}}\) at 465 nm). Therefore the EDTA and carbonate ternary complexes may be distinguished spectrally whereas the nitrilotriacetic acid complexes have the same \(\lambda_{	ext{max}}\) as the carbonate complexes (1).

A number of anions were able to perturb the iron-ovotransferrin-EDTA complex including phosphate, sulfate, and nitrate. This was seen by decreases in absorbance at 490 nm over a 5-min period. Since chloride, perchlorate, and nitrate did not decrease the visible absorbance significantly, effects due to changes in the ionic strength of the solution were not important. To verify this, when sodium chloride was added to the same ionic strength as NaH\(_2\)PO\(_4\) or Na\(_2\)SO\(_4\) (i.e. 300 mM) no perturbation of iron-ovotransferrin complex was observed. These results suggested that a number of anions were able to bind to the protein and in some way change the iron-ovotransferrin-EDTA complex.

When nitrilotriacetic acid was added to a solution of iron-ovotransferrin-EDTA, different spectral changes (compared to the other anions studied) were observed. Formation of an iron-ovotransferrin nitrilotriacetic acid complex can be inferred from the increases in the absorbance of the solution at 470 nm immediately after nitrilotriacetic acid was added.

---

**FIG. 4.** The changes in the amount of iron bound to ovotransferrin after the addition of HCO\(_3\)- to the iron-protein-EDTA complex. The concentrations of ovotransferrin and 57Fe-EDTA were 0.19 and 2.94 mM, respectively. The HCO\(_3\)- concentration was 100 mM. The solid curve shows the change in absorbance at 470 nm versus time. The relative saturation of the protein with iron was followed by determining the amount of \(^{57}\)Fe bound to the protein at various times as shown in the dashed curve. The initial saturation of the protein with iron was estimated by the initial absorbance of the solution at 490 nm. Therefore, there is no data point at the zero-time value since no accurate radioactive determinations could be made at this time in the experiment.

**FIG. 5.** The displacement of EDTA from the iron-ovotransferrin-EDTA complex by HCO\(_3\)-. The concentrations of ovotransferrin and Fe-[^1]CIEDTA were 0.19 and 2.94 mM, respectively. The concentration of HCO\(_3\)- was 100 mM. The amount of [^1]CIEDTA bound to the protein was determined at various intervals (O—O—O). The increase in the iron-ovotransferrin-HCO\(_3\)- concentration in the same solution was followed at 470 nm (—). No data point is shown for the saturation of the protein with [^1]CIEDTA at zero time, because this value was determined by the absorbance at 490 nm and does not represent a specific radioactivity determination. The value was approximately 70% and is off scale on the figure as drawn.
Anion Binding to Iron Transferrins

Apparently nitrilotriacetic acid is able to compete with EDTA for coordination sites on the iron, and the iron-nitrilotriacetic acid can deliver iron to the binding site of the protein. When nitrilotriacetic acid was added, spectral changes were observed, both in the position of $\lambda_{\text{max}}$ and extinction of iron-EDTA solutions, supporting the conclusion that nitrilotriacetic acid does compete for iron. Complexes of iron-nitrilotriacetic acid are known to be very efficient kinetically for the donation of iron to apotransferrin (7). Also, nitrilotriacetic acid has been shown to catalyze the exchange of iron from transferrin and chelates (14). Clearly, interactions of nitrilotriacetic acid and iron-nitrilotriacetic acid with transferrins are different from those of other iron complexes and chelators.

At the present time, it is not clear which of the hydrated forms of CO$_3^-$, carbonate or bicarbonate, is specifically bound by transferrin. In the present work there was no correlation between the ability of the anion to interact with iron-ovotransferrin-EDTA complexes and the ability of the anion to form a ternary complex with iron and transferrin, but these studies do indicate that charge may be an important factor in the anion-protein interactions. Glycine (a slight negative charge, but close to its isoelectric point at pH 7.8), which does function as an anion substitute, did not displace iron-EDTA while another anion substitute, citrate ($-3$ charge), was active. Also, sulfate ($-2$ charge) and phosphate ($-2$ charge), which do not form ternary complexes with iron and transferrin, were active, while the mononegative chloride, perchlorate, and nitrate had no effect on iron-ovotransferrin-EDTA complexes. These results suggest that a negative charge of at least $2$ is important in stabilizing anion-transferrin interactions. This would imply that it is carbonate and not bicarbonate which binds to transferrin.

Studies on the interaction of carbonate with iron-ovotransferrin-EDTA complexes yielded some unexpected results. It is clear that the mechanism of conversion of the iron-ovotransferrin-EDTA complex to the complex with carbonate and iron, does not include a direct displacement of EDTA by carbonate in the complex. Both the spectral studies as well as $^{55}$Fe and $^{54}$Fe labeling experiments show that there are one or more intermediate species in the exchange of EDTA for carbonate in the complex.

The expected result in these experiments, analogous to other mechanisms for the formation of carbonate complexes reported in the literature, should include a direct displacement of EDTA by carbonate to form the normal physiological iron-transferrin-CO$_3^-$ species. During such a process, iron would remain bound to the protein (6, 7, 9, 11). The fact that iron is displaced as well as EDTA by carbonate is best explained by a scheme that includes the binding of carbonate to the apoprotein. This binding at the anion binding site would shift the equilibrium toward apoprotein-CO$_3^-$ by mass action.

Ultimately, in this system, the iron-transferrin-CO$_3^-$ complex is formed via a mechanism yet to be determined. The present results do imply that a binary transferrin-CO$_3^-$ complex may be the reactive intermediate in the incorporation of iron from iron-EDTA rather than the ternary iron-transferrin-EDTA complex. Such a mechanism implies that carbonate must bind prior to the binding of a metal by the protein. A recent review also includes this suggestion, and our experiments give additional support to such a notion (15). Evidence for anion binding specifically to apotransferrin is interesting since it has been generally assumed that there is no stable protein-anion interaction in the absence of a metal (5). One brief report suggests that carbonate and oxalate may bind to apotransferrin, a conclusion derived from dialysis experiments and protein fluorescence quenching titrations with bicarbonate (16).

Attempts to obtain a value for the carbonate-apoprotein binding constant were unsuccessful. There is an additional equilibrium in this system between iron-EDTA and its dimer ($K_d = 10^{-2}$ m). Although several approaches were taken, the complicated interactions of iron chelates in this system made meaningful quantitation of the carbonate-apotransferrin binding constant difficult.

The data in this work suggest that there is an anion-protein interaction between apotransferrin and the anions used in these experiments. It has not been demonstrated that the anions are acting as a competitive species with iron-EDTA for the anion binding sites on the protein, although this seems likely. Whatever mechanism is involved in these experiments, it is clear that there is an intimate relation between the binding of iron-EDTA and anions by transferrins.

Acknowledgments — We wish to thank Daniel C. Harris for his critical review of the manuscript. David T. Osuga for technical assistance, Laura Hayes and Chris Howland for editorial assistance, and Clara Robison and Gail Nilson for typing of the manuscript.

REFERENCES

Interaction of anions with iron-transferrin-chelate complexes.
T B Rogers, R E Feeney and C F Meares


Access the most updated version of this article at http://www.jbc.org/content/252/22/8108

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/252/22/8108.full.html#ref-list-1