Influence of sialylation on the binding of ferric ion to human serum transferrin

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Introduction

Human transferrin is a plasma glycoprotein that transports ferric ions in the blood. It is comprised of two globular lobes, C-lobe and N-lobe, each with one metal-binding site for the ferric ion. The *N*-glycan structures on the C-lobe can be bi- or tri-antennary and each of them terminates with sialic acid. Physiological and pathophysiological changes in transferrin sialylation may alter the thermodynamic and kinetic properties of iron binding.

In this study, the equilibrium constants for the two binding sites of human transferrin were determined at pH 5.6 and 7.4, in the presence of different synergistic anions: oxalate (ox.) and carbonate (carb.) and different sialylation patterns: for the native transferrin (Tf+s) and desialylated transferrin (Tf-s) [1].

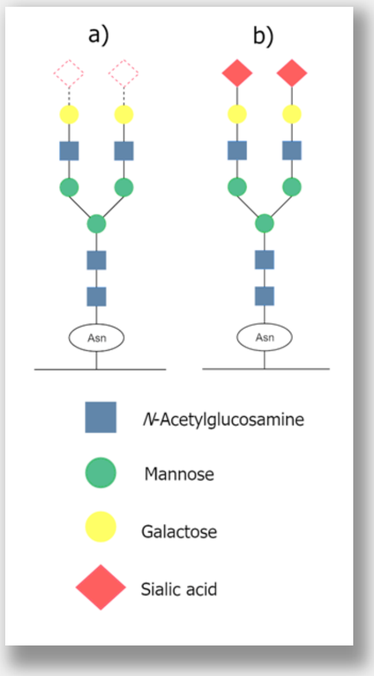

Figure 1. Schematic illustration of the most abundant *N*-glycans on C-lobe of the (a) desialylated (Tf-s) and (b) native human serum transferrin (Tf+s).

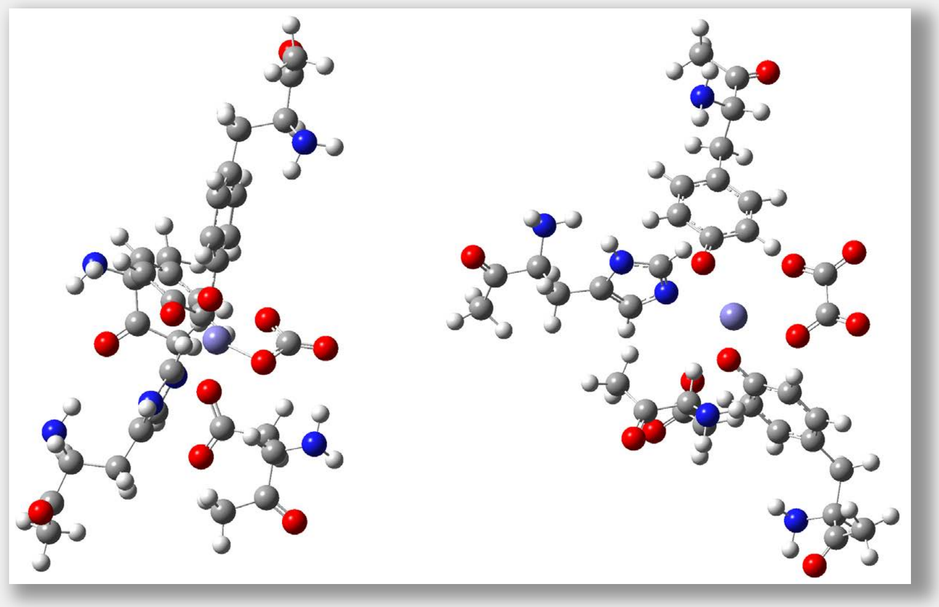

Figure 2. Common synergistic anions in the iron binding site of human serum transferrin: carbonate (left) and oxalate (right).

Results

Titrations of apotransferrin (transferrin containing no iron bound) with ferric ion presented in a nitrilotriacetate complex were performed and the extent of transferrin iron saturation was monitored spectrofluorometrically. Binding constants were calculated from the observed fluorescence intensities using HypSpec2014 software [2-5].

Table 1. Calculated binding constants for the binding of ferric ion to human serum transferrin.

	pH 7.4		pH 5.6
	log(K_1)	**log(K_2)**	**log(K_1)**
Tf+s carb.	18.45±0.21	19.77±0.44	14.10±0.06
Tf-s carb.	20.46±0.34	19.24±0.43	12.85±0.05
Tf+s ox.	19.65±0.26	18.97±0.30	13.64±0.04
Tf-s ox.	19.47±0.97	20.54±1.81	13.95±0.05

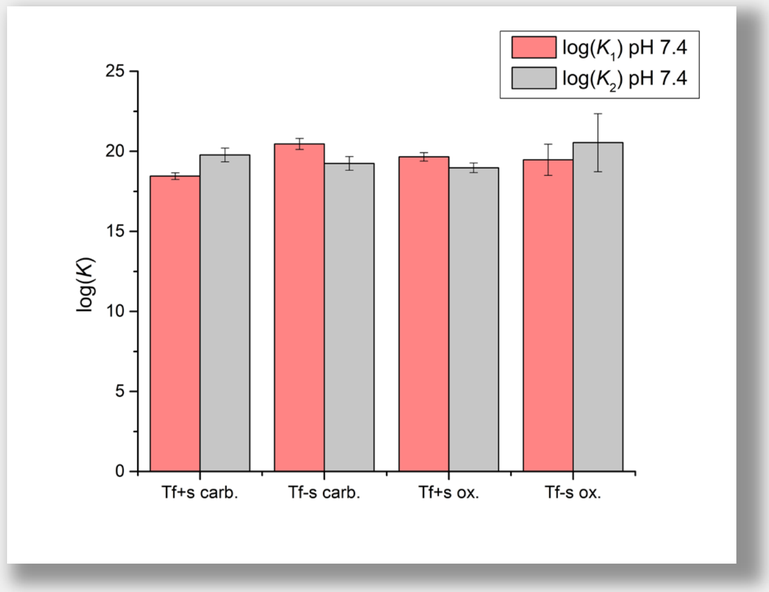

Figure 3. Schematic representation of the calculated Fe-Tf binding constants at pH 7.4.

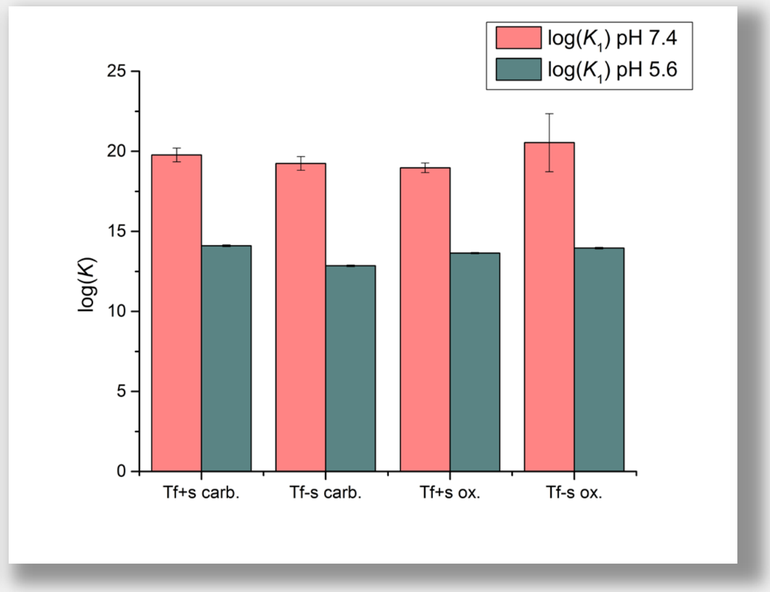

Figure 4. Schematic representation of the calculated binding constants for the binding of first ferric ion to transferrin at pH 5.6 and pH 7.4.

Conclusion

At pH 7.4 in the presence of carbonate as a synergistic anion, the calculated equilibrium constant for the binding of the second ferric ion to Tf+s is greater than the constant for the first ferric ion ($K_2 > K_1$), and in the presence of oxalate as a synergistic anion it is reverse ($K_2 < K_1$). A similar effect is observed for the binding of the ferric ion to Tf-s: in the presence of carbonate the first ferric ion binds to transferrin stronger than the second ferric ion ($K_2 < K_1$), and in the presence of oxalate it is reverse ($K_2 > K_1$). Such inverse affinities for the binding sites might be attributed to steric effects due to the difference in size between carbonate and oxalate. At pH 5.6 only one ferric ion binds to apotransferrin with a lower affinity than at pH 7.4 [6]. The difference in binding constants for Tf-s in the presence of oxalate and carbonate indicates the increased influence of sialylation on the preferred carbonate binding site. The results strongly suggest that the degree of sialylation significantly affects one of the transferrin iron binding sites, presumably on the C-lobe which also contains the *N*-glycan binding sites.

References

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