The effect of oxalate on the thermodynamic parameters for the binding of iron to human serum transferrin

Valentina BORKO[a,*], Tomislav FRIGANOVIĆ[a], Tin WEITNER[a]
[a]University of Zagreb, Faculty of Pharmacy and Biochemistry, Ante Kovačića 1, 10 000, Zagreb, Croatia
*vborko@pharma.hr

Introduction

Human transferrin is a serum glycoprotein that has ability to bind, transport and release ferric ions with two binding sites for metal ions, the C-site and N-site.
Each bound ferric ion is hexa-coordinated: four ligands to the metal are provided by the transferrin amino acid residues, while the remaining two are provided by an external anion, termed the synergistic anion. This synergistic anion is carbonate in vivo, however, other anions (such as oxalate, pyruvate, glycine, etc.) with similar structure can substitute for carbonate in vitro. [1]

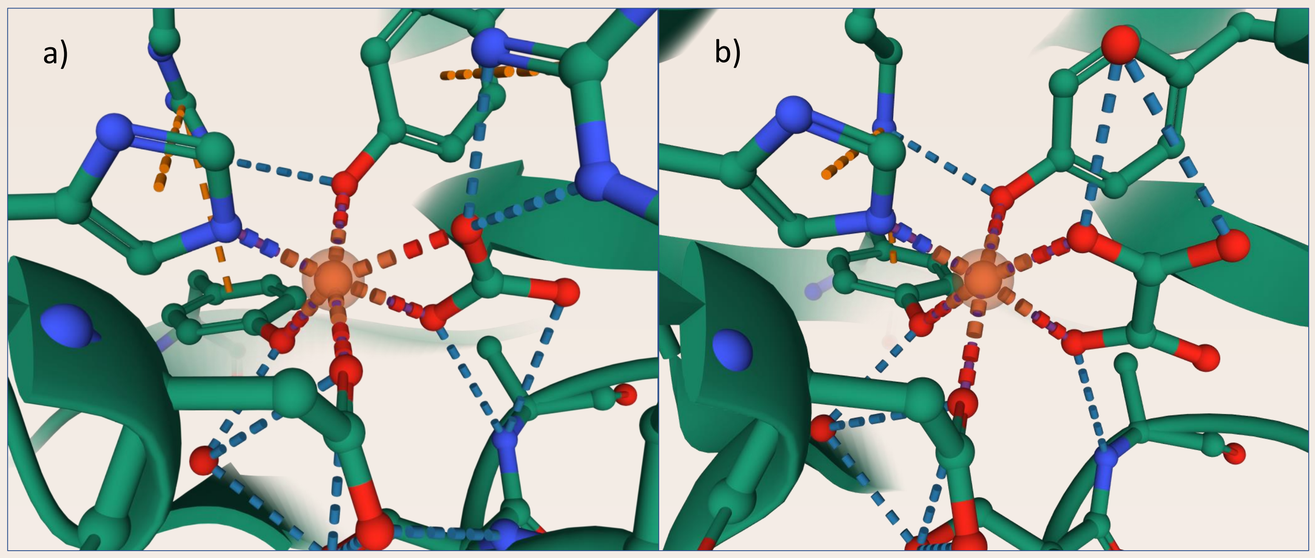

Figure 1. Human serum transferrin active site with a) carbonate b) oxalate as synergistic anions. [2-5]

Experimental

Isothermal titrations were performed using human serum transferrin in the presence of two different synergistic anions: carbonate (**Tf carb.**) and oxalate (**Tf ox.**) at pH 7.4. Ferric ion was introduced in the titration solution as nitrilotriacetate (NTA) complex with molar ratio of Fe : NTA = 1 : 2. The concentrations of the working solutions (apotransferrin and FeNTA) were determined spectrophotometrically prior to the measurement.

Results

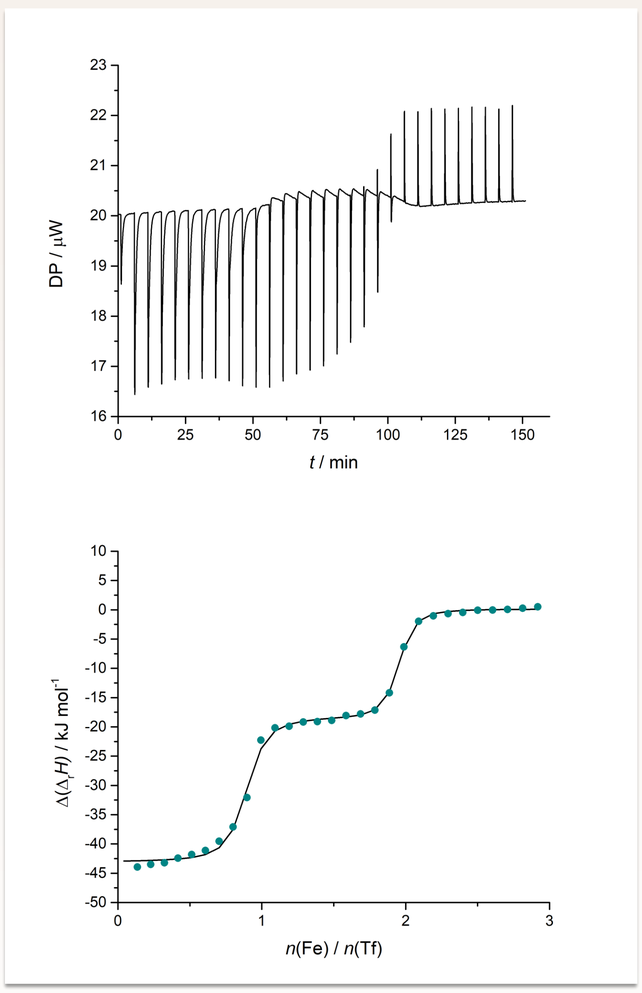

Figure 2. ITC data obtained from titration of human apotransferrin with FeNTA in the presence of carbonate (Tf carb.).

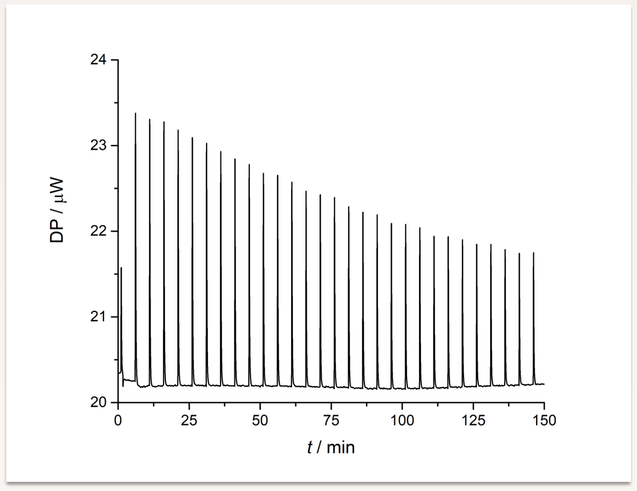

Figure 3. Control experiment (FeNTA + buffer).

Table 1. Thermodynamic parameters calculated from ITC titrations of transferrin with FeNTA in the presence of carbonate/oxalate.

Tf carb.		Tf ox.
C-lobe		
0.849	N	0.894
2.37e-9	K_d/ kJ mol^{-1}	1.36e-9
-43.2	$\Delta_r H$ / kJ mol^{-1}	-36.1
N-lobe		
1.06	N	1.09
4.51e-07	K_d/ kJ mol^{-1}	8.07e-08
-18.6	$\Delta_r H$ / kJ mol^{-1}	-17.5

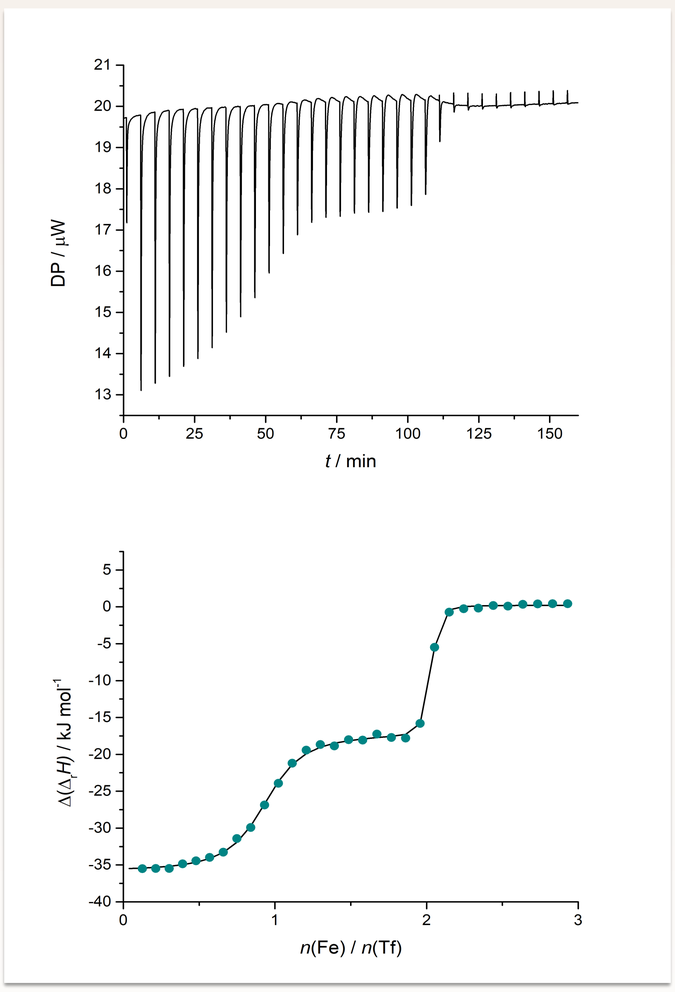

Figure 4. ITC data obtained from titration of human apotransferrin with FeNTA in the presence of oxalate (Tf ox.).

Resulting ITC curves displayed two inflection points typical for a model of two binding sites. Data analysis of the curves was based on the model of two sets of independent sites and best-fit parameters were calculated using Marquardt algorithm. Different slope in the two binding regions on the titration curve can be attributed to different binding affinities of ferric ion to human serum transferrin in the presence of different synergistic anions, suggesting lower affinities in the presence of oxalate. Also, the observed differences in the heat released in the first part of titration suggest different binding enthalpy for the C-site of transferrin. Similar effect on the two binding sites having different metal binding properties in the presence of different synergistic anions is known. [6]

References
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