

Influence of sialylation on the thermodynamic parameters for the binding of ferric ion to human serum transferrin Valentina BORKO¹, Tomislav FRIGANOVIĆ¹, Tin WEITNER¹

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Introduction

Human serum transferrin is a glycoprotein that consists of two structurally similar but functionally different lobes, referred to as N- and C-lobe, each with one iron binding site. Transferrin structure has two N-glycans covalently attached to residues ANS 413 and ASN 611.

This study investigated the influence of transferrin sialylation on the thermodynamic parameters for the binding of ferric ion to human serum transferrin using isothermal titration calorimetry (ITC).



Experimental

Isothermal titrations were performed using two different types of human serum transferrin: native apotransferrin (Tf+s) and desialylated apotransferrin (Tf-s). Desialylated apotransferrin was prepared by incubating native apotransferrin with immobilized neuraminidase enzyme. [3] Ferric ion was introduced in the titration solution as nitrilotriacetate 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.

N-Acetylglucosamine

Mannose

Galactose

Sialic acid

Congenital disorders of glycosylation (CDG)

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Pathological states

Figure 1. Factors influencing glycosilation in human serum transferrin.

Figure 2. Human serum transferrin structure (with attached carbohydrate) focused on the iron binding site on the Clobe. [1-2]

Figure 3. Shematic illustration of the most abundant N-glycans on C-lobe of the (a) native and (b) desiallyated transferrin.

Results







native human apotransferrin (Tf+s) with FeNTA.

Table 1. Thermodynamic parameters calculated from ITC titrations of Tf+s and Tf-s with FeNTA.

Tf+s		Tf-s
C-lobe		
0.601	N	0.869
3.66e-12	K _d / kJ mol-1	6.52e-10
-53.0	$\Delta_r H / kJ mol-1$	-44.5
N-lobe		
0.697	N	1.34
3.98e-10	K _d / kJ mol-1	1.83e-09
-23.2	Δ _r H /kJ mol-1	-31.4

desialylated human apotransferrin (Tf-s) with FeNTA.

ITC curves displayed two inflection points typical for a model of two binding sites. The first injections showed the binding of ferric ion to the C-site which saturates first, whereas the N-site saturates after the C-site. [4] Data analysis was based on the model of two sets of independent sites and best-fit parameters were calculated using Marquardt algorithm. The calculated parameters suggested that the degree of sialylation affects the binding thermodynamics of ferric ion to human serum transferrin, particularly the C-site.

References

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[2] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, Nucleic Acids Research, 28 (2000) The Protein Data Bank 235-242

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