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Note

Heat of saturation of apo and apo desialylated human serum transferrin at 298.15 K

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Abstract

To better evaluate the role of sialic acid residues in transferrin saturation, two different forms of human serum transferrin, namely the apo and the corresponding apo desialylated forms, were saturated with Fe^{3+} and the heats of saturation measured at 298.15 K. In both cases an exothermic reaction was observed, the value being larger for the apo form. The presence of sialic acid is believed to facilitate the saturation process in human serum transferrin.

Keywords: Adiabatic; Heat of reaction; Heat of saturation; Isothermal; Transferrin

1. Introduction

The transferrins are a class of homologous glycosylated single-chain metal-binding proteins (approx. 80 kDa) which function in the transport of iron to cells or as bacterostatic agents in a variety of biological fluids [1] and contain 6% (w/w) carbohydrate [2]. The transferrin molecule consists of two genetically related domains (N and C lobes) of similar molecular weight, connected by a short bridging peptide; each lobe is capable of binding a high-spin ferric ion (Fig. 1) and a synergic anion, usually bicarbonate in physiological situations [3]. The polypeptide chain of human serum transferrin (hTf) contains 679 amino acid residues and two glycosylation sites

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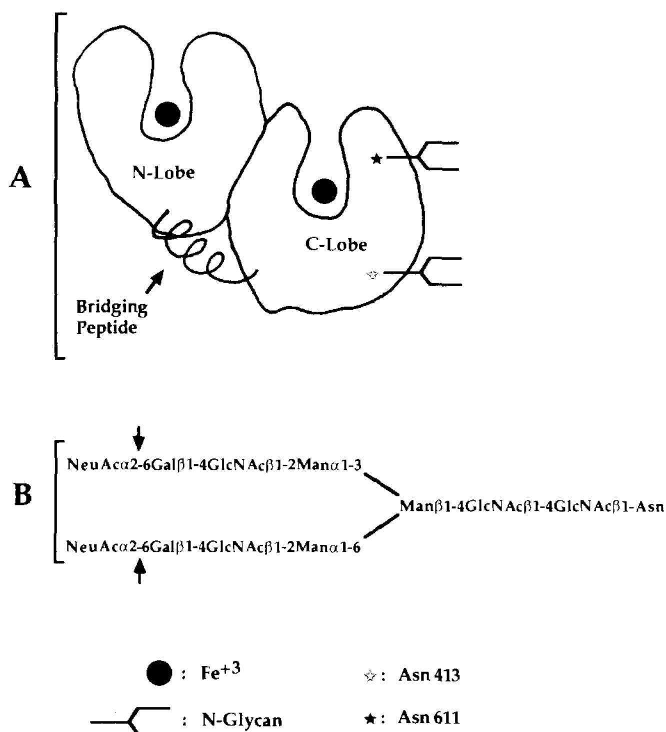

Fig. 1. A. Schematic representation of hTF. B. Primary structure of the major N-glycan present in hTf; Asn, asparagine; GlcNAc, *N*-acetylglucosamine; Man, mannose; Gal, galactose; NeuAc, *N*-acetylneuraminic acid (sialic acid). Greek letters and adjacent figure(s) give further information about the type of glycosidic bonds between residues. Arrows in B show the bonds hydrolyzed by neuraminidase.

both situated in the C lobe (Asn 413 and Asn 611) [4], each of which brings an oligosaccharide side chain of N type (N-glycan) [5]. The two N-glycans consist mainly of a biantennary structure with sialic acid as the terminal residue (Fig. 1) [5]. To gain further insight into the possible role of sialic acid in the saturation process of hTf, the apo form of hTf was treated with neuraminidase, a highly specific exoglycosidase which splits off the terminal sialic acid residues, then both the apo form and the corresponding desialylated one were separately saturated with Fe^{3+} presented as Fe-NTA (Fe complexed with nitrilotriacetate) and the heats of saturation measured. Both forms gave an exothermic reaction with a ΔH value of about -24.19 kJ mol^{-1} in the case of the apo hTf and -19.12 kJ mol^{-1} for the apo desialylated hTf. Our results suggest that sialic acid residues might play a physiological role in the saturation of hTf.

2. Experimental

Apo hTf was from Behring (Scoppito, L'Aquila, Italy) and was further purified by one-step immunoaffinity chromatography as previously reported [6]. The desialyla-

tion procedure was carried out as follows: a solution of apo hTf (100 mg in 10 ml of 60 mM Tris buffer, pH 7.4, containing 5 mM $NaHCO_3$), was treated with 0.4 U neuraminidase (Sigma Chemical Co., St. Louis, MO, USA) attached to agarose beads. The reaction was allowed to proceed for 48 h at 37°C and it was terminated by removal of the enzyme by centrifugation [7]. Subsequently, the supernatant was subjected to gel filtration on a Sephadex G-25 column (1.2 × 28 cm) equilibrated and eluted with protein buffer. The column eluate was monitored spectrophotometrically for its protein content (280 nm) and then the protein fractions recovered and pooled. The removal of sialic acid was judged by polyacrylamide gel electrophoresis in non-denaturing conditions (data not shown). After calibration, weighed amounts of protein solution were loaded in the reaction vessel of a Tronac 450-458 isoperibol calorimeter; then, pre-determined and appropriate amounts of Fe-NTA to give 100% saturation were added by means of a calibrated micro-titration buret. In all cases, thermal equilibrium was usually obtained within 20 min after charging the apparatus with buffer or protein solution, and about 10 min was allowed for the reaction to reach completion after adding Fe-NTA. Stock solutions of chelated ferric ion (Fe-NTA) were prepared by mixing 1 volume of 0.5 M $FeCl_3$ (in 0.05 M HCl) with 2 volumes of 0.5 M NTA in water and then the pH adjusted to 6 using a small amount of NaOH solution. The temperature changes in the vessel were measured using a thermistor and a Fluka model 8810A digital multimeter, and both reaction and cooling curves were recorded by means of an Olivetti M24 computer and a Hewlett Packard HP3396A integrator. Computer programs (in BASIC) containing calculation methods for the determination of saturation heats and reaction in isothermal and quasi-adiabatic calorimeters were used [8–10].

3. Results and discussion

Several reports have indicated that plasma hTf may be found partially desialylated mainly in alcoholic patients [11], and the degree of desialylation can also be correlated with iron-storage diseases in the liver [12]; thus, desialylated hTf could represent a good index for such physiological conditions. Moreover, it is possible that desialylated hTf, showing terminal galactosyl residues (Fig. 1), is removed from the blood circulation by asialoglycoprotein receptors of hepatocytes that are lectin-like molecules recognizing the exposed galactosyl residues of the glycoproteins [13]. Then, to investigate a possible role of sialic acid residues in hTf saturation, apo hTf and apo desialylated hTf were separately fully saturated with ferric ion and the heats of saturation were calculated by processing the thermal curves with a specific computer program. Before experiments, the heat of reaction of Fe-NTA in 60 mM Tris buffer, pH 7.4, containing 5 mM $NaHCO_3$, was determined at around 298.15 K, the reaction being endothermic with a ΔH value around $+4.03$ kJ mol^{-1} (Fig. 2 and Table 1). Figs. 3 and 4 show typical thermal curves obtained also at around 298.15 K, but they are related to the saturation of the two apo forms of hTf. In both cases one can see that during the saturation process an exothermic reaction occurs, being slightly more pronounced in the case of apo hTf (-24.19 kJ mol^{-1} for apo hTf and -19.12 kJ mol^{-1} for apo

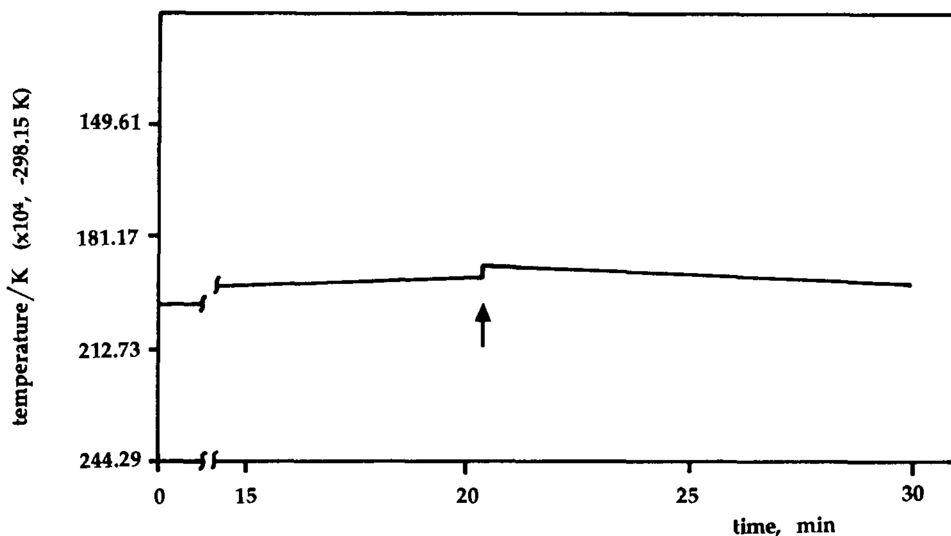

Fig. 2. Thermal curve obtained in Tris buffer, pH 7.4. Arrow shows the point of addition of Fe-NTA.

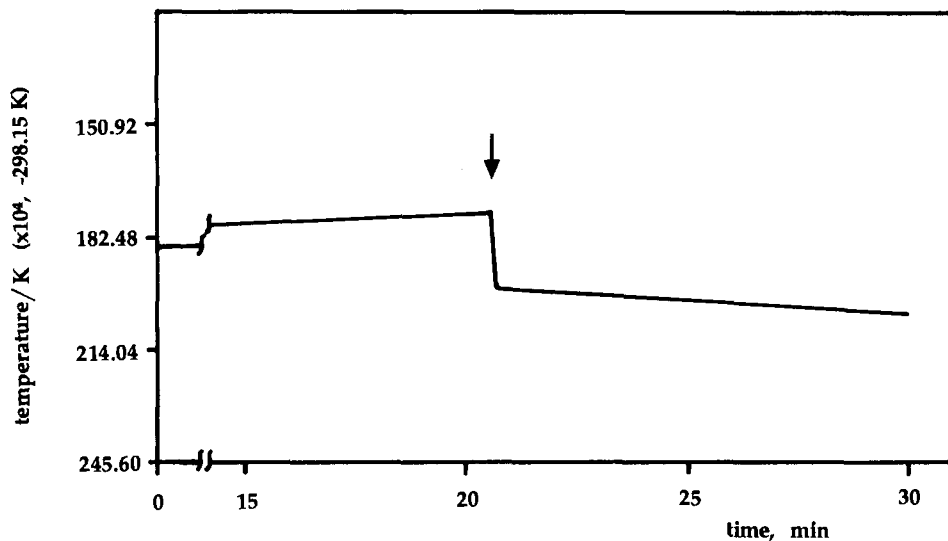

Fig. 3. Thermal curve obtained with a solution of apo hTf. Arrow shows the point of addition of Fe-NTA.

desialylated hTf). Conversely, when a solution of Fe-NTA was mixed with the protein buffer, an endothermic reaction was recorded (Fig. 1). This finding implies that the net heat saturation for both apo hTf forms should be 4.03 units larger (-28.22 kJ mol^{-1} versus -23.15 kJ mol^{-1}, respectively). Table 1 shows the enthalpies of reaction/saturation obtained using the same amount of Tris buffer solution or protein solution (2.5 g) and the same amount of Fe-NTA (6.25 molar in Fe^{3+}). Interestingly,

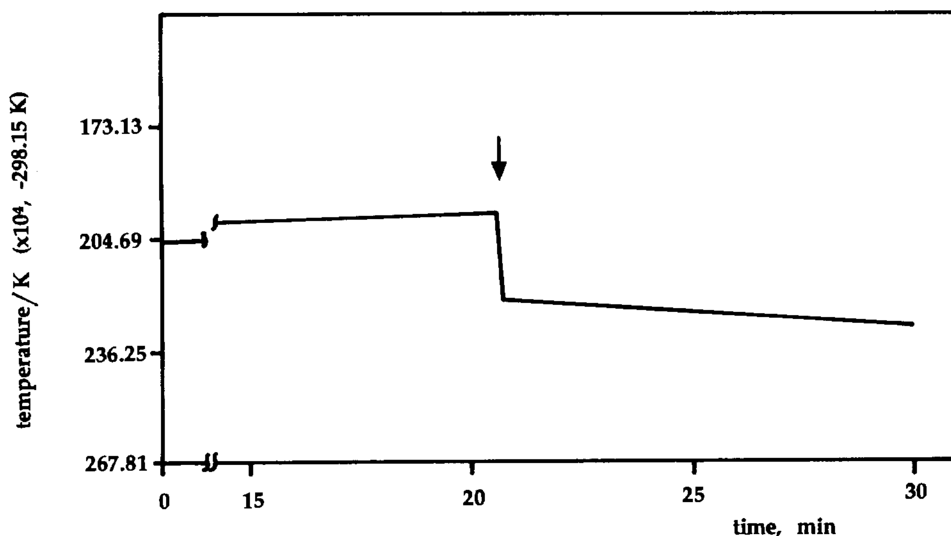

Fig. 4. Thermal curve obtained with a solution of apo desialylated hTf. Arrow shows the point of addition of Fe-NTA.

Table 1
Change in enthalpy on dilution of Fe-NTA in Tris buffer and on saturation of apo hTf and apo desialylated hTf

Solvent/protein solution[a]/(g)	Fe-NTA/ ($mol \times 10^7$)	ΔH/ ($kJ\ mol^{-1}$)	Average	Standard deviation
Tris buffer, pH 7.4				
2.5	6.2492	+4.01		
2.5	6.2498	+4.04	+4.03	0.019
2.5	6.2502	+4.04		
Apo hTf				
2.5	6.2503	−23.56		
2.5	6.2508	−23.98	−24.19	0.809
2.5	6.2514	−25.03		
Apo desialylated hTf				
2.5	6.2497	−18.56		
2.5	6.2506	−18.76	−19.12	0.858
2.5	6.2511	−20.04		

[a] hTf concentration was $10\,mg\,ml^{-1}$ in Tris buffer (60 mM Tris/HCl, pH 7.4, containing 5 mM $NaHCO_3$).

a more negative value of ΔH was obtained when apo hTf was saturated in comparison with the apo desialylated hTf. This behavior could be partly explained by considering that at physiological pH values, sialic acid residues are negatively charged; then, one can hypothesize the action of a certain driving force due to the presence of sialic acid in the two N-glycans. As far as both N-glycans are present in the C lobe of hTf, studies are

in progress to find out whether the two lobes are independent of one another in the saturation process or if they communicate as a result of ligand-dependent changes in the heat of saturation. Intriguingly, since hTf is normally found in the plasma as four forms under various degrees of saturation (holo, apo, N-monoferric, and C-monoferric) [13], there may be an advantage in investigating the physiological functions of these four forms and the corresponding desialylated ones.

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