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Corrigendum

Corrigendum to "Crystal structure of trypsin–turkey egg white inhibitor complex" [Biochem. Biophys. Res. Commun. 313 (2004) 8–16]

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In the above-referenced article, we reported the crystal structure of trypsin with the second domain of the ovomucoid turkey egg white inhibitor. This structure showed a continuous density near the calcium-binding loop, which was identified as a heptapeptide from the first domain of the inhibitor. However, reinterpretation of the electron density map clearly showed the presence of a disaccharide (di NAG) attached to Asn 11**I** of the inhibitor.

Turkey egg white ovomucoid inhibitor is N-glycosylated and the sugars (mannose and hexosamine) form 20–25% of the overall molecular weight (28,800 Da) [1] and a trace amount of carbohydrate must have been present in the inhibitor used for crystallization. The NAG molecules appeared at $1-3\sigma$ level in the final $F_o - F_c$ map (Fig. 1) and the introduction of di NAG reduced the R_{factor} from 0.19 to R_{factor} 0.17 and R_{free} 0.19. Some more density, which remained unexplained, was that of a tetrapeptide which was part (Res. 1 to 4 Seq: TNEE) of the heptapeptide previously identified to be from the first domain of the trypsin inhibitor. The density covered the peptide at 1σ level in the $(F_o - F_c)$ map. The peptide and the di NAG were stable during refinement. The average B factor for the peptide and di NAG was 94 and 68 Å^2, respectively. The refined coordinates are deposited in the Protein Data Bank (PDB code: 1Z7K).

N-glycosylated proteins have two NAG units attached to a few mannose units, and the first NAG is covalently linked to Asn residue of the glycosylated protein [2] as shown below.

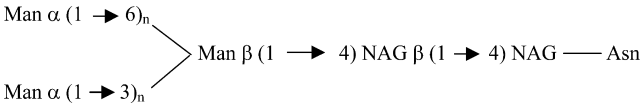

Table 1 shows that NAG has strong interactions both with the inhibitor and with the tetrapeptide fragment. In fact, the NAG units act as a mediator between the tetrapeptide and the inhibitor. The interactions between the peptide and the sugars mimic a β-sheet-like arrangement (Fig. 2). Remaining mannose residues were not present in the structure; presumably removed during purification. The tetrapeptide is held strongly by the NAG fragments and is situated on the surface near the calcium-binding loop of trypsin. The peptide interacts with His 71, Asn 72, Asp 74, Ser 153, and Leu 154 (Fig. 2) of the symmetry related trypsin molecule. The loop starting from 71 to 80 (calcium-binding loop) of trypsin deviates significantly (r.m.s. deviation 1.07 Å) from the native trypsin conformation to facilitate interactions with the tetrapeptide and thus stabilizing it. This is the first observation of a protein–sugar–peptide interaction in crystalline state.

A comparison with other N-glycosylated proteins was made to check the nature of interactions. The comparison revealed three different types of sugar interactions:

1. Sugars interacting with symmetry related molecules.
2. Sugars buried into the protein molecules.
3. Sugar molecules which are solvent exposed.

In general, N-linked sugars have strong interactions with the protein as well as symmetry related molecules. The interaction is either direct or through solvent. In the present structure, the sugar is attached to the Asn

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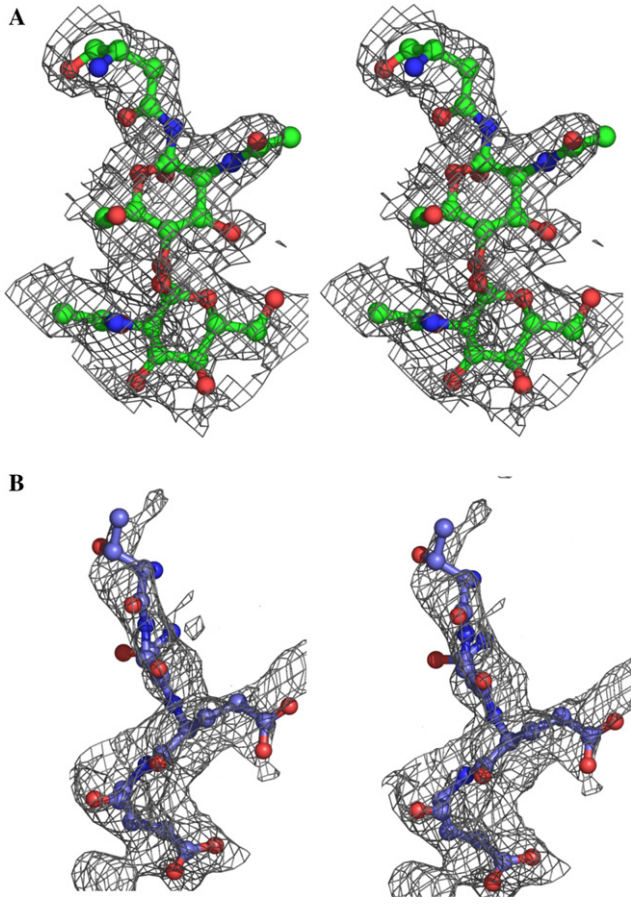

Fig. 1. Stereo view of the electron density of β-(1,4)-linked *N*-acetylglucosamine (NAG) dimer and the tetrapeptide. (A) NAG-1 appears at 3σ level and NAG-2 appears at 1.0σ level and (B) tetrapeptide at 1σ level in $(F_o - F_c)$ map, respectively.

11**I** of the inhibitor and the other end has a few weak interactions with the tetrapeptide, which is held by the calcium-binding loop of symmetry related trypsin molecule (Fig. 2).

Similar interactions are found in the crystal structure of an anti-neuraminidase–antigen complex [3] (PDBID:1A14, Fig. 3). It can be seen from Fig. 3 that four mannose residues attached to di NAG lie at the interface of symmetry related molecules and are involved in direct interactions with them. In the case of class II MHC molecule [4] (PDBID:1A6A), two NAG molecules are bound at different locations and one of them has direct interactions with the symmetry related molecule whereas other is solvent exposed.

In the case of erythrina corallodendron lectin and its complexes with mono- and disaccharides [5] (PDBID:1AX1), one end of the branched sugar containing mannose, fructose, xylinose, and di NAG interacts with the protein, while the other end is free. Similar observations have also been made in the crystal structure of the mouse glandular kallikrein-13 [6] (PDBID:1A05, Fig. 4). Finally, some of the O-linked sugars are found to be buried in the protein molecules [7].

In the present study, the free end of the di NAG interacts with the tetrapeptide through weak interactions. Similar interactions may be the cause for the aggregation of glycosylated hemoglobins and also the cell–cell recognition role of glycoproteins [8].

Table 1
Interactions of NAG with trypsin inhibitor and the peptide fragment

Res. No	Res. name	Atom	Res. No	Res. name	Atom	Dist.
Interactions with inhibitor						
7	Ser	OG	2	NAG	C6	3.68
7	Ser	OG	2	NAG	O5	3.47
7	Ser	OG	1	NAG	O3	3.38
7	Ser	OG	2	NAG	C5	3.50
7	Ser	OG	2	NAG	O6	2.85
7	Ser	C	1	NAG	O6	3.92
7	Ser	O	1	NAG	O6	3.06
11	Asn	CG	1	NAG	O5	2.71
11	Asn	OD1	1	NAG	C2	3.87
11	Asn	OD1	1	NAG	C5	3.89
11	Asn	OD1	1	NAG	O5	2.62
11	Asn	ND2	1	NAG	N2	2.66
11	Asn	ND2	1	NAG	C7	3.30
11	Asn	ND2	1	NAG	C3	3.58
11	Asn	ND2	1	NAG	O7	3.47
11	Asn	ND2	1	NAG	C5	3.52
Interactions with peptide fragment						
1	Thr	O	2	NAG	C8	3.45
2	Asn	O	2	NAG	O3	3.59
4	Glu	O	2	NAG	O4	3.54

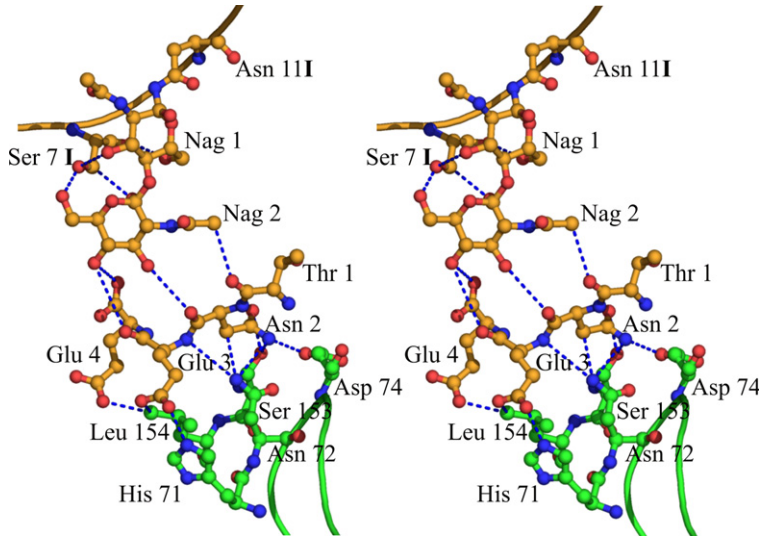

Fig. 2. Interactions of β-(1,4)-*N*-acetylglucosamine with tetrapeptide and trypsin inhibitor. The other end of the tetrapeptide interacts with the calcium-binding loop of the symmetry related trypsin molecule.

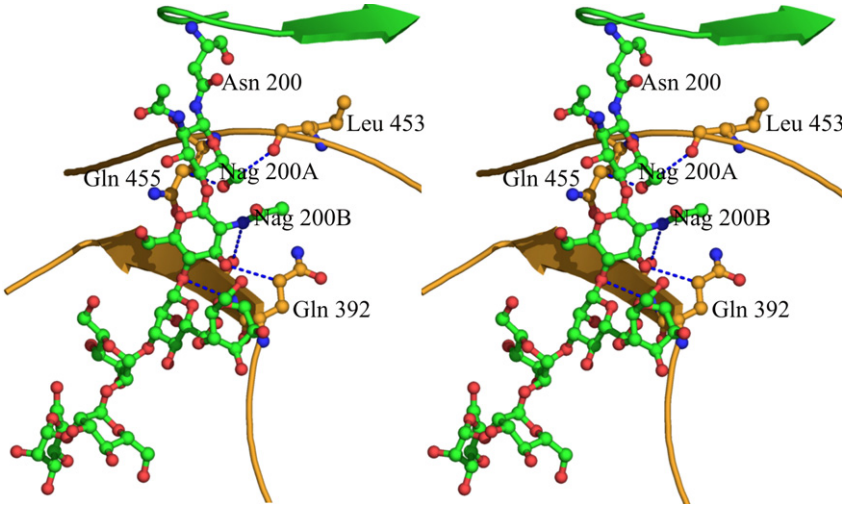

Fig. 3. The interactions of di NAG in the neuraminidase complex structure. Symmetry related molecule is shown in yellow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

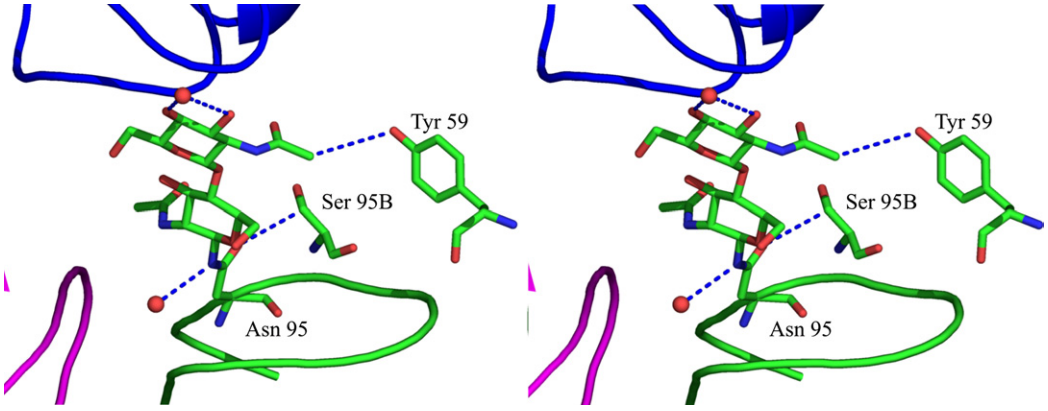

Fig. 4. The interaction of N-linked di NAG with kallikrein. The other end of the sugar is solvent exposed.

Acknowledgments

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