# 東北医科薬科大学

# 審査学位論文（博士）要旨

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| 氏名（本籍） | パン　　スンリ<br>潘　　順　麗　（中国） |
| 学位の種類 | 博士（薬科学） |
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| 学位授与の要件 | 学位規則第 4 条 1 項該当 |
| 学位論文題名 | Each *N*-glycan on human IgA and J-chain uniquely affects oligomericity and stability<br>（ヒト IgA および J 鎖の N-型糖鎖修飾が複合体形成や安定性に及ぼす影響） |
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**Each *N*-glycan on human IgA and J-chain uniquely affects oligomericity and stability**

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**Background:** Immunoglobulin A (IgA) plays a pivotal role in various immune responses, especially that of mucosal immunity. IgA is usually assembled into dimers with the contribution of J-chains, which bind to the secretory component (SC) of the polymeric Ig receptor (pIgR) and transported to the mucosal surface. There are two *N*-glycosylation sites in human IgA1-Fc, Asn263, Asn459 and one in the J-chain, Asn49. There is no consensus as yet on the functional role of the *N*-glycosylation.

**Aims:** The aim of this study is to understand the structural and functional role of *N*-glycosylation in J-chain and IgA.

**Methods:** To gain a better understanding of their role, we designed a series of IgA1-Fc plasmids (Wild Type, C471S, C471S/N263Q/N459Q, N263Q/N459Q, N263Q, N459Q, C471S/N263Q and C471S/N459Q) and J-chain plasmids (Wild Type, C15S/C69S, C15S/C69S/N49Q and N49Q), which were recombinantly expressed alone or in combination. The glycan structure was analyzed by LC-MS/MS and lectin blotting. Thermofluor assay and ANS (8-anilino-1-naphthalenesulfonic acid) fluorescence assay were performed to examine the melting temperature ($T_m$), hydrophobicity of each protein. NMR analysis of metabolically $^{13}$C-labaled Fc was conducted to determine flexibility of each *N*-glycan.

**Results:** IgA1-Fc without the J-chain, was predominantly expressed as a monomer, and in its presence dimers and some polymers appeared. N263 (Fc Cα2), N459 (Fc tailpiece) and N49 (J-chain) were shown to be site-specifically modified with *N*-glycans by lectin blotting and mass spectrometry analysis. Mutant IgA1-Fc N459Q failed to form a proper dimer in the presence of the J-chain, instead higher-order aggregates appeared. ANS fluorescence experiments suggest that the N459-glycans cover a hydrophobic surface at

the Fc tailpiece that prevents other Fc molecules from approaching the dimeric IgA. A thermofluor assay revealed that the *N*-glycans at N263 (Fc) and N49 (J-chain) both contribute in different ways to the thermal stability of the Fc-J-chain complex. NMR analysis of $^{13}$C-labeled Fc suggests that the N459-glycan is relatively flexible while the N263-glycan is more rigid.

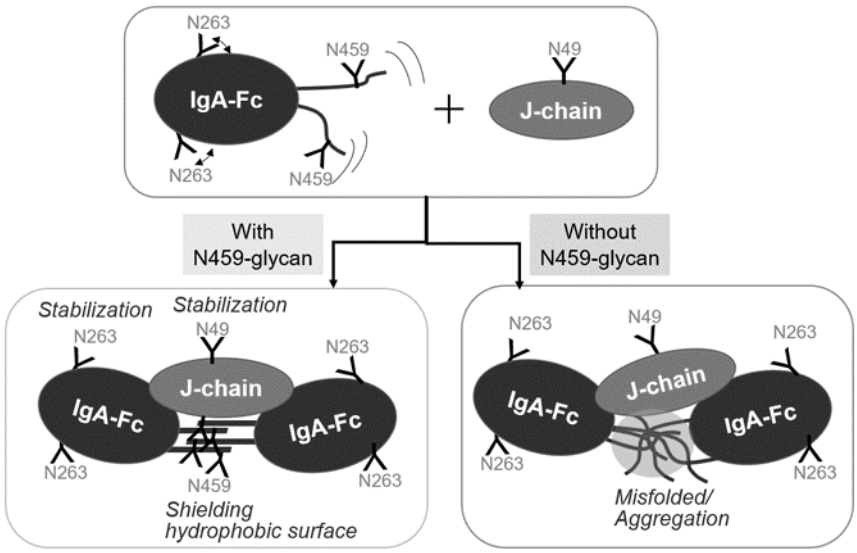


**Fig. 1. Role of each *N*-glycan in IgA1-Fc-J-chain assembly and stability**

**Conclusions:** We conclude that the N459-glycan of IgA1-Fc is essential for initial dimer formation and prevention of higher-order aggregates while those at N263 (Fc) and N49 (J-chain) stabilize the Fc-J-chain complex (Fig. 1).

＜参考文献＞　主論文（原著論文）

**Shunli Pan**, Noriyoshi Manabe, Shiho Ohno, Sachiko Komatsu, Tsutomu Fujimura, Yoshiki Yamaguchi. Each *N*-glycan on human IgA and J-chain uniquely affects oligomericity and stability. *Biochimica et Biophysica Acta (BBA) - General Subjects* 1868(2), 130536 (2024)