Synthesis and Immunological Evaluation of *Escherichia coli* O1-Derived Oligosaccharide–Protein Conjugates toward Avian Pathogenic *Escherichia coli* O1 Vaccine Development

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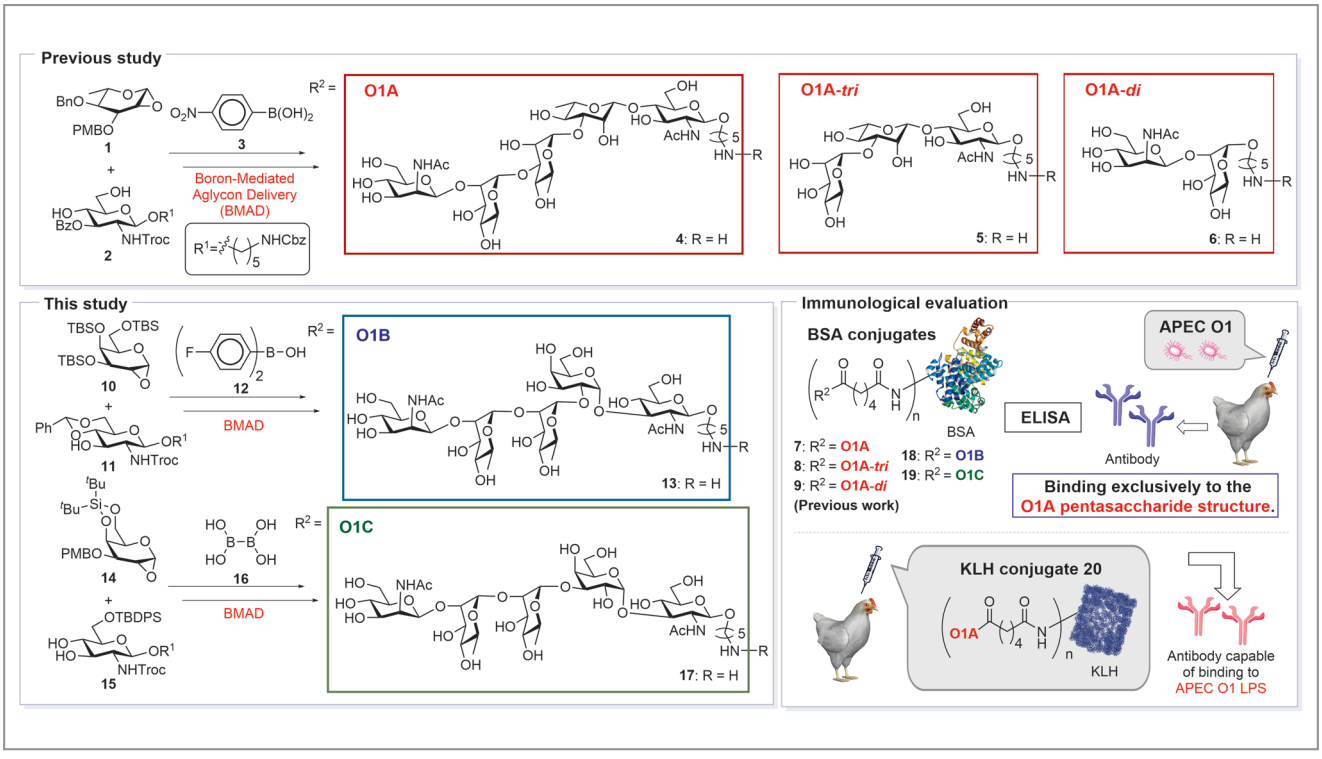
Avian *Escherichia coli* O1 (APEC O1) is a pathogenic bacterium that causes significant economic losses to the poultry industry and is also feared to cause zoonotic infections due to its genomic similarity to human pathogenic *E. coli*. In addition, the emergence of drug-resistant strains is a concern and the development of a glycoconjugate vaccine with a high safety profile would be strongly desirable. However, the glycan structures of APEC O1 antigen have not been clarified yet, which does not help the development of such a vaccine.

The group of Professor Kazunobu Toshima and Associate Professor Daisuke Takahashi at Keio University (Yokohama, Japan) has been studying this area, focusing on the three glycan structures O1A, O1B, and O1C, reported to be present in *E. coli* O1. Professor Takahashi said: "We hypothesized that the O1A antigen structure, which has been reported to be pathogenic, may be important as a potential glycotope for APEC O1."

"In our previous studies, we first synthesized complex glycoconjugates with O1A pentasaccharide, trisaccharide, and disaccharide structures using our boronic acid catalyzed BMAD (boron-mediated aglycon delivery) method (*Angew. Chem. Int. Ed.* **2015**, *54*, 10935–10939; *Chem. Commun*. **2017**, *53*, 3018–3021; *J. Am. Chem. Soc.* **2018**, *140*, 3644–3651; *Angew. Chem. Int. Ed.* **2018**, *57*, 13858–13862; *Nat. Commun.* **2020**, *11*, 2431; *Chem. Eur. J.* **2020**, *26*, 10222–10225; *Angew. Chem. Int. Ed.* **2023**, in press, DOI: 10.1002/anie.202307015)," said Professor Takahashi. He continued: "We evaluated their immunological activities to identify candidate glycans for the APEC O1 antigen, and revealed for the first time that the O1A



Scheme 1 State of the art and main results of the study

pentasaccharide structure is the glycotope of APEC O1 (*Angew. Chem. Int. Ed.* **2021**, *60*, 1789–1796)."

In this study, the authors first synthesized complex glycoconjugates with O1B and O1C pentasaccharide structures using borinic acid catalyzed and diboron-catalyzed BMAD methods (for borinic acid catalyzed methods see: *Org. Lett.* **2016**, *18*, 2288–2291; *Org. Lett.* **2016**, *18*, 5030–5033; *J. Org. Chem.* **2018**, *83*, 7281–7289; for diboron-catalyzed method see: *J. Org. Chem.* **2020**, *85*, 16254–16262) and evaluated their immunological activities. Professor Takahashi remarked: "Interestingly, it was clearly shown that only the O1A pentasaccharide, incorporating a β-rhamnoside structure, is important as a potential glycotope for APEC O1." He continued: "Furthermore, we also synthesized a KLH-O1A pentasaccharide conjugate and evaluated its antigenicity. ELISA tests confirmed the efficient production of antibodies capable of binding to both APEC O1 LPS and O1A-pentasaccharide structures, indicating that the glycoconjugate with O1A pentasaccharide is a promising vaccine candidate against APEC O1."

Professor Takahashi told SYNFORM: "One of the most impressive results is that we were able to demonstrate that only the O1A pentasaccharide containing the β-rhamnoside structure among O1A, B, and C is useful as an epitope, as per the working hypothesis. The other remarkable result is that KLH-O1A pentasaccharide conjugate produced antibodies that bind to APEC O1 LPS and O1A-pentasaccharide structures."

The authors believe that since the vaccine candidate against APEC O1 discovered in this study is not made from the pathogen, but has chemically synthesized homogeneous sugar chains that are less likely to cause adverse reactions, this makes it potentially valuable from a practical standpoint.

"We were able to demonstrate the viability of our BMAD methods through the efficient synthesis of O1B and O1C pentasaccharides. Therefore, the broad application of this method to the synthesis of antigenic glycan candidates holds great promise for the development of new glycoconjugate vaccines against various pathogens," said Professor Takahashi. He concluded: "In addition, if APEC with O1B and O1C pentasaccharide structures are expressed in the future, the glycoconjugates synthesized in this study may become vaccine candidates."

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Prof. K. Toshima

Kazunobu Toshima received his Ph.D. in 1988 from Keio University (Japan) under the supervision of Professors Mitsuhiro Kinoshita and Kuniaki Tatsuta. He spent one year in Professor K. C. Nicolaou's group at the University of Pennsylvania (USA) as a postdoctoral fellow. He was appointed as Lecturer of the Department of Applied Chemistry at Keio University in 1989. There, he was promoted to Assistant Professor in 1994, Associate Professor in 1996, and Full Professor in 2003. He was awarded The Chemical Society of Japan Award for Young Chemists in 1995, Taro Yamashita Academic Award in 1996, The Chemical Society of Japan Award for Creative Work in 2014, and SSOCJ Daiichi-Sankyo Award for Medicinal Organic Chemistry in 2015.

Prof. D. Takahashi

Daisuke Takahashi received his Ph.D. in 2006 from Tokyo Institute of Technology (Japan) under the supervision of Professor Takashi Takahashi. He spent two years in Professor Ole Hindsgaul's group at the Carlsberg Laboratory in Denmark as a JSPS fellow and as a postdoctoral fellow. He was appointed as a research associate of the Department of Applied Chemistry at Keio University (Japan) in 2008, where he was promoted to Assistant Professor in 2012 and Associate Professor in 2016. He was awarded the Incentive Award from the Japanese Society of Carbohydrate Research in 2016, the Incentive Award from Synthetic Organic Chemistry, Japan in 2016, and The Carbohydrate Research Award in 2021.

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