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Site-Selective Unnatural Amino Acid Incorporation at Single or Multiple Positions to Control Sugar-Protein Connectivity in Glycoconjugate Vaccine Candidates

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Abstract: *In cellulo* site-specific unnatural amino acid incorporation based on amber stop codon reassignment is a powerful tool to modify proteins at defined positions. This technique is herein applied to the selective functionalization of the Pneumococcal surface adhesin A protein at three distinct positions. N^{ϵ}-propargyloxycarbonyl-L-lysine residues were incorporated and their alkyne groups reacted using click-chemistry with a synthetic azido-functionalized tetrasaccharide representative of one repeat unit of the *Streptococcus pneumoniae* serotype 14 capsular polysaccharide. Anti-PsaA antibody response induced in mice by the trivalent glycoconjugate was determined in comparison with corresponding monovalent and randomly functionalized conjugates. Our results suggest that controlled was superior to random conjugation for preserving antigenicity. In definitive, the reported strategy offers a unique opportunity to study the impact of carbohydrate antigen-carrier protein connectivity on immunogenicity.

Introduction

Glycoconjugates vaccines formed of bacterial surface polysaccharides grafted on a carrier protein are among the most efficient means to fight against bacterial infection. The introduction of commercial glycoconjugate vaccines active against meningococcus, pneumococcus or *Haemophilus influenzae* type b has considerably reduced the incidence of these pathogens both in terms of infections and carriage.[1,2,3] Induced humoral response depends on the administered dose, the choice of both the carbohydrate antigen and the carrier protein, the coupling chemistry but also on parameters such as the length of the carbohydrate antigen, the carbohydrate antigen/protein ratio and the connectivity, that is, positions at which the carbohydrate antigen is linked to the carrier protein.[4] The relationship between the carbohydrate hapten length and the sugar/protein ratio has been documented and the general trends are now well-established. It usually looks like a bell curve: the anti-carbohydrate humoral response increases with the sugar/protein ratio to reach a maximum and then diminishes if further carbohydrate haptens are grafted onto the carrier protein. Moreover, the maximum is observed at a higher sugar/protein ratio when reducing the carbohydrate length.[5,6,7] This means that one can use short rather than long synthetic oligosaccharide haptens at the expense of a higher grafting to induce a similar immune response level. At the same time a high sugar/protein ratio masks or denatures carrier protein B epitopes giving rise to a low anti-carrier protein antibody (Ab) response.[8] This can be regarded as an advantage to avoid carrier protein/carbohydrate hapten competition referred to as carrier-induced epitopic suppression[9] except for proteins with dual role of carrier and antigen. For such purpose and also because it is now demonstrated that the carbohydrate antigen can activate $CD4^+$ T cell subsets (referred to as Tcarb) when presented on the surface by Major Histocompatibility Class II (MHCII) molecules covalently linked to a T-helper peptide from the carrier,[10] connectivity is certainly equally important. However, less is known about the influence of the connectivity because fully controlled site-specific conjugation methods are lacking. Site-specific conjugation can be achieved both enzymatically and chemically: The revolutionary bacterial protein glycan coupling technology makes possible the direct transfer of polysaccharides to a protein catalyzed by an oligosaccharyltransferase *in cellulo*.[11] Alternatively the enzymatic transfer can be carried out *in vitro* using transglutaminases.[12] Chemical bioconjugation techniques can also be applied. These ap-

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proaches usually exploit the rarity of disulfide bonds or surface-exposed tyrosines to master the connectivity.[12,13] However, these strategies often require extensive sequence engineering (*e.g.*, to introduce consensus sequences) or do not allow to direct the conjugation to the desired domain. Carrier protein mutagenesis to incorporate a cysteine[14,15] or an unnatural amino acid (UAA) bearing a biorthogonal functional group, followed by their selective coupling with a carbohydrate antigen,[16] can be accomplished with minimal modifications and offer a higher flexibility. On the one hand, UAA can be introduced according to a residue specific strategy which consists in replacing an amino acid – usually methionine – by a bioisostere UAA. This implies the use of a strain auxotrophic for this amino acid, its systematic replacement and possibly extensive mutagenesis to introduce or remove this amino acid from the sequence of the protein of interest.[17] To our knowledge, this approach has been tested once for the preparation of a glycoconjugate candidate vaccine based on the replacement of a unique methionine introduced in the Qβ bacteriophage capsid protein sequence. Of note multivalency was reached thanks to the self-association of the modified capsid protein in a Virus-Like Particle.[14] On the other hand, one can rely on the site specific strategy whereby an UAA is incorporated at a position corresponding to an amber stop codon, introduced by mutagenesis in the protein gene sequence.[18] According to this approach full-length protein biosynthesis is only achieved if the UAA is effectively incorporated, an important feature for quality control process and protein purification. Hence it has been applied both for cell free and *in cellulo* glycoconjugate vaccine candidates.[19,20] Only recently Kapoor *et al.* reported a multiple incorporation of *p*-azidomethylphenylalanine into a carrier protein using cell free protein synthesis platform.[21] We report herein the *in cellulo* incorporation of an UAA at three distinct positions within a protein sequence as a complementary strategy. To this aim, surface-exposed lysine residues of a pneumococcal protein were replaced with an UAA bearing a bioorthogonal alkyne functional group. The modified protein was next further conjugated using copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) reaction with a synthetic azido-functionalized tetrasaccharide related to the repeating unit of *Streptococcus pneumoniae* type 14 capsular polysaccharide (Pn14TS) to afford the corresponding glycoconjugate. The humoral response evaluated in mice for this conjugate compared to controls randomly modified at three positions or modified at a single chosen position outlined how carbohydrate antigen- protein connectivity influences the level of this response.

Results and Discussion

Conjugate design and mPsaA mutant preparation

Pneumococcal surface adhesin A (PsaA) was selected as the carrier protein. This highly-conserved protein, expressed by >99% pneumococcal serotypes,[22,23] is involved in Mn^{2+} ion transport and beyond, in host epithelial cell adherence.[24,25,26] For these reasons, it has been suggested that PsaA could be included in investigational pneumococcal vaccines: PsaA showed significant protection against colonization[27] and remained immunogenic when used as a carrier protein in mice models.[28,29,30] In addition, that PsaA B- and T-epitope mapping has been documented experimentally[31,32,33] was considered as an asset to select the positions to be grafted and to explore the effect of carbohydrate antigen/protein connectivity on immunogenicity. We previously reported the preparation of homogeneous mature PsaA (mPsaA)-based conjugates thanks to cysteine mutagenesis carried out at positions K237, K247 (close and within a T-helper epitope) and K309 (the C-terminal residues distant from known epitopes).[15] Adopting the site-specific incorporation of unnatural amino acid (UAA) strategy,[18] we decided to introduce amber stop codon TAG at three distinct positions of the mPsaA sequence, coding for surface-exposed lysine residues namely residues K32, K213 and K237 which differ from previously investigated ones. They are respectively located within very strong/strong predicted mouse H2-IAd/b epitopes ($PsaA^{29–43}$),[34] an experimentally proven B- and T-epitope ($PsaA^{209–219}$) and close to but outside a T-helper epitope region ($PsaA^{243–257}$) (Figure 1). Conjugates at the first two positions were thus expected to induce a stronger anti-Pn14TS response according to the mechanism unraveled by Avci *et al.*[10]

Adopting a stepwise strategy, every mutation was investigated separately prior to be combined with the others. Along this line, *E. coli* BL21(DE3) expression strain was first transformed with a pEVOL plasmid coding for the *Methanosarcina mazei* pyrrolysyl-tRNA synthetase/$tRNA_{CUA}$ pair[35] known to transfer the non-canonical amino acid N^{ϵ}-propargyloxycarbonyl-L-lysine (PrK).[36] Then the bacteria were transformed with either a pET11 coding for each mPsaA mutant with a *N*-terminal hexahistidine-tag or a pET24d coding for each mPsaA mutant with a C-terminal hexahistidine-tag. Mutant mPsaA harboring

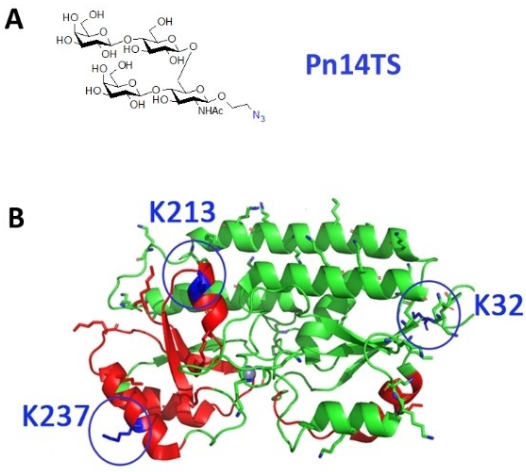


Figure 1. (A) Structure of tetrasaccharide antigen from *S. pneumoniae* serotype 14 capsule (Pn14TS); (B) Ribbon diagram of PsaA (green). Lysine side-chains are represented in green except the three lysines targeted for mutagenesis (in blue); Known T-epitopes are represented in red. Representation based on the 1PSZ PDB file, with a resolution of 2.0 Å.[24]

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N-terminal histidine tags were expressed under optimized conditions (see Supporting Information) to check the specificity of UAA incorporation. As revealed by Western-Blot analysis (Figure 2A), full length mutants are only detected when PrK was added to the growth culture medium (blue arrow, 34.3 kDa). In absence of PrK, only truncated forms of the K213 and the K237 mutants were expressed (red arrow, 23.2 and 25.9 kDa) but the 2.8 kDa truncated K32 mutant was not visible. These observations confirmed that the TAG codon remained partially recognized as a stop codon. Most importantly the *M. mazei* pyrrolysyl-tRNA synthetase/$tRNA_{CUA}$ pair was orthogonal to the cell protein biosynthesis machinery and unable to use a canonical amino acid in place of PrK in Bl21 (DE3) strain. In a second series of experiments, the three targeted mutant proteins were expressed and produced with a histidine tag modification at their C-terminus. As observed previously, both full-length and truncated forms were produced as a mixture (Figure 2B, blue – 34.5 kDa – and red arrows – 24.2 and 21.5 kDa). However, both forms could be separated using Ni affinity chromatography as exemplified for mutant K213 (Figure 2C), with a yield of 4 mg of modified protein per liter of culture.

The strategy was next applied to the production of the mPsaA mutant combining the three K32*-K213*-K237* mutations. However, presence of the full length triple PsaA mutant could not be detected even by using sensitive Western-Blot analysis (Figure 3A). We reasoned that this failure might be due to unfavorable competition between the Prk-loaded tRNA and the release factor 1 (RF1) which terminates protein translation

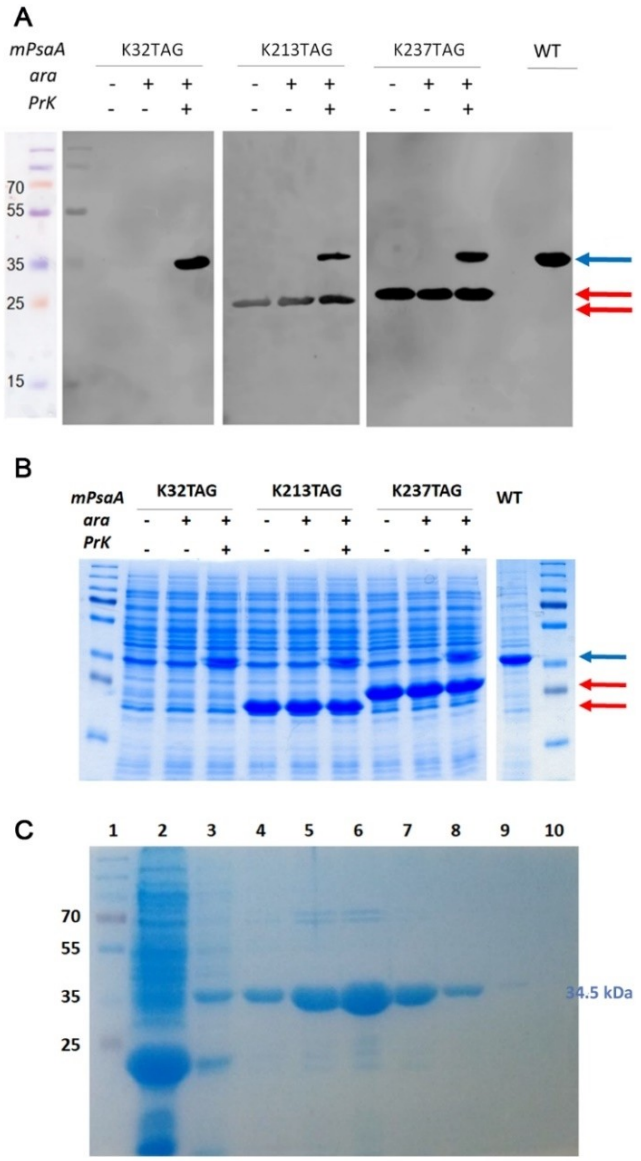


Figure 2. Analysis of mPsaA expression. (A) Western-Blot analysis of cell lysates from mutant strains grown in the presence or absence of PrK and arabinose (ara) revealed with a mAb anti-histidine tag. mPsaA mutants are tagged with a *N*-terminal 6-histidine; (B) SDS-PAGE analysis (12% acrylamide gel stained with coomassie blue) of raw protein extracts from mutant strains grown in the presence or in the absence of PrK and arabinose. mPsaA mutants are tagged with a *C*-terminal 6-histidine; (C) SDS-PAGE analysis (12% acrylamide gel stained with coomassie blue) of the PsaA-K213PrK mutant purified by affinity chromatography on a nickel column. Lane 1: protein marker; Lane 2: flowthrough fraction; Lanes 3–10: eluted fractions with increasing concentration of imidazole (10 to 300 mM of imidazole). Blue and red arrows indicate full-length and truncated proteins, respectively.

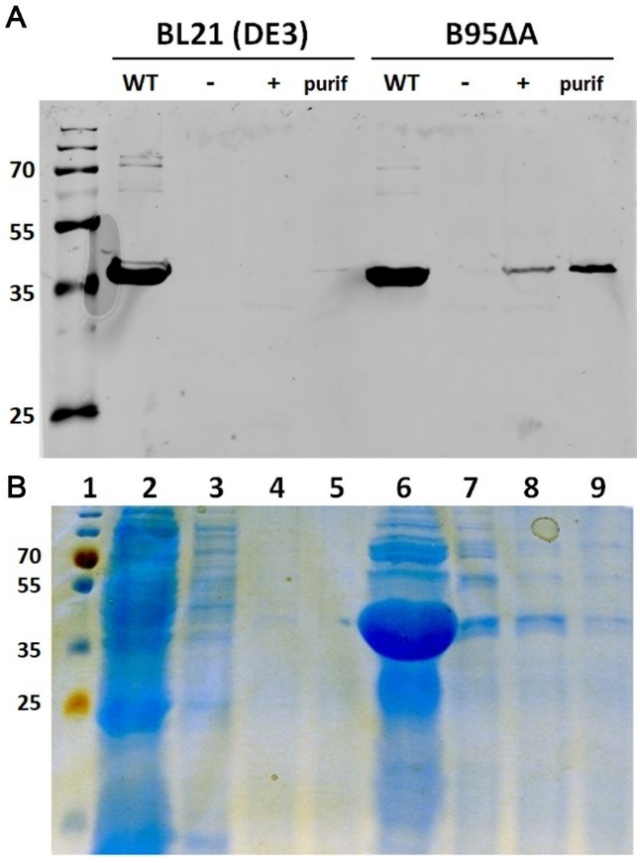


Figure 3. (A) Western-Blot analysis of multiple site incorporation of PrK in PsaA produced in BL21(DE3) (left) or in B95ΔA (right) in the absence (–) or the presence (+) of Prk and after affinity chromatography (purif), revealed with a mAb anti-histidine tag; (B) SDS-PAGE analysis (12% acrylamide gel with coomassie blue) of the PsaA triple mutant purified by affinity chromatography on a nickel column. Lane 1: protein marker, lane 2: flowthrough fraction, lane 3–5 wash buffer, lane 6–9 elution fractions.

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upon recognizing the amber stop codon. To test this hypothesis, we decided to switch to an *E. coli* expression strain deprived from RF1. In our hands, the use of the C321ΔAexp strain, knock-out for the gene *prfA* which codes for RF1 and in which every TAG codon has been replaced by the TAA codon,[37] proved unsuccessful noticeably due to growth default. Indeed, reading frame overlapping in *E. coli* is about 18% and the replacement of a TAG stop codon by another stop codon may be deleterious. We thus turned our attention to the BL21(DE3)-derived B95ΔA strain developed by Mukai et al.[38] This strain is also characterized by RF1 suppression but the substitution of only 95 TAG codons by TAA or TGA codons mostly targets genes essential for survival and growth of bacteria. Improved viability and its use for UAA incorporation at multiple sites was further confirmed.[39] Compared to BL21(DE3), the B95ΔA strain proved less efficient in incorporating a single PrK, for example at position 32 (Figure S1 in Supporting Information). Opposite result was observed regarding the triple mutant production since its presence was detected in cell lysates by both Western-Blot and SDS-PAGE analysis (Figure 3A and B). PsaA triple mutant was actually obtained (11 mg/L of cellular culture) after Ni-affinity purification (Figure 3B).

The identity of the triple mutant mPsaA was confirmed by mass spectrometry experiments. Molecular weight of the wild type mPsaA has been calculated equal to 34,311 Da or 34,443 Da (depending on the presence or absence of the *N*-terminal methionine). Consequently, theoretical calculated average mass of the triple mutant mPsaA should be greater by 3×82 Da (i.e., three times the MW difference between PrK and K), and thus equal to 34,557 or 34,689 Da. In line with these calculations, the recombinant protein appeared in the form of two peaks at 34,549 Da and 34,698 Da, with or without N-terminal methionine, respectively (Figure 4B). Tryptic in-gel digestion of the triple mutant protein was next carried out to ascertain the positions where the PrK have been inserted. The proteolytic mass analysis confirmed the identity of the protein (89% PsaA protein sequence coverage, 96% based on mPsaA protein sequence). As expected, PrK residues were detected in digested fragments $PsaA^{26–34}$, $PsaA^{210–237}$ and $PsaA^{214–242}$ which comprise targeted positions 32, 213 and 237, respectively (See Supporting Information). Of note, unmodified fragments were detected for mutated position K32 (24% of the total peptide event detected) but not for positions K213 nor K237, located downstream in the sequence. Partial propargylcarbamate deprotection of the PrK32 during analysis cannot be ruled out but unlikely: indeed, trypsin is highly specific, cutting at the carboxyl side of arginine and lysine residues. Moreover, Prk is stable in the digestion conditions (aqueous ammonium hydrogenocarbonate, 100 mM, 55 °C, 1 h) (data not shown). A hypothesis is that the lack of RF1 in B95ΔA strain renders the system more permissive and yet not totally orthogonal in incorporating canonical lysine rather than PrK when the TAG codon is encountered (at least early during the translation).[38,40]

Conjugate preparation and characterization

The three mono mPsaA mutants were reacted with known tetrasaccharide Pn14TS[30] according to a previously reported CuAAC procedure.[41,20] Crude glycoconjugates were purified using size-exclusion chromatography and further analyzed for their purity by protein electrophoresis and identity by HPLC-HRMS (Figure S2). As a result, introduction of a single tetrasaccharide antigen on each monovalent mutant was confirmed, the glycoconjugates being obtained in >90% purity.

The click-reaction was first attempted on the triple mPsaA mutant with azido-functionalized fluorescein dye to test its reactivity (see Supporting Information and Figure S3) and next applied to Pn14TS with larger excess of Pn14TS substrate and increased concentration of reactants to favor a complete transformation of the protein. The resulting glycoconjugate

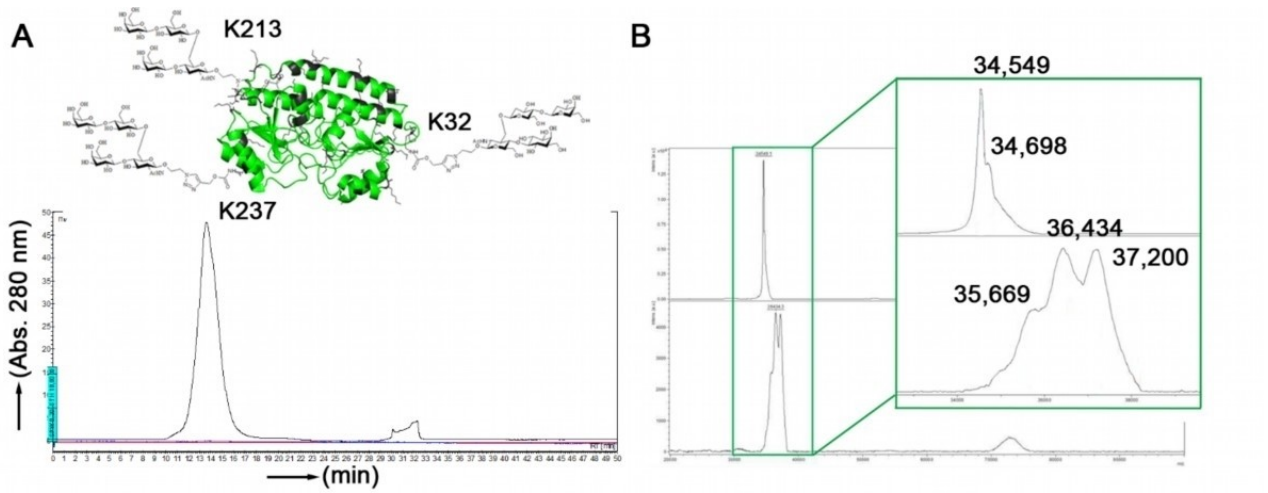


Figure 4. A) Size exclusion chromatography profile of triple mutant mPsA–Pn14TS conjugate. HiLoad 10/300 SuperdexTM 75 pg (GE Healthcare) column and PBS 0.1 M, pH 7.3 as eluent; B) MALDI-TOF mass spectra of triple mutant mPsA before (top panel) and after conjugation (bottom panel). Zoom of the 32–40 kDa region (insert).

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(75% recovery from conjugation reaction) was further analyzed by mass spectrometry after gel filtration purification (Figure 4). As expected, the glycoconjugate was detected at a higher mass in comparison with the triple mutant in the MALDI-TOF mass spectrum. This spectrum revealed the presence of three main species roughly separated from each other by 777 Da, reflecting the coupling of one (minor form found in the sample), two or three tetrasaccharides. Moreover, each peak shifted by 171 Da compared to the theoretical value suggesting that on average 10–11 residues of the mPsaA are oxidized. Tryptic digest of both glycoconjugate and triple mutant mPsA was further carried out to complete these data. Oxidized residues are the three histidine H57, H67 and H139, the three tryptophan W99, W171 and W123 and the three methionine M262, M295, M296 as well as, possibly, *N*-terminal methionine and histidine of the tag. While present in the triple mutant mPsaA in its non-conjugated form, probably due to treatments associated with proteolytic mass analysis, it seems that oxidative modifications are accentuated by the CuAAC reaction known to generate reactive oxygen species which can degrade proteins.[42] For example, H57/H67 in peptide digest fragments 54–77/78 were oxidized in 12 out of 97 but only in 9 out of 154 detected events after or before the conjugation step, respectively (See Table S1 for complete analysis).

Identification of peptides bearing the Pn14TS modification was more tedious. It seems that the conjugation preferentially took place at position 213 then at position 237 on the basis of recorded events by mass analysis. However, it is not known whether conjugated Pn14TS can impact the tryptic digestion and bias the results.

So as to determine in what extent the Pn14TS/mPsaA connectivity influences the antigenicity of the glycoconjugates, a control conjugate was prepared. Lysine side-chain amino groups of wild type mPsaA were derivatized with hexanoic acid succinimidyl ester and further randomly conjugated to Pn14TS to provide a conjugate displaying an average of three Pn14TS at variable positions (See Supporting Information and Figure S4).

Humoral/immune response

The pneumococcal conjugates obtained with mPsaA mutated at positions K32, K213 and K237 and the combined mutations as well as the random conjugate and wild type mPsaA were encapsulated in chitosan nanoparticles and adjuvanted with α-galactosylceramide to be administered subcutaneously to groups of 5 male C57/BL6 J mice three times at two weeks intervals. Both anti-pneumococcal capsular polysaccharide serotype 14 (CP14) and anti-mPsaA Ab response was determined one week after the third and the second immunization, respectively. None of the conjugates induced an anti-CP14 Ab response in an ELISA experiment using CP14 as the coated antigen (data not shown). An unwanted anti-triazole response could not be totally ruled out although this ring was reported as being only marginally immunogenic. The sera were thus tested against a control conjugate made of BSA first derivatized with propargyl chloroformate and then conjugated to Pn14TS by click chemistry (See Supporting Information). The ELISA assay proved negative indicating that triazole ring does not interfere with the immune response and is not part of a neoepitope. Contrasting with these results, an anti-mPsaA response was raised by all conjugates but the negative control (PBS) in mice secondary sera (Figure 5). Noticeably, the IgG response induced by the conjugates based on PrK-modified mPsaA was equivalent to that induced by wild-type mPsaA. This observation suggests that neither PrK incorporation nor the conjugation step significantly impact the antigenicity of the mPsaA.

The highest anti-mPsaA response was observed for the conjugate obtained with mutant mPsaA K237. The response was statistically significant in comparison with PBS and the random conjugate ($P < 0.05$) and with the two mono mutants K32 and K213 ($P < 0.01$) but not with the triple mutant Pn14TS-mPsaA conjugate.

This observation seems to confirm that not only the extent of conjugation[8] but also the connectivity does impact the anti-carrier humoral response.[15] As mentioned earlier, mutations at K32 and K213 differ from mutation K237 as they target mPsaA regions that contain known T-helper or both T-helper and B-epitopes, respectively. Observed results were therefore not unexpected. However, further studies are necessary to ascertain their origin, notably to establish that diminution of the anti-carrier response due to alteration of protein B-epitopes or recruitment of T-helper to trigger Tcarbs, possibly correlates with an increase in the anti-carbohydrate antigen response. In the past, we did observe an anti-CP response at 3 μg/dose of Pn14TS but the Pn14TS-mPsaA were obtained using a different – thio/maleimide – coupling chemistry and at a final 5.4 mol/

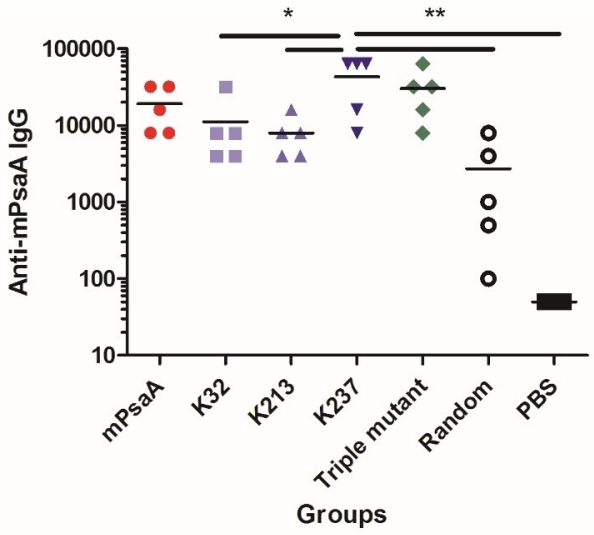


Figure 5. The anti-mPsaA Ab response. The immunization was performed twice at day 0 and 14 and the serum antibody response in the mice was determined at day 21. The obtained results are represented as anti-PsaA IgG response. mPsaA and the 3 mono-mutant conjugates were administered at 130 μg/dose; triple mutant and random conjugates at 43 μg/dose. Statistical difference between the groups is *, $P < 0.05$; **, $P < 0.01$. Data represent means ± SD ($n = 5$).

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mol carbohydrate antigen/protein ratio.[30] Maintaining a 1:1 ratio is essential to investigate the effect of connectivity on both anti-CP and anti-mPsaA humoral response. A straightforward strategy to satisfy this criterion while observing anti-CP Ab response consists in increasing the length of the oligosaccharide. In the present case, the use of a dodecasaccharide representing three repeat units of the CP serotype 14 appears as a suitable candidate from the literature.[6] Now considering the anti-mPsaA IgG response induced by either the triple mutant or the random conjugate, it is apparent that the response is more heterogeneous and the Ab titres lower for the latter. It is possible that derivatization and conjugation steps impacted the protein structure of the random conjugate although mPsaA proved very stable in previous studies[30] and herein fully resistant to CuAAC experimental conditions (data not shown) as determined by circular dichroism analyses. Nevertheless, if this hypothesis is true, it reinforces the need for developing controlled, targeted conjugation strategies such as the incorporation of non-canonical amino acids bearing bioorthogonal functional groups.

Conclusion

The preparation of a glycoconjugate for vaccine purpose relying on the *in cellulo* multiple site-specific non-canonical amino acid incorporation in the carrier protein is reported for the first time. This strategy paves the way to the access to homogeneous glycoconjugate with full control of the connectivity. Obvious advantages compared with the 'classical', randomly obtained glycoconjugates are an easier physico-chemical characterization of the actual conjugate, a diminution of the number of production process steps thus saving high-added value material and associated costs. It provides a better control of the crucial conjugation step and preservation of the carrier protein antigenicity. Moreover, it will provide unique tools to study structure/immunogenicity relationship and beyond to control actual immune properties of the glycoconjugate vaccines.

Experimental Section

Expression and purification of triple mPsaA mutant: The mPsaA triple PrK protein was expressed and purified from an *E. coli* B95ΔA strain[38] (provided by the RIKEN BRC through the National BioResource Project of the MEXT/AMED, Japan) freshly transformed with a pEt24d-mPsaA-HHHHHH plasmid[20] containing TAG mutations at positions K32, K213 and K237 of the mPsaA gene and a pEvol PylRS plasmid (a kind gift from Edward Lemke (EMBL)) containing two genes of the Wild type *Methanosarcina mazei* Pyrrolysyl tRNA synthetase (*Mm*PylRS) and its cognate tRNA. An overnight 5 mL preculture in LB medium was diluted in 500 mL of autoinducible medium supplemented with 0,02% arabinose, 25 μg/mL Kanamycine, 30 μg/mL Chloramphenicol and 2 mM PrK and the culture was incubated for 27 h at 37 °C. Bacterial pellet was lysed by sonication in lysis buffer (NaH_2PO_4 50 mM, NaCl 150 mM, Imidazole 5 mM, lysozyme 1 mg/mL, PMSF 1 mM and DNAse 1 μg/mL). The mPsaA mutant protein was then purified by affinity chromatography from the clarified lysate using NiNTA beads and an imidazole gradient.

Conjugate synthesis using click-chemistry : All solutions were degassed and prepared under argon to minimize oxidation. A ratio of 1:5 was used between the protein and the tetrasaccharide with 31,6 nmol of mPsaA-3PrK for 600 nmol Pn14TS-N_3 to which was added copper(II) sulfate at 0.1 mM, *tris*((1-hydroxy-propyl-1H-1,2,3-triazol-4-yl)methyl)amine (THPTA) at 0.5 mM, aminoguanidine hydrochloride at 5 mM, aqueous sodium ascorbate at 1 mM, prepared extemporaneously, completed with distilled water at final volume of 1.1 mL. The reaction was left to proceed at room temperature overnight protected from light. The next day 200μL of EDTA at 500 mM was added and the reaction was then applied on a Zeba™ spin desalting column from Thermo Fisher scientific. The resulting conjugate was further purified on a HiLoad 10/300 Superdex™ 75 pg (GE Healthcare) column using PBS 0.1 M, pH 7.3 as eluent. Collected fraction were concentrated under centrifugation and then freeze-dried.

All the animal experiments were approved by the French Comité d'Ethique en Expérimentation Animale (CEEA) under the accreditation number 7897.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: glycoconjugate · immunology · *Streptococcus pneumoniae* · synthetic biology · unnatural amino acid

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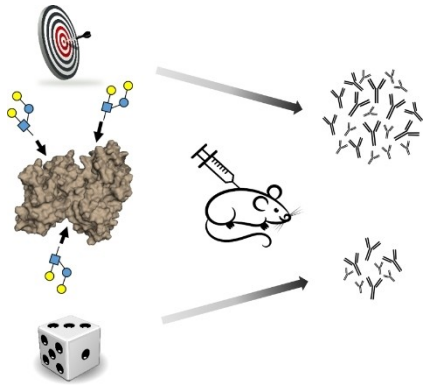
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RESEARCH ARTICLE

Antigen-carrier connectivity, if controlled, can help improving immunological response to glycoconjugate vaccines. In this work, it is shown that a site-selectively triple conjugated vaccine candidate against *Streptococcus pneumoniae* presents a better antigenicity than its randomly conjugated counterpart.



Dr. T. Violo, Dr. A. Lambert, Dr. A. Pillot, M. Fanuel, J. Mac-Béar, Dr. C. Broussard, Dr. C. Grandjean, Dr. E. Camberlein**

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Site-Selective Unnatural Amino Acid Incorporation at Single or Multiple Positions to Control Sugar-Protein Connectivity in Glycoconjugate Vaccine Candidates

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