



Expanding polysaccharide–protein coupling of glycoconjugate vaccines

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Francesco Berti*

From the GSK Vaccines S.r.l., Technical R&D, Siena, Italy

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For the preparation of glycoconjugate vaccines, polysaccharide antigens can usually be chemically modified to generate reactive functional groups (e.g., the formation of aldehyde groups by periodate oxidation of adjacent diols) for covalent coupling with proteins. In a recent issue of JBC, Duke *et al.* showed that an alternative agent, galactose oxidase (GOase) isolated from the fungus *Fusarium* sp. can generate aldehyde groups in a unique chemoenzymatic approach to prepare a conjugate vaccine against *Streptococcus pneumoniae*. These findings introduce a new strategy for the design and development of glycoconjugate vaccines.

Vaccination in the modern era, which began when Edward Jenner started inoculating people with a nonvirulent form of the smallpox virus isolated from cows, has leveraged many different kinds of antigens, including attenuated pathogens, killed bacteria, inactivated toxins, bacterial polysaccharides, recombinant proteins, and more recently, the DNA or RNA viral genetic code (1). In this context, over the last 40 years, polysaccharide–protein conjugate vaccines have had a tremendous benefit in human health by contributing to the prevention of bacterial infections caused by *Haemophilus influenzae* type b (Hib), *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Salmonella* serovar Typhi (2). A variety of conjugation chemistries have been used to couple carbohydrate antigens to protein carriers. Different functional groups that are already present (e.g., hydroxyl groups), or that can be introduced in polysaccharide antigens (e.g., aldehyde groups), have been used for further covalent coupling to proteins (3). Monosaccharides constituting the repeating units of polysaccharides possess often vicinal hydroxyl groups, both in the saccharide rings and in linear chains (e.g., in the glycerol-like chain of sialic acid). These groups readily react with sodium periodate to form aldehydes that can be coupled to the lysine residues of carrier proteins by reductive amination.

Several vaccines licensed for human use have been successfully prepared *via* periodate oxidation of diols available on the saccharide units. For example, Pfizer has used periodate chemistry to develop Hib (4), group C meningococcal (now the property of Nuron Biotech), and pneumococcal conjugate vaccines (5). The pharma company Baxter also applied

periodate treatment after the removal of O-acetyl groups from the structure of serogroup C meningococcal polysaccharides (now proprietary of Pfizer) (5). In addition, conjugate vaccines against group B *Streptococcus* (GBS), tested in clinic by both Pfizer and GSK Vaccines, have been developed using periodate-controlled oxidation of the sialic acid side chains and subsequent coupling by reductive amination to protein carriers (5).

To minimize the impact on polysaccharide structure and immunological epitopes, the periodate oxidation approach is commonly applied in poorly controlled stoichiometry to generate an appropriate concentration of aldehyde groups for further protein coupling. The oxidation of diol systems, as can be found on vicinal hydroxyl groups of saccharide rings (with the *trans* conformation being more reactive than the *cis* conformation (6)) or aliphatic chains (e.g., the glycerol-like C₇–C₉ chain of sialic acid), provokes structural modifications of polysaccharide antigens such as the irreversible opening of the saccharide ring or generation of the pseudo-sialic acid structure. For instance, opening several saccharide rings along the polysaccharide antigen might result in degradation phenomena, as well as in a reduced antibody cross-reactivity with the native polysaccharide on the surface of the infecting bacterium (7).

Oxidation by the GOase enzyme, successfully confirmed by Duke *et al.* (8) on *S. pneumoniae* serotype 14 capsular polysaccharide (Pn14p), offers an exciting approach to overcome some of these challenges. In appropriate polysaccharides, the GOase enzyme generates a site-specific aldehyde motif for conjugation. Distinct from the periodate oxidation, it generates only one aldehyde group at position C₆ of galactose, which is completely reversible to the original primary alcohol group (*i.e.*, by reduction with sodium borohydride). To verify the selective oxidation of Pn14p by GOase, as well as its reversibility to the native form of the polysaccharide (reduction by NaBH₄), Pn14p was characterized by mono- and bi-dimensional NMR analyses. Furthermore, to assess that the enzymatic derivatization of Pn14p is less destructive than chemical oxidation, CRM₁₉₇ protein–polysaccharide conjugates were prepared and tested for their capability to induce a humoral immune responses (serotype-specific IgM and IgG antibody titers) and functional protection activity (opsonophagocytic killing potential assay (OPKA) titers and *in vivo* lethality challenge) in a mouse model.

* For correspondence: Francesco Berti, francesco.x.beriti@gsk.com.

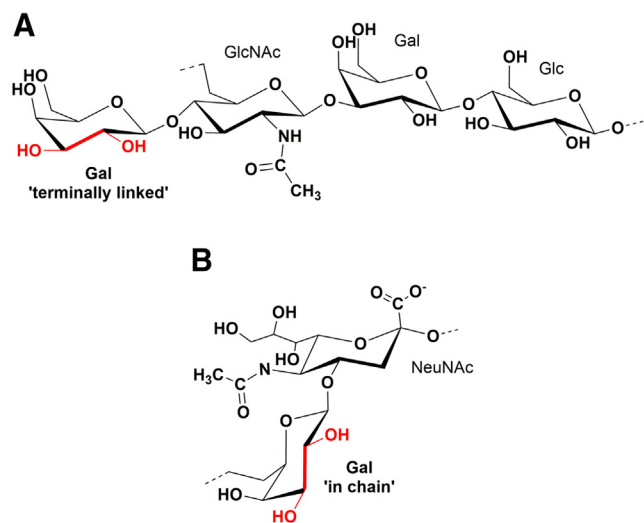


Figure 1. Highlighting the structures of polysaccharide with Galactose units “terminally linked” and “in chain”. Schematic representation of (A) *Streptococcus pneumoniae* serotype 14 and (B) *Neisseria meningitidis* serogroup W polysaccharide repeating units with Galactose residues “terminally linked” and “in chain,” respectively.

These experiments showed that GOase conjugates elicited a statistically significantly higher level of IgM as compared with the NaIO_4 -oxidized group on each day tested. Notably, the GOase conjugate elicited robust levels of IgG at the last time-point compared with the NaIO_4 -oxidized group, with an almost fourfold higher antibody titer when using plates coated with Pn14p (and twofold higher when using fixed serotype 14 bacteria). On the other hand, a comparable degree of OPKA titers was measured for the chemical or chemoenzymatically generated conjugate vaccines, with both glycoconjugates killing a statistically significant amount (>50%) of serotype 14 *S. pneumoniae* compared to adjuvant injection control. Furthermore, in the *in vivo* lethal challenge model, all adjuvant-immunized mice were moribund within 24 h after administration and subsequently euthanized, while all conjugate vaccine-administered mice showed minimal symptoms relative to adjuvant mice, with a 100% survival rate over the course of study.

Taken together, these results show that a novel chemoenzymatic approach for reversible activation of Pn14p can be harnessed to form a glycoconjugate vaccine that is site-specific and nondestructive for the competent polysaccharide antigen and is capable of providing a more robust humoral response with equal protection to the commercial preparation. Considering that many other have identified bacterial polysaccharide antigens not only against *S. pneumoniae* but also *N. meningitidis*, Group B *Streptococcus*, and *Shigella flexneri*, the feasibility of finding this conformation on other polysaccharide targets, both “terminally linked” (one glycosidic bond through C_1 only) and “in chain” (two glycosidic bonds), can provide added value to this approach (9) (Fig. 1). In addition, as a result of the presence of a terminal galactose

residue within the repeat unit, GOase is predicted to be able to readily activate 9 of the 24 *S. pneumoniae* serotypes included in current commercially available pneumococcal vaccines (10). In conclusion, the key chemoenzymatic differences in this novel approach that were lacking in previous chemical methods lay in its ability to be both site-selective and reversible, without dependence on deleterious oxidation strategies that can result in structural changes. This GOase chemoenzymatic approach provides a strong contribution to the toolbox for preparing conjugate vaccines through oxidation/reductive amination chemistry.

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Conflict of interest—F. B. is an employee of the GSK group of companies. F. B. is listed as an inventor on patents owned by the GSK group of companies.

Abbreviations—The abbreviations used are: GBS, group B *Streptococcus*; GOase, galactose oxidase; Hib, *Haemophilus influenzae* type b; OPKA, opsonophagocytic killing potential assay; Pn14p, *S. pneumoniae* serotype 14 capsular polysaccharide.

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