IMMUNOCHEMISTRY OF MENINGOCOCCAL GROUP B OLIGOSACCHARIDE-PROTEIN CONJUGATES

by

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RESUME

IMMUNOCHIMIE DES CONJUGUES PROTEINE-OLIGOSACCHARIDE MENINGOCOCCIQUES DU GROUPE B.

Le polysaccharide méningococcique du groupe B est connu pour être une exception du point de vue immunogénicité parmi les polysaccharides méningococciques immunogènes spécifiques de groupe.

Il est connu aussi que le polysaccharide méningococcique du groupe B ne voit pas son pouvoir immunogène accru chez les modèles animaux même après conjugaison par liaison covalente avec des protéines porteuses.

Afin d'étudier le rôle de la polydispersion du poids moléculaire du polysaccharide et le rapport stoechiométrique convenable entre carbohydrate et protéine dans un glycoconjugué caractéristique, les auteurs ont préparé des oligomères à partir de leurs polysaccharides à poids moléculaire élevé par dégradation partielle de la polydispersion moléculaire jusqu'à obtention d'unités d'environ 4 à 8 monomères.

Les oligosaccharides ont été isolés par chromatographie en gel et analysés par chromatographie liquide à haute performance et par résonance magnétique nucléaire au 13_C.

Ces oligomères se sont montrés capables d'inhiber les anticorps méningococciques spécifiques du groupe B.

Les oligomères ont aussi été conjugués à des protéines porteuses CRM 197 et à de l'anatoxine tétanique pour des études immunologiques sur modèles animaux.

In some cases of polysaccharide-protein conjugates, such as pneumococcal type 6A and meningococcal group B capsular polysaccharides, unsuccessful attempts have been made to improve immunogenic characteristics of these antigens after conjugation with a protein carrier by covalent bond.

In a previous work we prepared several kinds of conjugates between 6A polysaccharide and two different protein carriers, bovine serum albumin and cross-reacting material (CRM_{197}), a non-toxic mutant protein serologically related to diphteria toxin. Physicochemical and serological characteristics of these polysaccharide-protein conjugates involved high molecular weight molecules with a stechiometry ranging between 0.2 and 1 to 1 (mol per mol) in the ratio polysaccharide/protein. Both polysaccharide and protein moieties had their antigenic determinants exposed, since they were cross-reactive with their corresponding reference antigens tested against homologous antibodies.

The quantitative antibody analysis of animal sera obtained after immunization by these conjugates showed the conversion of CRM_{197} to an effective antigen after glycosylation (in comparison to the untreated protein), able to raise antitoxic antibodies with a higher titer than the minimum immune response estimated for man (Fig. 1).

Regarding the immune response to the polysaccharide, only a few treated animals showed a significant increase in specific antibodies, although these antibodies were directed against the common determinants of the reference polysaccharide. In light of these results, we have considered other aspects of the immunochemistry involved in the preparation of these molecules, which recently have had a revival of interest as a promising approach to manipulate the immunological response against saccharide antigens.

In particular, at least two main points deserve a better understanding at molecular level: first, if the molecular weight of the saccharide hapten represents an important factor in conjugated molecules; and second, if the stechiometry of conjugated molecules (on a molar basis) is favorable to the saccharide moieties instead of the protein carrier. Regarding the latter, recent works seem to indicate a positive answer. However, in order to investigate this second point, several experiences demonstrated dif-

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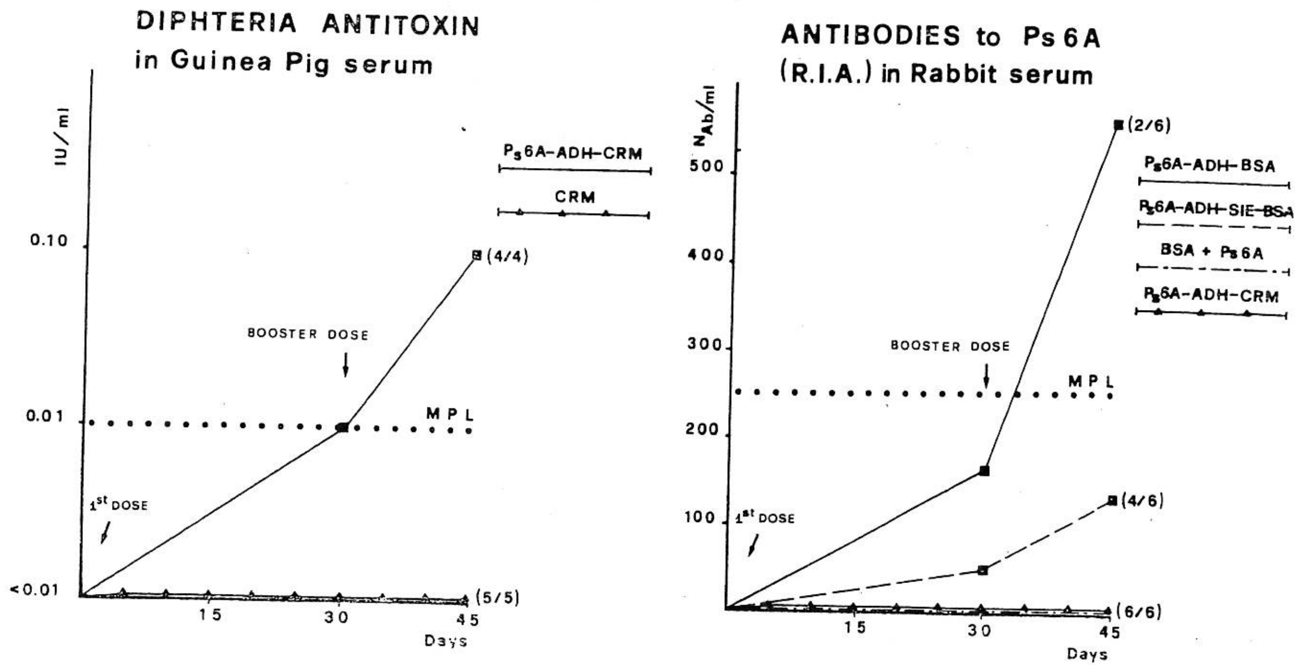


FIGURE 1

ficulties in obtaining a stechiometric ratio between polysaccharide and protein exceeding 2 to 1 on a molar basis and this could be explained by the larger molecular weight polydispersion of the polysaccharides. Thus, oligosaccharides derived from native homologous polysaccharides were considered for the preparation of oligosaccharide protein conjugates both for type 6A pneumococcal and group B meningococcal capsular antigens. In this communication I will refer to the preparation and the characterization of group B meningococcal oligosaccharides. The use of oligosaccharide-derived haptens involves knowing were the breaking of the linkage occurs in the polymer and what is the minimum molecular structure still recognized by the specific antibodies. Since the group B carbohydrate is composed of repeating units of N-acetyl-neuraminic acid, mild acid hydrolysis under mild temperature conditions was used to perform depolymerization of the polysaccharide.

High performance liquid chromatography spectra (Fig. 2) showed that a time-dependent depolymerization of the structure occurred, but polydispersed systems were still obtained when very low molecular weight haptens were formed.

R represents the ratio between the saccharide molecular weight distribution recovered before Kd = 0.5 in respect to that which is recovered after this limit in a Sephadex G-50 gel chromatography.

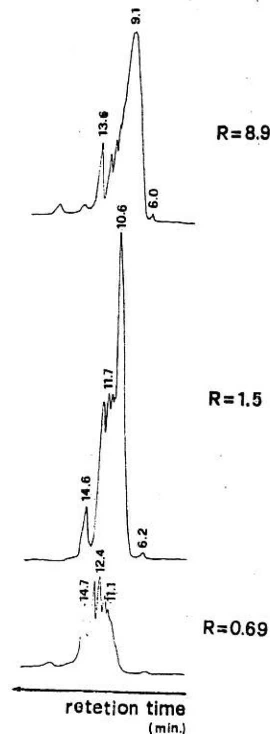


FIGURE 2

Preliminary results of ^{13}C-NMR spectroscopy (Fig. 3) evidenced the retention of the N-acetyl group in the depolymerized structure and an approximate polymerization degree corresponding to 4-6 monose

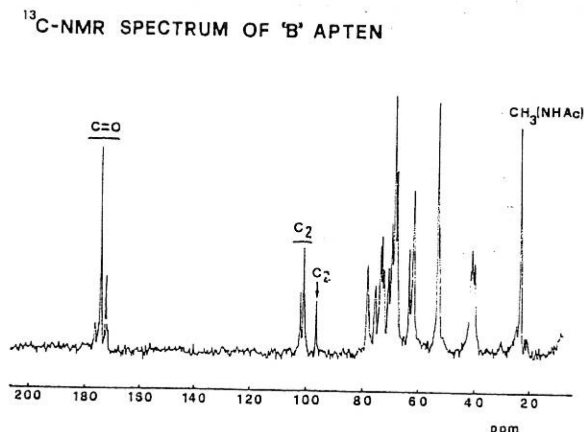


FIGURE 3

units. More thorough examinations of the hydrolyzed linkage are in progress using oligosaccharides reduced by sodium borohydride. Because antipolysaccharide immunoglobulins raised in animals against whole bacterial cells are heterogenous with respect to different sizes of a molecular weight polydispersion, quantitative measures of antibody specificity to different

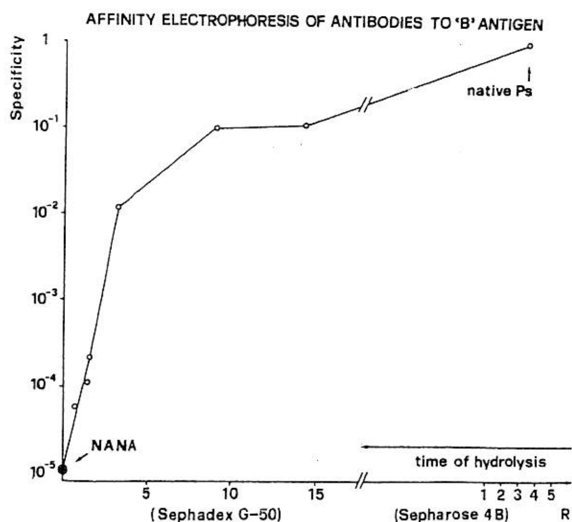


FIGURE 4

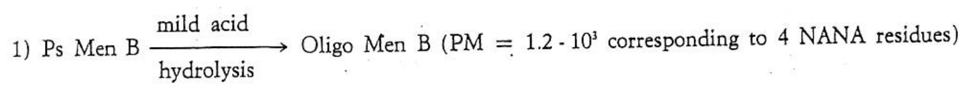
molecular sizes of a polydispersion were performed by a variant of affinity electrophoresis at pH = 8.6 (Fig. 4) using the B antigen in different stages of hydrolysis performed in mild acid conditions. Specificity is the expression of the ratio between the Minimal Inhibiting Concentration (MIC) of the native Group B antigen in respect to that of the hydrolysis-derived B haptens, while R is the ratio between the amount of the saccharide molecular weight distribution recovered before Kd=0.5, in respect to that recovered after this limit in Sepharose-4B or G-50 gel chromatography. Quantitative measures of antibody specificity to different molecular sizes of the B antigen, obtained in various steps of hydrolysis, showed that haptens of very low molecular weight were still recognized by antibodies to B polysaccharide and N-acetyl-neuraminic acid was effectively indicated as the immunodominant sugar, since inhibition of antibodies occurred both with pure sialic acid and with sialic acid obtained by complete acid hydrolysis of B polysaccharide.

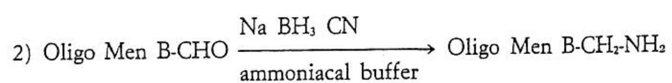
Interestingly, a similar value of inhibition was also shown when sialic acid was tested against the group C meningococcal polysaccharide and this finding could be explained on the basis that the C polysaccharide contains approximately 25% of sialic acid without O-acetylation in its molecular structure.

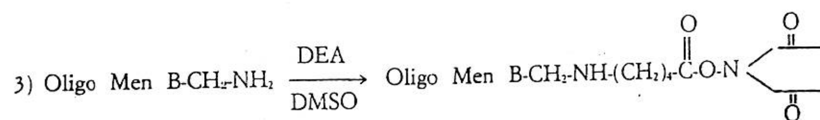
However, it should be pointed out that in working with oligosaccharide haptens, correct measurement of the dimensions of the immunodeterminant group of group B antigen can only be obtained by measuring the affinity constant value using monoclonal antibodies and we are now planning studies with these reagents. In the present study we used oligomers constituted by 4-6 residues of NANA, which is a value close to the theoretical one generally described as determinant group for polysaccharides with single-unit side chains. By using these group B oligomers, glycoconjugates were prepared with the protein carriers CRM_{197} and tetanus toxoid. Since a direct coupling of the haptens to the proteins, via reductive animation involving the hemiketal group of the terminal sialic acid residue, yielded a poor amount of conjugate product, activation of the B oligomers was performed according to the procedure shown in scheme. The succinimidyl ester-derived oligomers were then conjugated to the amino groups of the proteins carriers. At present, experiments involving the immunological characteristics of the conjugate molecules are in progress using animal models.

SCHEME

Préparation and activation of meningococcal group « B » oligosaccharides.







DEA: Disuccinimidyl ester of adipic acid.