CHEMICAL REAGENTS FOR THE ACTIVATION OF POLYSACCHARIDES IN THE PREPARATION OF CONJUGATE VACCINES

Reference to Related Applications

This application claims priority to U.S. Provisional Application No. 61/287,593 of the same title filed December 17, 2009, the entirety of which is hereby incorporated by reference.

Background

1. Field of Invention

This invention is directed to reagents and methods of conjugating proteins to carbohydrates and, in particular, the processes and chemicals used in the manufacture of vaccines, and also the vaccines manufactured by the process.

2. Description of the Background

Vaccines that contain protein covalently linked to carbohydrate have proven remarkably successful in inducing an immune response to the carbohydrate moiety. Examples of such vaccines, known as "conjugates" are available for *Haemophilus influenzae* type b (e.g., ActHib, Hiberix), *Neisseria meningiditis* types A C W and Y (e.g., Menactra) and *S. pneumoniae* (e.g., Prevnar, Synflorix). In order for the protein to be linked to the carbohydrate, the latter generally needs to be activated so that it can be reacted with the protein, either directly or via a spacer (Dick, W.E. Jr and Beurret, M. Glyconjugates of bacterial carbohydrate antigens. A survey and consideration of design and preparation factors. In: *Conjugate Vaccines* (Eds Cruse, J.M. and Lewis, R.E.). Karger, Basel, 1989. One means of activation is through oxidation of the carbohydrate to produce aldehydes, which are then linked to lysines on protein through reductive amination. In other cases, the protein is first functionalized with hydrazides or aminooxy groups, which are subsequently reacted with aldehydes on the carbohydrate (Lees, A. Use of amino-oxy functional groups in the preparation of protein-polysaccharide (PS) conjugate vaccines United States Patent Publication No. 2005/0169941; which is incorporated by reference). Another method for activating polysaccharides is with the use of cyanogen bromide, to form a cyano-ester on the polysaccharide which is subsequently reacted with a spacer molecule such as adipic dihydrazide. The functionalized polysaccharide is then reacted with the protein. Improved methods for

Claims:

1. A process of conjugation of a carbohydrate comprising:

   Mixing 1-cyanoimidazole (1-CI) with the carbohydrate to create an activated chemical compound; and

   mixing the activated compound with a second compound to create a conjugate.

2. The process of claim 1, wherein the carbohydrate is a natural or synthetic carbohydrate, polysaccharide, oligosaccharide, or combination thereof.
3. The process of claim 1, wherein the second compound is a peptide, a polypeptide or a protein.
4. The process of claim 1, wherein the second compound is an antigenic molecule.
5. The process of claim 1, wherein conjugation is direct or indirect with the additional of functional groups that facilitate conjugation.
6. The process of claim 1, further comprising removing components with a lower molecular weight than the conjugate by dialysis, filtration, chromatography, or a combination thereof.
7. The process of claim 1, wherein the conjugate is a vaccine or a diagnostic agent.
8. The process of claim 1, wherein the steps are performed together.
9. The process of claim 1, further comprising including a linking compound between the activated compound and the second compound.
10. A vaccine or diagnostic agent produced by the process of claim 1.

ACTIVATION OF POLYSACCHARIDES VIA THE CYANYLATING AGENT, 1-CYANOIMIDAZOLE (1-CI) IN THE PREPARATION OF POLYSACCHARIDE/PROTEIN CONJUGATE VACCINES

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Claims

1. A process of conjugation of a carbohydrate comprising:

mixing 1-cyanoimidazole (1-CI) with the carbohydrate to create an activated chemical compound; and

mixing the activated compound with an antigenic second compound to create a conjugate, wherein the second compound is a peptide, a polypeptide or a protein.

2. The process of claim 1, wherein the carbohydrate is a natural or synthetic carbohydrate, polysaccharide, oligosaccharide, or combination thereof.

3. The process of claim 1, wherein conjugation is direct or indirect with the addition of functional groups that facilitate conjugation.

4. The process of claim 1, further comprising removing components with a lower molecular weight than the conjugate by dialysis, filtration, chromatography, or a combination thereof.

5. The process of claim 1, wherein the conjugate is a vaccine or a diagnostic agent.

6. The process of claim 1, wherein the steps are performed together.

7. The process of claim 1, further comprising mixing the activated compound with hexanediamine, ethylenediamine, hydrazine, adipic dihydrazide, or 1,6-diaminooxyhexane to form a linking group between the activated compound and the antigenic second compound.

8. A vaccine or diagnostic agent produced by the process of claim 1.