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Preparation of multifunctional glyconanoparticles as a platform for potential carbohydrate-based anticancer vaccines

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Dedicated to the memory of Professor Nikolay K. Kochetkov

Abstract—A novel platform for anticancer vaccines has been prepared using glyconanotechnology recently developed in our laboratory. Ten different multifunctional gold glyconanoparticles incorporating sialylTn and Lewis^y antigens, T-cell helper peptides (TT) and glucose in well defined average proportions and with differing density have been synthesised in one step and characterised using NMR and TEM. Size and nature of the linker were crucial to control kinetics of S–Au bond formation and to achieve the desired ligand ratio on the gold clusters. The technology presented here opens the way for tailoring polyvalent anticancer vaccines candidates and drug delivery carriers with defined average chemical composition.

Keywords: Gold glyconanoparticles; Carbohydrate antigens; Synthesis; Multifunctional nanoparticles; Synthetic vaccines

1. Introduction

Metallic nanoclusters functionalised with biomolecules have been a subject of sustained interest for several years.[1] Integrated nanoparticle–biomolecule multifunctional systems constitute useful tools to mimic the behaviour of biomolecules in cells thus helping to explore the mechanisms of biological processes with a variety of potential applications.[2] The preparation of gold nanoparticles protected with self-assembled monolayers of carbohydrate antigens (glyconanoparticles, GNPs) was first reported by us in our continuous search for multivalent systems to prove and evaluate carbohydrate–carbohydrate interactions.[3] These GNPs, which are easily constructed by reducing a gold salt[4] in the presence of thiol functionalised synthetic neoglycoconjugates, are extremely small, water soluble, stable to glycolytic enzymes and can be manipulated as biological macromolecules.[3] Interestingly enough, these GNPs show a permanent magnetism at room temperature, the origin of which is presently being investigated.[5] A review on the preparation, characterization and applications of GNPs has recently appeared.[6]

GNPs provide a multivalent glycocalix-like carbohydrate display with a well defined average chemical composition. They have been used to prove[3a,7] and to quantify[8] adhesion forces between carbohydrate antigens and to interfere with carbohydrate–carbohydrate interaction mediated biological processes.[9] The simple and versatile method of preparation of these biofunctional gold nanoclusters[3,4] allowed to prepare constructs in which the metallic core is protected with mixed monolayers of different carbohydrate and noncarbohydrate ligands including fluorescence probes.[3b] Other authors have used the same synthetic approach to prepare nanoparticles also comprising of a mixed monolayer with functional groups for specific binding to macromolecules.[10]

We now have explored the scope of this preparative strategy by constructing multifunctional gold GNPs

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protected with mixed monolayers of different tumour associated carbohydrate antigens and immunogenic peptides, in well defined average proportions and with differing density, as a new platform for potential anticancer vaccines. To our knowledge, these multifunctional GNPs are the most complex biofunctional nanoclusters that have been prepared so far. In this communication, we report on the preparation and characterization of these multifunctional nanostructures and present preliminary data on the properties of these constructs as potential anticancer vaccine candidates.

Carbohydrates are T cell independent antigens and most carbohydrate based vaccines are conjugate systems in which oligosaccharide or polysaccharide antigens are covalently linked to immunogenic structures.[11] Polysaccharide–protein conjugates are being effectively used in vaccination strategies against bacterial infections.[12] On the other hand, intensive work has been underway for several years to exploit the over expression of tumour associated oligosaccharides to develop anticancer vaccines.[13] In this case, synthetic tumour associated oligosaccharide epitopes are conjugated to a carrier protein, which provides T cell help required for antibody production.[11] A variety of strategies to present these oligosaccharide epitopes for inducing sufficiently strong helper T cell responses have been developed and impressive advances have been achieved in the synthesis of tumour associated glycopeptide and glycolipid structures to construct potential vaccine candidates.[13e,14] While for glycolipids or glycopeptides with large tumour associated oligosaccharide epitopes, single molecule presentation seems to be sufficient for antibody recognition, short haptenic molecules, such as the disaccharide antigen sialyl-Tn (sTn), appear to require being presented as clusters.[15] It has also been observed that even for larger molecules, such as oligosaccharides containing the tetrasaccharide Lewis^y epitope, clustering of the glycodomain is important for antibody production.[16] In this connection, it has been anticipated that multivalent structures may provide more antibody density than monovalent immunizing agents, and that increasing the number of tumour epitopes should result in a broader degree of protection against multiple cancers.[17] Also, incorporating different antigens in a single clustered format has been proposed for the construction of unimolecular vaccines of defined chemical structures.[18]

It has been shown that antigens covalently conjugated to solid core carboxylated polystyrene microspheres of narrowly defined size (0.04–0.05 μm) induce high antibody titres in mice.[19] We envisioned that GNP technology[3] could provide a platform for potential anticancer vaccines if conditions were found to include, in a controlled manner, tumour associated oligosaccharide epitopes and T cell helper peptidic components in the self-assembly process. Therefore, we have investigated the self-assembly of gold nanoclusters comprising mixed monolayers of sTn and Lewis^y antigens in various proportions, a peptide from tetanus toxoid (TT) and glucose as an inert component to control the density of the antigens in the final construct. The sTn epitope is a mucin associated antigen expressed on a variety of epithelial cancer cells[20] and Lewis^y has been identified as an antigen for eliciting antibodies against colon, liver, prostate and ovarian carcinomas.[21]

2. Results and discussion

Neoglycoconjugates **1**–**3** (Chart 1) were synthesised and equipped with an appropriate thiol ended spacer group as previously reported.[3] A C_2 aliphatic spacer for the glucose neoglycoconjugate **1** and a C_5 aliphatic linker for the tumour associated carbohydrate epitopes (**2** and **3**[22]) were chosen. The peptide ligands **4** and **5**, comprising of the sequence FKLQTMVKLFNRIKNNVA, linked through the amino terminal group to a thiol ended C_{11} aliphatic spacer (**4**) or to a mixed hexaethylene glycol-C_{11} aliphatic spacer (**5**), were synthesised on solid phase. The first two amino acids of this polypeptide were included for their ability to be hydrolysed by lysosomal proteases[23] and the remaining 16 amino acids represent αα89–105 from TT.

Neoglycoconjugate **2** was synthesised containing an α oriented thiopentyl spacer group at the reducing end of the disaccharide compound. To this purpose the synthesis was carried out from easily available 2-acetamido-2-deoxy-D-glucose instead of from more expensive *galacto*-derivative. Inversion of configuration at C-4 position of the *gluco*- compound[24] led to pentenyl galactopyranoside **9** in good yield (Scheme 1). Finally, acceptor **10** was prepared by addition of thiolacetic acid to the olefin **9**, catalysed by azobisisobutyronitrile (AIBN), also in good yield.

For α-sialylation[25] of compound **10**, chloride donor **11**[26] was used as depicted in Scheme 2. The yield for the α-anomer was poor and attempts to improve yield and stereoselectivity using more elaborated sialic acid donors,[27] also failed. However, the obtained pure material was enough to continue with the preparation of **2** and we concentrated on the preparation and characterization of the GNPs rather than improving this synthetic procedure. Deprotection of fully protected compound **12** was accomplished in aqueous basic media to obtain neoglycoconjugate **2** in good yield (Scheme 2).

Using these components (**1**–**4**) in different ratios, 10 GNPs (**GNP1**–**GNP10**) protected with self-assembled mixed monolayers comprising the different ligands in various proportions (Chart 2 and Table 1) were prepared. Glucose was a major component of all GNPs and all of them contained a low density of TT peptide ligand (3% of the total monolayer). The proportions of the two carbohydrate epitopes in the mixed monolayer