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Glycopeptide-functionalized gold nanoparticles for antibody induction against the tumor associated mucin-1 glycoprotein

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ABSTRACT

We report the preparation of gold nanoparticle (AuNP)-based vaccine candidates against the tumor-associated form of the mucin-1 (MUC1) glycoprotein. Chimeric peptides, consisting of a glycopeptide sequence derived from MUC1 and the T-cell epitope P30 sequence were immobilized on PEGylated AuNPs and the ability to induce selective antibodies in vivo was investigated. After immunization, mice showed significant MHC-II mediated immune responses and their antisera recognized human MCF-7 breast cancer cells. Nanoparticles designed according to this report may become key players in the development of anticancer vaccines.

Introduction

Mucin-1 (MUC1) is a top-priority ranking cancer antigen and thus a relevant target for the development of therapeutic cancer vaccines.[1] The membrane-bound MUC1 glycoprotein is extensively overexpressed on epithelial tumor cells. Concomitant underglycosylation of the MUC1 extracellular domain results in the formation of tumor-associated antigens with exposure of the peptide backbone and presentation of Tn, T, sialyl-Tn and sialyl-T structures.[2] Nevertheless, the immunogenicity of these tumor-associated glycopeptide antigens is too low to override the endogenous tolerance of the immune system. Therefore, MUC1 cannot directly be used as an anti-tumor vaccine. Instead a number of efforts are required to enhance the immunogenicity of the MUC1 antigens, for example, by covalent coupling of synthetic *O*-glycosylated MUC1 glycopeptides to suitable immune carrier proteins, lipid immunostimulants or peptide T-cell epitopes.[3]

Gold nanoparticles (AuNPs) have carrier properties suitable for development of cancer vaccines.[4] AuNPs are inert, non-toxic and readily endocytosed by antigen presenting cells (APCs).[5] Owing to the multiple presentation of the antigen on their surface, AuNPs can improve antigen delivery and up-take by highly efficient APCs such as the dendritic cells.[6] AuNPs carrying multiple copies of both a protein antigen[4d] and CpG oligonucleotides,[4f] melanoma antigen derived peptides,[4e] carbohydrates[7] and glycopeptides[8] have previously been prepared for evaluation as potential cancer vaccines. In a very recent report, the synthesis of AuNPs functionalized with MUC1-glycopeptides was described.[9] However, there are currently no immunological reports of AuNP-based vaccine candidates targeting the tumor-associated MUC1-1 glycoprotein.

Furthermore, only in few cases the reported nanoparticle-based vaccine candidates were subsequently investigated in their ability to produce antibodies in vivo.[4d,f,7b,8b] The system utilizing CpG oligonucleotides as adjuvant was developed choosing red fluorescent protein as the model antigen, a non-natural antigen.[4f] Very recently the same group reported the use of the extra domain B of fibronectin as the tumor-associated antigen.[4d] For the other two systems based on carbohydrates or glycopeptides only moderate immune responses were reported.[7b,8b] Moreover, all to date reported AuNP-based vaccine candidates were prepared by direct reduction of the gold precursor with $NaBH_4$ in the presence of thiol-terminated glycopeptides[7a,8] or glycosylated polymers.[7b] Although this one-pot synthesis strategy might be attractive, it introduces limitations in vaccine design.

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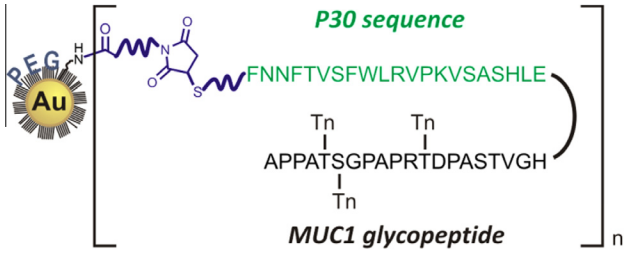


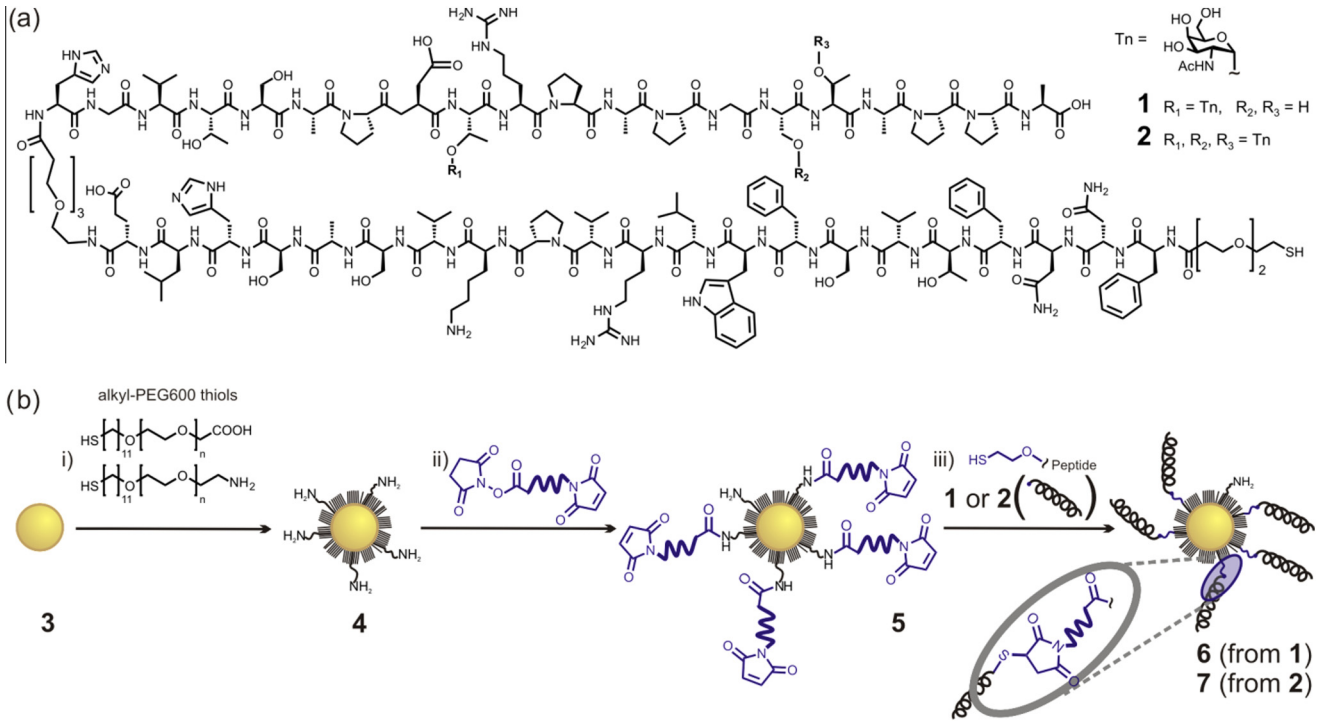
Figure 1. Schematic overview of the immunized three-component AuNP–P30–MUC1 vaccine candidate that contains three Tn glycosylation sites.

Nanoparticle size and degree of functionalization (i.e., epitope loading) are tightly interconnected and only a limited range of stable colloidal solutions may be achieved.[8b] We therefore envisioned the conjugation of glycopeptides to PEGylated AuNPs.

Here, we describe three-component vaccine candidates that combine MUC1 glycopeptides covalently linked to CD4 T-cell peptide epitopes (P30 from Tetanus Toxoid) and PEGylated AuNPs (Fig. 1). Our design aims at promoting antigen presentation on APCs, increased T-cell activation, and MHC II-restricted T- and B-cells cooperation. To evaluate the presentation efficacy and mode of action of the MUC1–P30–AuNP-based formulation, a control formulation was prepared for direct comparison, which consisted of a previously reported MUC1 glycopeptide—P30 T-cell epitope conjugate (vide infra).[3c]

Results and discussions

MUC1–P30–AuNP three-component vaccine candidates were prepared according to the route outlined in Scheme 1. The preparation of two vaccine candidates was investigated to demonstrate the feasibility of the approach. Briefly, two different MUC1 tandem repeat glycopeptides containing the tumor associated Tn-antigen and extended with the P30 T-cell epitope, were synthesized on solid-phase resulting in peptides **1** and **2**. At the N-terminus of both glycopeptides a spacer ending with a free thiol group was incorporated for conjugation to the maleimido groups on PEGylated AuNPs. Freshly prepared citrate-capped AuNPs (**3**) with a diameter of 13 nm, were passivated (i.e., formation of a self-assembled monolayer of thiol-ligands on their surface) by treatment with a mixture of amino- and carboxy-terminated alkyl-PEG600 thiols, with a molar fraction of the amino terminated derivative $x_{NH_2} = 0.20$.[10] A heterobifunctional linker ($SM(EG)_2$), with a maleimido group on one side and a *O*-succinimide reactive ester on the other, was used to couple the P30–MUC1 glycopeptide **1** (or **2**) to PEGylated AuNPs **4** (Scheme 1b) via the intermediate **5**.[10] Purification by ultracentrifugation gave the MUC1–P30–AuNP three-component vaccine candidates **6** and **7** (Scheme 1b). The peptide loading (peptide copies/AuNP) was determined by two approaches, differing in the method used to measure the concentration of peptide in a given sample. In the first approach, peptide concentration was determined by amino acid analysis, while in the second approach it was determined by fluorescence detection, based on the intrinsic fluorescence of a single tryptophan residue in the P30 sequence. AuNP concentration was determined by ICP-OES and the ratio between peptide concentration and AuNP concentration afforded the peptide loading. Both methods indicated that the AuNP-based vaccine candidates **6** and **7** are characterized by high peptide loadings ranging between 1200–1400 peptides/AuNP by amino acid analysis or between 500–700 peptides/AuNP as determined by Trp fluorescence (Figs. S8 and S9). The discrepancy between these two intervals may be a consequence of a suboptimal calibration for the fluorescence-based determination. Nonetheless, the data show that two totally different quantification methods give peptide loadings, which only differ by approximately a factor of 2. Considering that dose–response relationships are generally observed on a logarithmic scale, the accuracy in estimating the



Scheme 1. (a) Structure of the P30–MUC1 tandem repeat glycopeptides **1** and **2**. (b) Synthetic route for the AuNP-based vaccine candidates: (i) passivation of AuNPs **3** with alkyl-PEG600 thiols gives PEGylated AuNPs **4**, (ii) coupling of a NHS/maleimido heterobifunctional linker affords AuNPs **5**, (iii) coupling of the MUC1–P30 peptides **1** or **2** results in the three-component vaccines candidate **6** and **7**, respectively.