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A versatile approach for the immobilization of amines via copper-free "click" reaction between azido self-assembled monolayer and alkynyl Fischer carbene complex. Application to the detection of staphylococcal enterotoxin A antibody

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ABSTRACT

A copper-free "click" reaction between an azido-terminated self-assembled monolayer (SAM) chemisorbed on planar gold-coated glass sensors and an alkynyl Fischer carbene complex yielded functionalized surfaces onto which facile and swift grafting of amine-containing molecules was achieved via aminolysis of the Fischer carbene moieties. The course of this process was conveniently monitored by Polarization-Modulation Infrared Reflection-Absorption Spectroscopy (PM-IRRAS), contact angle measurements and X-ray photoelectron spectroscopy (XPS). A study of the parameters involved in the covalent grafting of the model protein bovine serum albumin (BSA) to the Fischer carbene modified SAM was carried out. As an application, the bacterial toxin staphylococcal enterotoxin A (SEA) produced by some pathogenic strains of *Staphylococcus aureus* was immobilized on the gold chips and immunocapture of a polyclonal antibody raised against SEA was detected by PM-IRRAS so as to ultimately construct an optical immunosensor for the detection of this toxin in food samples.

1. Introduction

Formation of functional organic thin films at the surface of metals or metal oxides is a key step in the elaboration of "smart" materials that find applications in various areas. When the thin film contains a biologically active molecule, it can provide important avenues of research in the field of biomaterials, biosensors and so on. Among the various pathways available, chemisorption of alkyl thiolates is a particularly useful strategy to create perfectly controlled and ordered monolayers of organic molecules at the surface of noble metals such as gold [1]. In this way surface-adsorbed molecules are chemisorbed in a well-defined orientation and functionalization can be further carried out, provided the sulfur derivative contains a reactive group at the other end of the alkyl chain. Similarly to the evolution recently observed in the bioconjugation methods carried out in solution [2], new strategies used to anchor biomolecules to surfaces have been recently put forward [3], among which azide–alkyne cycloaddition reactions appear as particularly useful and versatile [4–8]. This strategy is qualified as bioorthogonal as it involves functional groups not generally present in biomolecules and that do not react with biofunctionalities such as amines, alcohols and carboxylic acids. In their copper-catalyzed format, alkyne–azide cycloadditions have been successfully used to produce ordered monolayers of peptides [9], proteins [10], DNA [7,11] and sugars [12].

We previously reported the functionalization of gold nanoparticles and silica substrates (surface-oxidized silicon chips or glass slides) with Fischer-type alkoxymetallocarbene entities via cross-metathesis or Huisgen 1,3-dipolar cycloaddition reactions (in a copper-free format) with vinyl- or azide-terminated SAMs and made use of the electrophilic character of this entity to chemisorb amine-containing molecules by aminolysis reaction [13,14]. In this article, conditions for the covalent immobilization of the model protein bovine serum albumin (BSA) by aminolysis of surface-confined Fischer carbene entities were first optimized. The metallic surface was characterized at each step of its functionalization by PM-IRRAS, XPS and contact angle measurements. As an application the bacterial toxin staphylococcal enterotoxin A (SEA) was

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immobilized onto gold-coated glass chips and its ability to capture an anti-SEA antibody was detected by PM-IRRAS.

2. Results and discussion

2.1. Formation of alkoxycarbene-functionalized SAM on gold

The formation of the alkoxycarbene-terminated SAM on gold chips is illustrated in Scheme 1.

A glass slide coated with a thin layer of gold was flame-annealed then treated with a 1 mM ethanolic solution of bis(11-azidoundecyl)disulfide synthesized according to the literature [5,15] for 60 h at RT. After extensive washing, the gold surface (step A) was analyzed by PM-IRRAS (Fig. 1A, spectrum a). Assignment of the major vibration bands is given in Table 1.

The presence of a typical band at ca. 2100 cm^{-1} provided indisputable evidence that the gold surface was now covered with a monolayer of 11-azidoundecylthiolate. More generally, the surface IR spectrum of the SAM of 11-azidoundecylthiolate was very similar to the IR spectrum of the starting disulfide (Fig. 1B, spectrum a).

Next, the gold substrate was dipped by a solution of phenylacetylenylethoxycarbene pentacarbonyl tungsten in toluene at 50 °C for 4 h. After another extensive washing in toluene, the gold surface (step B) was once again submitted to PM-IRRAS analysis (Fig. 1A, spectrum b and Table 1). The asymmetric stretch of the azido group around 2100 cm^{-1} was no longer observed on the IR spectrum whereas three typical bands corresponding to the modes of vibration of the $LW(CO)_5$ entity (C_{4v} symmetry) were now present. Interestingly, the surface IR spectrum also displayed some marked differences in comparison to the IR spectrum of the alkynyl Fischer carbene complex (Fig. 1B, spectrum b). Indeed, it lacked the band at 2157 cm^{-1} assigned to the $\nu(C{\equiv}C)$ band. Moreover, a significant shift of the position of the $E + A_1$ modes from 1907 to 1937 cm^{-1} was also noticed (compare spectra a and b). Two other intense and rather broad bands were observed at ca. 1640 and 1450 cm^{-1}, that we tentatively assigned to the C=C and the N=N stretching vibrations of the formed triazole, respectively. All these features indicated that the Fischer alkoxycarbene entity had covalently bound to the SAM via 1,3-dipolar cycloaddition with the azidoalkylthiolate and was not simply adsorbed to the metal surface. The kinetics of azide–alkyne cycloaddition at the liquid/solid interface was studied by PM-IRRAS. The intensity of the $\nu_{as}(N{=}N)$ band was gradually seen to decrease over time while that of the main $\nu(C{\equiv}O)$ band increased gradually within the first 4 h then decreased during the next 2 h (Fig. 2).

Contact angle measurements were also carried on the gold-coated surfaces. While the contact angle θ of the annealed gold surface was found equal to 58.0 ± 0.5°, it increased to 70.8 ± 0.2° after step A in agreement with literature data [6] and slightly decreased to 66.7 ± 0.7° after step B. To confirm the chemical composition of the organic layer after step B, the gold surface was also submitted to XPS analysis. All the atoms expected to compose the organic layer at step B were eventually observed on the survey spectrum (not shown). High resolution spectra for C 1s, N 1s, O 1s and W 4f are depicted in Fig. 3. Each spectrum was decomposed into several components related to the various chemical environments of the atoms in the molecular layer (Table 2).

The C 1s peak was decomposed into 3 components of unequal intensity readily assigned to carbons in C–C, C–O/C–N and C=O bonds. Similarly, the O 1s peak was decomposed into three components even if only two kinds of O are expected from the molecular composition of the layer. The S $2p_{3/2}$ and S $2p_{1/2}$ spectra

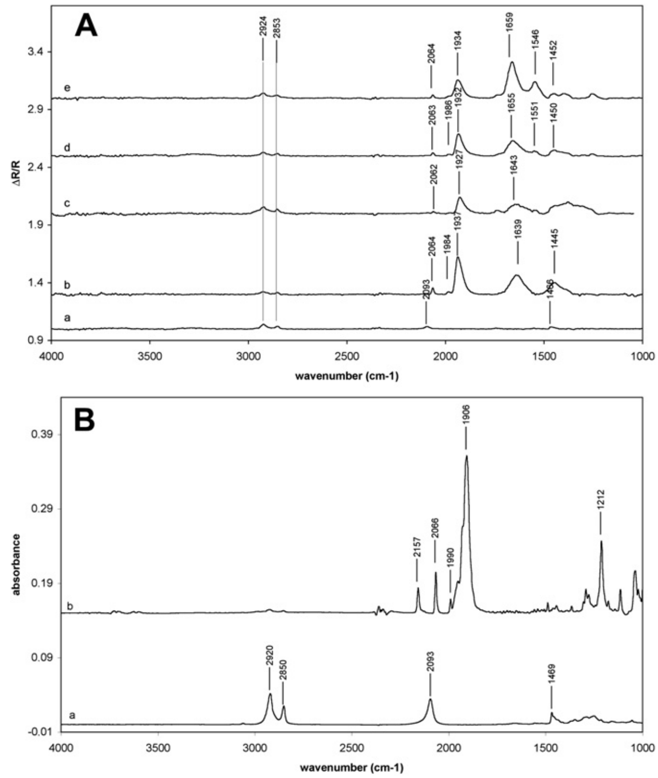
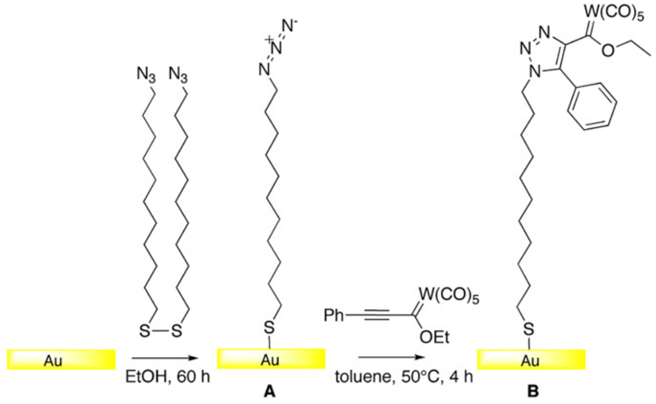


Fig. 1. A) PM-IRRA spectra of azidoundecylthiolate SAM (a), after reaction with 10 mM phenylacetylenylethoxycarbene pentacarbonyl tungsten at 50 °C (b), after reaction with 10 mM n-butylamine in water (c) or 100 mg/l BSA in phosphate buffer pH 8 (d) or 9.6 mg/l SEA in phosphate buffer pH 8 (e). B) ATR-IR spectra of bis(11-azidoundecyl) disulfide (a); phenylacetylenylethoxycarbene pentacarbonyl tungsten (b).



Scheme 1. Functionalization of gold-coated glass substrates.

Table 1
Band assignments of the surface IR spectra recorded after steps A, B and C.

Step	Wavenumber (cm^{-1})			
	A	B	C1	C2
$\nu_{as}(C{-}H)$	2924	2924	2922	2923
$\nu_s(C{-}H)$	2853	2853	2850	2850
$\nu_{as}(N{=}N)$	2093			
$\nu(C{\equiv}O)$ (A_1)		2064	2062	2063
$\nu(C{\equiv}O)$ (B_1)		1984 (sh)	1975 (sh)	1986
$\nu(C{\equiv}O)$ $(E + A_1)$		1937	1927	1932
$\nu(C{=}O)$ (amide I)				1655
$\nu(C{-}N) + \partial(N{-}H)$ (amide II)				1551
$\partial(C{-}H)$	1466			
$\nu(C{=}C)$		1639	1643 (br)	
$\nu(N{=}N)$		1445	1462 (br)	1450 (br)