Thioctic acid amides: convenient tethers for achieving low nonspecific protein binding to carbohydrates presented on gold surfaces†

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The thioctic acid amides of 2′-aminoethyl α-D-mannopyranoside and 2′-aminoethyl α-1,3-D-mannopyranosyl (α-1,6-D-mannopyranosyl)-α-D-mannopyranoside presented on both planar and nanoparticle gold surfaces give higher specific and lower non-specific protein binding than the related 2′-thioethyl glycosides.

The presentation of carbohydrates on planar gold sensor surfaces,[1] colloidal gold particles[2] and recently gold-based microarrays[3] has been the focus of considerable research interest for studies of carbohydrate recognition. In moving from model studies to biomedical applications, two considerations become critical *viz*.: non-specific protein binding; and the stability of the modified surface with respect to ligand desorption. Optimal presentation of ligands on planar gold surfaces is generally achieved using PEG-thiol-containing SAMs using the protocols developed by Whitesides *et al*.[4] However, less effort has gone into defining an optimal linker/presentation system for carbohydrates on gold nanoparticles. Recent studies from these[5] and other[6] labs have aimed to develop glyconanoparticles for the colorimetric detection of lectins. Such studies have often relied on the readily accessible 2′-thioethyl glycosides, such as **1a**, although short chain alkanethiols are known to form disordered SAMs.[7] Therefore, we have investigated other linker technologies that might prove convenient for applications that use either planar or nanoparticulate gold surfaces.

Thioctic acid-based systems have been used to present a variety of classes of molecule on gold surfaces,[8] but to the best of our knowledge they have not previously been used with carbohydrate ligands. Naturally occurring thioctic acid (lipoic acid) is potentially attractive for a number of reasons: the presence of two sulfur atoms obviates the need to engage in noxious sulfur chemistry, whilst also providing two potential points of attachment to gold.[9] The alkyl chain length in thioctic acid would be expected to result in a more ordered self-assembled monolayer than a simple mercaptoethyl tether, a factor that may be reinforced by amide coupling to sugar derivatives, which is also expected to give enhanced order and hence stability.[1c] In the current study, α-D-mannopyranoside and α-1,3-D-mannopyranosyl(α-1,6-D-mannopyranosyl)-α-D-mannopyranoside-based thiols (**1a** and **2a**) and thioctic-amides (**1b** and **2b**) (Fig. 1) (see ESI) were presented on both planar gold and on gold nanoparticles. Surface plasmon resonance (SPR) and UV-visible spectroscopy were used to evaluate specific and non-specific protein recognition by these surfaces.

Mannoside SAMs were prepared by immersion of gold coated SPR sensor chips (bare Au; Biacore SA) in 2 mM methanol/20% water solution of thiols **1a** or **2a**, or thioctic-amides **1b** or **2b**. The integrity of monolayers formed in this manner was determined by cyclic voltammetry (ESI). Surfaces modified with thiols **1a** and **2a** displayed characteristics similar to bare gold, indicating that a poorly ordered monolayer, with significant "holes", had been formed. In contrast, surfaces modified with thioctic-amides **1b** and **2b** showed a higher degree of insulation [reduced penetration of ferricyanide in solution giving a decreased current response] consistent with a more ordered surface.

The higher affinity of Con A for trimannoside than for mannoside has been previously established.[10] However, the SPR response obtained when Con A was passed over the mannoside **1b** surface was greater than that achieved from surfaces presenting the corresponding trimannoside **2b** (Fig. 2). Ligand density on surfaces is well known to have a profound impact on carbohydrate recognition selectivity and affinity.[1c,4] Both the **1b** and **2b** surfaces gave a substantially greater response to Con A than surfaces modified with the corresponding thiols **1a** and **2a**, which is presumably due to greater organisation of the monolayer, and hence more optimal ligand presentation with the thioctic acid derivatives. The "on" rate for binding to both trimannosides, **2a** and **2b**, is higher than compared to the corresponding mannosides, **1a** and **1b** (Fig. 2). These observations are consistent with the notion that recognition of the preferred trimannoside ligand is prevented by sub-optimal ligand density on these particular surfaces.[11]

Specific and non-specific binding of the trimannoside SAMs was evaluated by SPR using multivalent lectins [concanavalin A (Con A), *Tetragonolubus purpureas* lectin (TPL) and *Ricinus communis* agglutinin 120 (RCA_{120})] as well as two "sticky" proteins

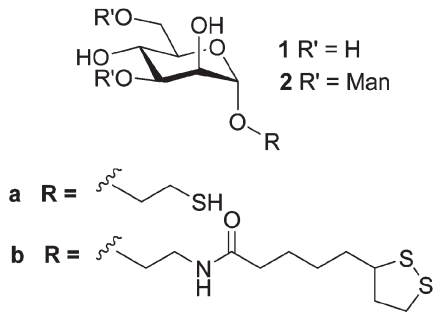

Fig. 1 Structures of the 4 mannoside derivatives.

† Electronic supplementary information (ESI) available: synthesis and characterisation of all compounds; cyclic voltammograms of monolayers; UV-vis. spectra of protein induced nanoparticle aggregation. See http://www.rsc.org/suppdata/cc/b5/b503843j/

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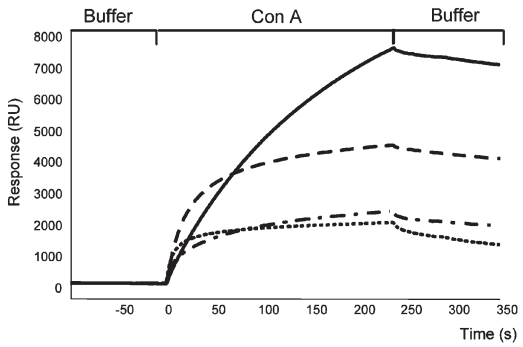

Fig. 2 SPR response curves for the binding of 250 μg/ml Con A to gold sensor chips coated with thiols **1a** (dash–dot) and **2a** (dot), thioctic-amides **1b** (solid) and **2b** (dash).

(fibrinogen and cytochrome c).[4] Surfaces prepared from trimannoside thiol **2a** showed high non-specific binding to three of the proteins tested (Fig. 3; Table 1). In spite of the different binding affinities, molecular weights and isoelectric points of proteins tested,[12] surfaces prepared with thioctic-amide **2b** showed low non-specific binding in all cases; less than 60 RU in all cases *cf.* 3000 RU response for the Con A (1 RU = 1 pg mm^{-2}; the surface area of the flow cell = 1.2 mm^2). Surfaces prepared with **2b** appear to show higher resistance to adsorption of fibrinogen and cytochrome c than ethylene oxide-containing mixed SAMs.[4] The lack of non-specific binding[13] to the thioctic-amide-based surface by a series of different proteins suggests that protein adsorption on these surfaces is dominated by the interfacial properties of the surface and not by specific properties of the proteins concerned.

Gold nanoparticles are characterised by a discrete surface plasmon absorption band in the visible electronic spectrum[14] which is dependent on, amongst other parameters, the average diameter of the particles. Hence gold nanoparticles characterised by interparticle distances greater than the average particle diameter appear red; when the interparticle distance in aggregates decreases to less than approximately the average particle diameter, the colour changes to blue.[14] These colorimetric properties make it possible to detect multivalent lectins following derivatisation of the gold surface with appropriate carbohydrate ligands.[5,6] In the current study, gold nanoparticles were prepared by citrate reduction of tetrachloroaurate, following the method of Turkevich,[15] and modified by interaction with 20 mM solutions of thiols **1a** or **2a**, or thioctic-amides **1b** or **2b**. The nanoparticles were purified by several centrifugation–washing steps and redispersed in Tris buffer, pH 7. Centrifugation and resuspension could be repeated several times without observable loss of colloidal stability; no observed colour change due to aggregation was detected following storage for 4 months at 4 °C. The average diameter of the particles prepared in this manner was determined by light-scattering and TEM[5] to be *ca.* 17 nm. UV-vis spectroscopy showed that derivatisation of gold particles with monolayers of mannosides **1a** or **1b** or trimannosides **2a** or **2b** resulted in only a small shift in A_{max} (typically from *ca.* 520 to 524 nm), indicating only a small change in the dielectric properties of the metal surface upon self-assembly.

All four sets of red coloured gold nanoparticle conjugates turned blue on addition of Con A due to particle aggregation (ESI). For all four sets of particles, A_{max} shifted from *ca.* 525 nm to longer wavelengths when the concentration of Con A was increased from 0 to 20 μg/ml (Fig. 4A) (and ESI). Particles derived from thioctic-amides **1b** or **2b** showed a more pronounced response at lower concentrations of Con A than those derived from thiols **1a** or **2a**. At 5 μg/ml Con A, formation of stable aggregates from particles derived from thioctic-amides **1b** and **2b** was essentially complete within 10 min; the resulting aggregates were stable for at least 18 h. In contrast, particles derived from mannoside-thiol **1a** failed to respond to Con A at 5 μg/ml, whilst particles derived from trimannoside-thiol **2a** achieved maximum aggregation within 18 h, *i.e.*, much slower than the thioctic-amide-derived particles (Fig. 4B). It is clear that the choice of tether has a profound impact on particle response to Con A, with thioctic-amide-based particles outperforming their thiol-derived counterparts.

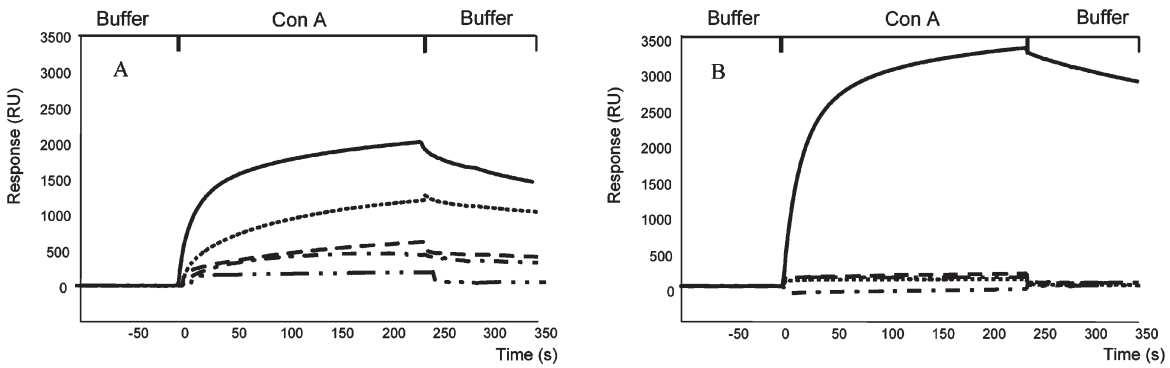

Fig. 3 Comparison of SPR responses after injection of 10 μg Con A (solid); 20 μg RCA_{120} (dash–dot–dot); 20 μg TPL (dash–dot); 20 μg fibrinogen (dot); and 20 μg cytochrome c (dash) over gold surfaces modified with: (A) trimannoside **2a**; (B) trimannoside **2b**.

Table 1 SPR response (RU) of gold surfaces modified with **2a** or **2b** to specific and non-specific protein binding

Ligand \ Protein	Con A	RCA_{120}	TPL	Fibrinogen	Cytochrome c
Thiol **2a**	1634 (100%)	47 (3%)	353 (22%)	1105 (68%)	434 (27%)
Thioctic-amide **2b**	3000 (100%)	4 (1.3%)	20 (0.7%)	46 (1.5%)	57 (1.9%)

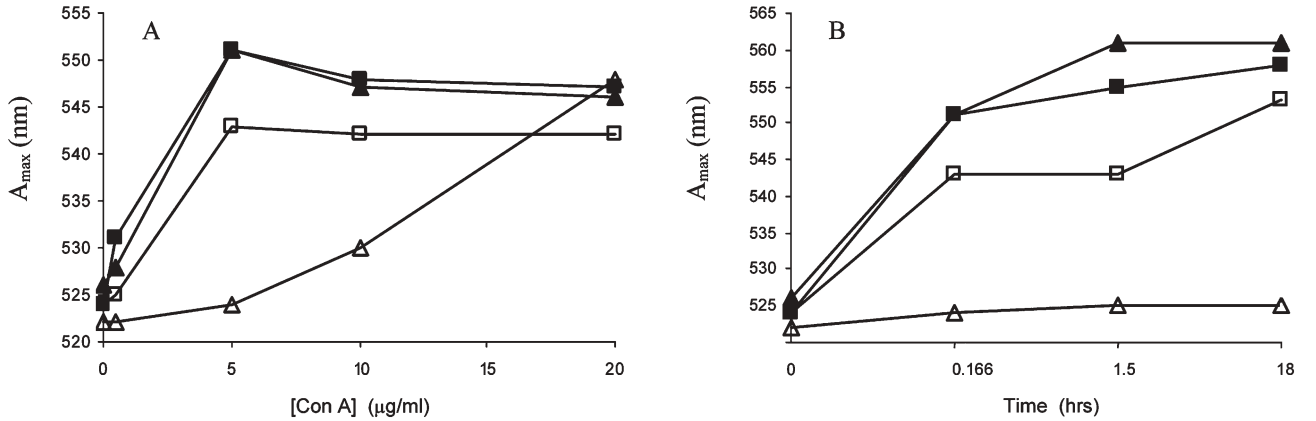

Fig. 4 Change in A_{max} of gold particles derivatised with **1a** (open triangles), **1b** (solid triangles), **2a** (open squares), **2b** (solid squares) with (A) 0–20 μg/ml Con A and; (B) with 5 μg/ml Con A after 10 min, 1.5 h and 18 h. (The lines on both A and B are shown for line of sight only.)

All four sets of particles were evaluated for non-specific binding using the set of proteins used previously in SPR studies at 50 μg/ml. Non-specific particle aggregation was assessed by a red shift of A_{max} (ESI). Mannoside-thiol **1a** derivatised particles showed a degree of non-specific binding to all proteins, whilst only fibrinogen induced aggregation of particles derived from trimannoside **2a**. Essentially no non-specific aggregation of particles derived from thioctic-amides **1b** and **2b** was observed; specific binding to Con A is substantially higher than non-specific binding to particles derived with the thioctic-amide-based ligands. These results are similar to those obtained for non-specific binding of the same proteins to SAMs on the SPR chips. Further, the induced aggregation of thioctic-amide **2b** derivatised particles by 2.5 μg/ml Con A in the presence of up to 50 μg/ml fibrinogen exhibited $<$ 10% competitive binding (ESI).

In conclusion, the thioctic-amide system serves as an accessible and effective method for carbohydrate presentation on gold surfaces in a manner that limits non-specific protein binding whilst enhancing specific binding of the target lectin.

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11 A linear response of carbohydrate-presenting surfaces to lectins is typically only seen at ligand coverage ($<$ 5%) (ref. 1b).

12 RCA_{120} m.w. 120 kDa, isoelectric point 7.5–7.9, binds α-D-galactose; TPL: isoelectric point 7.3, affinity for α-D-fucose; Fibrinogen: isoelectric point 5.5, a structurally complex, flexible molecule of approximately 340 kDa; Cytochrome c: isoelectric point 9.1–9.5, m.w. 12.4 kDa, displays a significant level of non-specific adsorption to surfaces presenting carboxylic acid groups: M. Collinson, E. F. Bowden and M. J. Tarlov, *Langmuir*, 1992, **8**, 1247; J. Lahiri, G. D. Fate, S. B. Ungash and J. T. Groves, *J. Am. Chem. Soc.*, 1996, **118**, 2347.

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