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Gold-phosphine binding to *de novo* designed coiled coil peptides

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ABSTRACT

The coordination of the therapeutically interesting [AuCl(PEt_3)] to the *de novo* designed peptide, TRIL23C, under aqueous conditions, is reported here. TRIL23C represents an ideal model to investigate the binding of [AuCl(PEt_3)] to small proteins in an effort to develop novel gold(I) phosphine peptide adducts capable of mimicking biological recognition and targeting. This is due to the small size of TRIL23C (30 amino acids), yet stable secondary and tertiary fold, symmetric nature and the availability of only one thiol binding site. [AuCl(PEt_3)] was found to react readily with the Cys side chain in a 1:1 ratio as confirmed by UV-visible, ^{31}P NMR and mass spectrometry. Circular dichroism confirmed that the coiled coil structure was retained on coordination of the $\{Au(PEt_3)\}^+$ unit. Redesign of the exterior of TRIL23C based on a biologically relevant recognition sequence found in GCN4, did not alter the coordination chemistry of [AuCl(PEt_3)]. To the best of our knowledge, this represents the first report on the coordination of gold(I) phosphine compounds to *de novo* designed peptides, and could lead to the generation of novel gold(I) phosphine peptide therapeutics in the future.

1. Introduction

The search for new and improved metal based drugs is fueled primarily by the success of the first platinum-based chemotherapeutic drug, cisplatin, and the second generation analogues such as carboplatin and oxaliplatin. Both the success of platinum drugs and the severe limitations associated with their use in the clinic have driven extensive research into alternative metal complexes for drug development. Gold is a promising non-platinum metal which has been investigated for the development of new therapeutics, and for which promising results are increasingly being reported. Notably the phosphine gold(I) complex auranofin (marketed as Ridaura) is currently used in the clinic for the treatment of rheumatoid arthritis. Furthermore, promising anticancer and anti-HIV activity have been reported for auranofin and related gold(I) complexes [1,2].

Various studies suggest that the phosphine gold(I) unit in auranofin is important for antitumor activity, but that the thiol carbohydrate moiety is replaced in vivo [2]. It has been recognized that this offers an opportunity to incorporate a bioactive ligand in its place [3–7]. One attractive class of bioactive and biocompatible ligands are peptides, which offer targeting and therapeutic opportunities, e.g. nuclear localization sequences (NLS), cell penetrating peptides (CPPs) and tumour targeting sequences such as RGD.

Reports exist in the literature of short peptide gold(I) phosphine complexes, which have been synthesized through the incorporation of the gold(I) phosphine unit into a non-natural amino acid. These are generally derivatives of the thiol containing amino acid cysteine (Cys), and are subsequently used in solid phase peptide synthesis as a means of incorporating the gold(I) phosphine into the peptide sequence [5]. This approach has been successfully employed; however, these synthetic procedures can potentially be time consuming, restricted by the harsh chemical synthesis conditions required and ultimately would be limited to peptide sequences that can readily be synthesized.

Long peptides and proteins can achieve biomolecular recognition through the formation of many favorable non-covalent interactions between amino acid side chains and the target. In addition to the primary structure, it is the correct secondary structure, such as the α-helix or β-sheet, which is crucial for the correct positioning of these side chains, thereby achieving strong and selective binding to the target. Not surprisingly biological recognition is achieved on a larger length scale (nm) than traditional small molecule chemistry (Å). It is therefore our intention to investigate the coordination of gold(I) triethylphosphine based on auranofin, to protein-type sequences with well defined secondary structure elements (nm). Ultimately the goal would be to develop targeted derivatives of auranofin, with improved biodistribution and bioactivity.

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A potential challenge associated with this approach is the large size and complexity of the natural proteins we are intending to mimic in our design. Therefore our intention is to exploit the advantages offered by *de novo* peptide design (from "first principles"), which provides an opportunity to study the coordination of gold(I) phosphine to simplified protein folds in aqueous solution. Due to the high affinity of gold(I) for soft ligands such as the thiol of Cys, it should be possible to readily incorporate the gold(I) phosphine into a peptide sequence under mild aqueous conditions, as has previously been reported for natural proteins including albumin [8], zinc fingers [9–11], glutathione reductase [12] and cyclophilin [13]. In this manuscript we will focus on *de novo* designed α-helical coiled coil peptides based on TRI, Ac-G(LKALEEK)$_4$G-NH$_2$, developed by Pecoraro, DeGrado and co-workers [14,15]. Cys derivatives of these have been shown to readily coordinate soft heavy metal ions such as mercury, cadmium, bismuth, arsenic and lead [14–23]. However, to the best of our knowledge, the study reported here represents the first on the coordination of gold(I) phosphine compounds to *de novo* designed peptides with well defined secondary and tertiary structure. This approach could ultimately result in the development of a new generation of gold(I) phosphine peptide therapeutics.

2. Results and discussion

2.1. Peptide design

TRI is a 30 amino acid peptide based on the heptad repeat approach, Ac-G($\mathbf{L_a}K_\mathbf{b}A_\mathbf{c}L_\mathbf{d}E_\mathbf{e}E_\mathbf{f}K_\mathbf{g}$)G-NH$_2$, which spontaneously self-assembles in water to generate a two stranded coiled coil at low pH, and a three stranded coiled coil at higher pH (>5.5) [14]. This self-assembly is the result of a hydrophobic core of leucine residues (**a** and **d** sites) and interhelical salt bridges (between **e** and **g** sites). Gold(I) complexes have been reported to bind to both histidine (His) and Cys side chains, though with a greater affinity for the latter. As such, a thiol metal binding site was designed in the interior of the coiled coil by replacing a leucine (Leu) residue (at the 23 position) with a Cys, to generate the previously reported TRIL23C, Ac-G-LKALEEK-LKALEEK-LKALEEK-CKALEEK-G-NH$_2$ [23]. A control peptide which lacked the necessary thiol group required for gold(I) coordination, was prepared. Tryptophan (Trp) was incorporated into this peptide sequence, Ac-G-WKALEEK-LKALEEK-LKALEEK-LKALEEK-G-NH$_2$ (TRIL2W), to aid in concentration determination.[1] These sequences were synthesised, purified and characterized by routine methods [24].

2.2. UV-visible spectroscopy

Starting with the more reactive chloride derivative of auranofin, [AuCl(PEt$_3$)], its ability to bind to TRIL23C under mild conditions and in aqueous solution, was investigated by UV-visible spectroscopy. Aliquots of a stock solution of [AuCl(PEt$_3$)] were titrated into a 30 μM solution of TRIL23C monomer in 10 mM phosphate buffer pH 8. This resulted in the steady increase in the absorbance at 250 nm (Fig. 1A), which plateaus on addition of 30 μM [AuCl(PEt$_3$)] (Fig. 1B), indicating a 1:1 ratio between the gold(I) complex and the peptide monomer TRIL23C. The UV-visible spectra obtained (Fig. 1A) are not dissimilar to those previously reported for [AuCl(PEt$_3$)] binding to cysteine, penicilamine and small model peptides based on zinc finger proteins [10,25]. The total metal concentration was used to calculate an extinction coefficient of λ_{250} 2935 M^{-1} cm^{-1} for the complex [Au(PEt$_3$)(TRIL23C-*S*)]. This compares well to similar species in the literature [25].

In order to establish if binding was indeed through the intended Cys side chain, the concentration of available thiols was measured prior to, and at the end of, the titration using the Ellman's test [26,27]. This confirmed 30 μM of available thiols prior to the addition of any [AuCl(PEt$_3$)]. However, once the absorbance at 250 nm had reached its plateau, the Ellman's test indicated that no available thiols were present in solution. This observation is further supported by an analogous titration performed with a peptide sequence which does not contain a Cys residue, TRIL2W.[1] The titration of a stock solution of [AuCl(PEt$_3$)] into a 30 μM solution of TRIL2W monomer in 10 mM phosphate buffer pH 8, did not result in any increase in the absorbance at 250 nm (Fig. 1B).

These results and the ratio of 1 Au(I): 1 TRIL23C monomer are consistent with substitution of the chloride group with the deprotonated thiol of Cys, so as to generate [Au(PEt$_3$)(TRIL23C-*S*)]. In contrast, soft metal ions such as mercury, cadmium, bismuth, arsenic and lead are capable of binding in the interior of the three stranded coiled coil, (TRIL23C)$_3$, to all three equivalent Cys side chains, one from each peptide chain [14–23]. Despite reports of three and four coordinate gold(I) complexes [2], linear two coordinate gold(I) is more common and we observe the replacement of the highly labile chloride ligand by a single thiol from one peptide chain. This suggests that the triethylphosphine ligand remains bound (vide infra) and prevents the gold(I) from being accommodated in the interior of the coiled coil, as a result of steric clashes.

2.3. pH dependent binding

In order to assess the pH dependence of gold(I) complexation, the pH of an aqueous solution containing 30 μM [AuCl(PEt$_3$)] and 30 μM

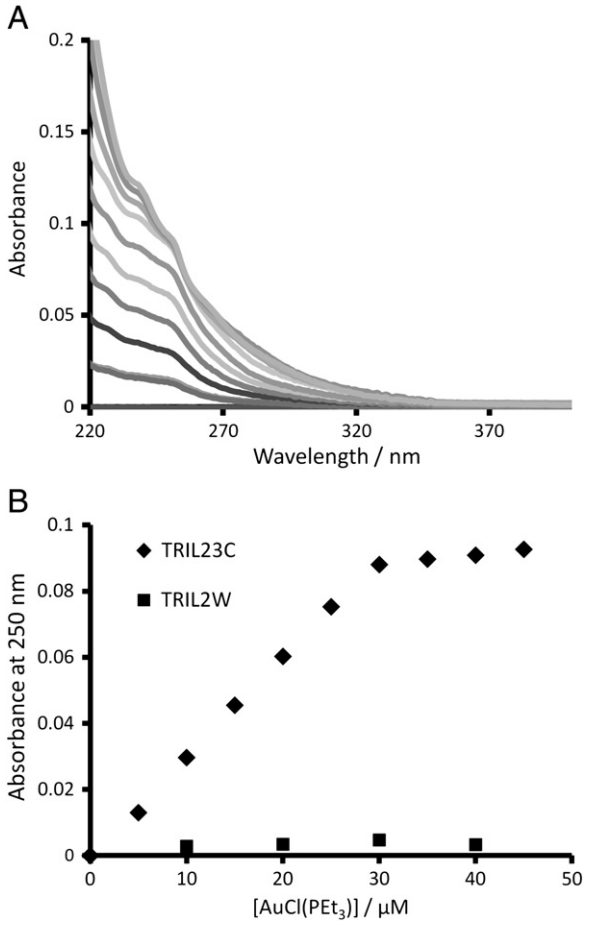

Fig. 1. (A) UV-vis spectra for a titration of [AuCl(PEt$_3$)] into a solution containing 30 μM TRIL23C in 10 mM phosphate pH 8. (B) A plot of the absorbance at 250 nm as a function of [AuCl(PEt$_3$)]/μM titrated into a 30 μM solution of TRIL23C and TRIL2W, respectively.

[1] In the absence of a Cys thiol side chain, a single Trp residue is required to determine the peptide concentration by monitoring the absorbance at 280 nm.