Experimental Section

A series of Mo/HZSM-5 catalysts were prepared as described in ref. [4b]. HZSM-5 powders were impregnated with aqueous solutions containing a given amount of ammonia heptamolybdate (AHM), then dried at RT and 373 K for 12 and 8 h, respectively. After calcined at 773 K for 5 h, the catalysts were crushed and sieved to yield granules of 20–60 mesh.

A specially designed device was built for the MAS NMR study. It contains a gas flow section, a reaction and sample preparation section, and an on-line mass spectrometer (Figure 1). After the reaction (the temperature can be as high as 1123 K), the reaction and sample preparation section can be taken off and turned through 90°. Then, in the reaction atmosphere the sample can be transferred into a NMR-rotor with the aid of a tamper (to pack the sample into the tube) that is mounted in a bellows attatched to the upper side of the flange. The rotor is then sealed with a cap in a rack with the aid of tamper. A spin rate as high as 12 kHz can be reached following this sample filling method. In the present case, the samples were treated with a methane flow ($SV = 1500\ mL\,g^{-1}\,h^{-1}$, 1 atm) for 1 h at 573, 673, and 873 K. Subsequently, the catalysts were subjected to reactions of 10 min, 0.5 h, 1 h, 3 h, and 6 h. In all the cases, the temperature was raised at a rate of $15\ ^{\circ}C\,min^{-1}$.

Proton MAS NMR spectra were recorded in 4 mm ZrO_2 rotors at 400.13 MHz on a Bruker DRX-400 spectrometer fitted with a MAS probe. For each spectrum, a $\pi/10$ pulse and a 4 s relaxation delay were used, and 400 scans were accumulated. Samples were spun at 8 kHz, and the DSS (DSS = sodium 4,4-dimethyl-4-silapentane sulfonate) signal was taken as the reference for the ^1H chemical shifts. The deconvolution of the spectra was conducted using WINNMR, supplied by the spectrometer manufacturer.

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[12] In the TPSR (temperature program surface reaction) profile of this reaction (detected by the on-line mass spectrometer) water was shown to be produced when the temperature reached 973 K, with a maximum at about 13 min after the reaction began. Two minutes later (15 min after the start of the reaction) the start of benzene formation is observed. The formation of water stopped about 1 h later at the same temperature, but that of benzene increases slowly in the first hour, and then appears to be constant. This indicates that the water formation is a stoichiometric (not catalytic) reaction [see Eq. (1)], where as benzene formation is a catalytic reaction.

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First Crystal Structure of a Medicinally Relevant Gold Protein Complex: Unexpected Binding of $[Au(PEt_3)]^+$ to Histidine**

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Antiarthritic gold(I) compounds such as the injectable drugs aurothiomalate[1] (Myochrysine) and aurothioglucose (Solganol),[2] and the oral drug[3] [$Au^I(PEt_3)$ (2,3,4,6-tetra-*O*-acetyl-β-D-thioglucose-*S*)] (auranofin) are "prodrugs", which undergo facile ligand displacement reactions.[4, 5] The critical target sites are thought to be thiolate sulfurs (cysteine residues) in proteins and enzymes,[5] Au^I being a very "soft" (class 'b') metal ion.[6, 7] However, there is a lack of structural data on adducts of antiarthritic Au^I complexes with proteins.[8] We report here investigations of reactions between the enzyme cyclophilin and the antiarthritic complex[9, 10] [$Au(PEt_3)Cl$] (**1**), and the first X-ray crystal structure of a protein adduct with a Au^I phosphane complex. Unexpectedly, in cyclophilin crystals, Au^I binds to the nitrogen atom of an active site His residue, despite the presence of four Cys thiol groups. The results have implications for understanding the biological chemistry of gold antiarthritic complexes. Additional interest in this work arises from the need for site-specific heavy-atom reagents for X-ray crystallography.[8]

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Cyclophilins are ubiquitous proteins involved in protein folding, transport, and assembly, and are linked to general cellular stress responses.[11] They are peptidyl prolyl isomerase (PPIase) enzymes, and cyclophilin A (an immunophilin) is the major intracellular receptor for the immunosuppressive drug, cyclosporin A (CsA). We chose cyclophilin-3 (Cyp-3, one of the most abundantly expressed cyclophilin isoforms in the free living nematode *Caenorhabditis elegans*) for study, since it has a known X-ray structure[12] and possesses four free Cys thiol groups which are potential Au^I target sites.

First the reaction between Cyp-3 and **1** was studied in solution by mass spectrometry. For a molar ratio of Cyp-3:**1** of 1:3.5, the sample after one day revealed three new peaks with masses of 18612.0 u (calcd 18615.5 u), 18736.8 u (calcd 18733.6 u), and 18932.9 u (calcd 18930.6 u), corresponding to {Cyp-3 + Au}, {Cyp-3 + [$AuPEt_3$]}, and {Cyp-3 + Au + [$AuPEt_3$]}, respectively (Figure 1). For a molar ratio Cyp-3:**1** of 1:7, the sample after five days revealed that the peaks for

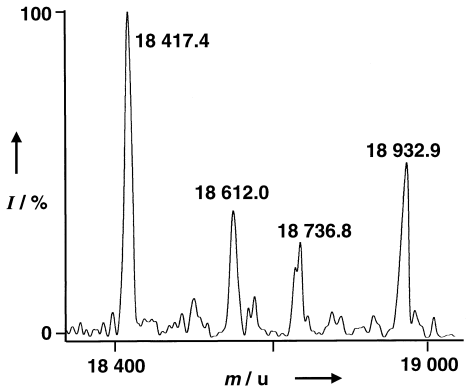

Figure 1. LC-ESI-MS of Cyp-3 (13.5 μM) after incubation with **1** (47.3 μM) for 24 h at 291 K. The peaks correspond to Cyp-3 (18417.4 u, theoretical mass: 18419.2 u); {Cyp-3 + Au} (18612.0 u, theoretical mass: 18615.5 u); {Cyp-3 + [$Au(PEt_3)$]} (18736.8 u, theoretical mass: 18733.6 u); {Cyp-3 + Au + [$Au(PEt_3)$]} (18932.9 u, theoretical mass: 18930.6 u).

Cyp-3 and {Cyp-3 + [$AuPEt_3$]} had disappeared, peaks for {Cyp-3 + Au} (18618.8 u), and {Cyp-3 + Au + [$AuPEt_3$]} (18930.8 u, major peak) were still detectable, and new peaks appeared at 19242.5 u corresponding to {Cyp-3 + Au + 2[$AuPEt_3$]} (calcd 19245.7 u) and 18812.8 u corresponding to {Cyp-3 + 2Au} (calcd 18812.4 u). These results appeared to be consistent with chloride displacement and $[AuPEt_3]^+$ binding to the protein, followed by PEt_3 displacement (and oxidation to $OPEt_3$). Such a displacement might be facile in the region of Cys40 and Cys168 (highly conserved in all divergent loop cyclophilins) since their sulfur atoms are within 5.5 Å and could readily provide linear S-Au-S coordination for Au^I.

Reactions of Cyp-3 with the disulfide 5,5′-dithio-bis(2-nitrobenzoic acid (DTNB) also suggested that all four Cys thiol groups are accessible. By UV/Vis spectroscopy, the average thiol content of Cyp-3 was determined to be 4.2 mol thiol per mol Cyp-3. The reaction was also investigated by LC-ESI-MS. The chromatographic peak for Cyp-3 alone corresponded to a mass of 18418.5 u (mean value, theoretical mass 18419.2 u).[13] This peak disappeared after reaction of Cyp-3 with DTNB, and the major new chromatographic peak had a mass of 19210.6 u (Figure 2), corresponding to {Cyp-3 + 2DTNB} (i.e. $4 \times 1/2$ DTNB, calcd: 19211.2 u). This implies that all four Cys thiol groups form mixed disulfides with DTNB and are therefore accessible.

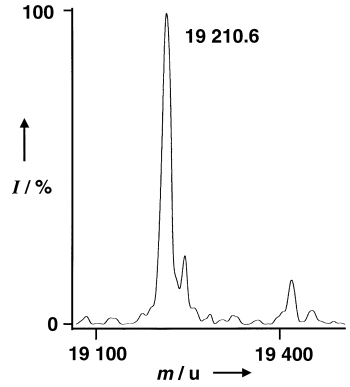

Figure 2. LC-ESI-MS of Cyp-3 after reaction with excess DTNB. The major peak (19210.6 u) corresponds to four half DTNB species bound to Cyp-3, that is all four Cys residues blocked as mixed disulfides (theoretical mass 19211.2 u).

To identify the gold coordination sites, we treated single crystals of Cyp-3 with complex **1** for periods of 0.42, 2, 4, 6 h, and two weeks. The crystals were then frozen and examined by X-ray diffraction. All crystals soaked with **1** changed their appearance. They became cracked and lost their sharply defined edges. A crystal structure determined after 0.42 h soaking did not show the presence of gold in the calculated electron density map, and after soaking for 6 h did not diffract at all. After samples had been soaked for two weeks, cracking was severe. The electron density map from crystals soaked for 2 or 4 h contained a large peak corresponding to a single gold site (Figure 3). The structure was solved by using isomorphous replacement since that of unliganded Cyp-3 is known.[12] In the first difference map, Au was clearly seen together with additional electron density in the region expected for a ligand *trans* to His133 Nε2. This was interpretable as PEt_3 as indicated by the shape of the electron density. The structure was refined with SHELXL97[14] using 20154 unique data (5% for the calculation of R_{free}) and initial constraints on the Au–N and Au–P bond lengths of 2.05 and 2.27 Å, respectively, values similar to those in crystalline [Au(2-isopropylimidazole)(Pcy_3)][15] and [Au(phthalimido)(PEt_3)][16]. The final R factor was 18.48% and R_{free} 24.68%.

Surprisingly, the gold ion was not bonded to a Cys sulfur atom, but instead to the Nε2 nitrogen atom of His133 (Au–N 2.06 Å) and to PEt_3 (Au–P 2.27 Å). The linear geometry (N-Au-P angle 167.7°) is characteristic of Au^I. The temperature factors for the complex (Nε2 13.86, Au 43.77, P 79.45, C(av) 74.96) suggest that the PEt_3 group is undergoing rotational motion.

Of the four His residues in Cyp-3, the imidazole rings of only His54 and His133 are solvent accessible, both in a solvent channel running between two Cyp-3 molecules related by a twofold screw axis parallel to the *b* axis. Cys168 also appears to be readily accessible and the sulfur atom of Cys163

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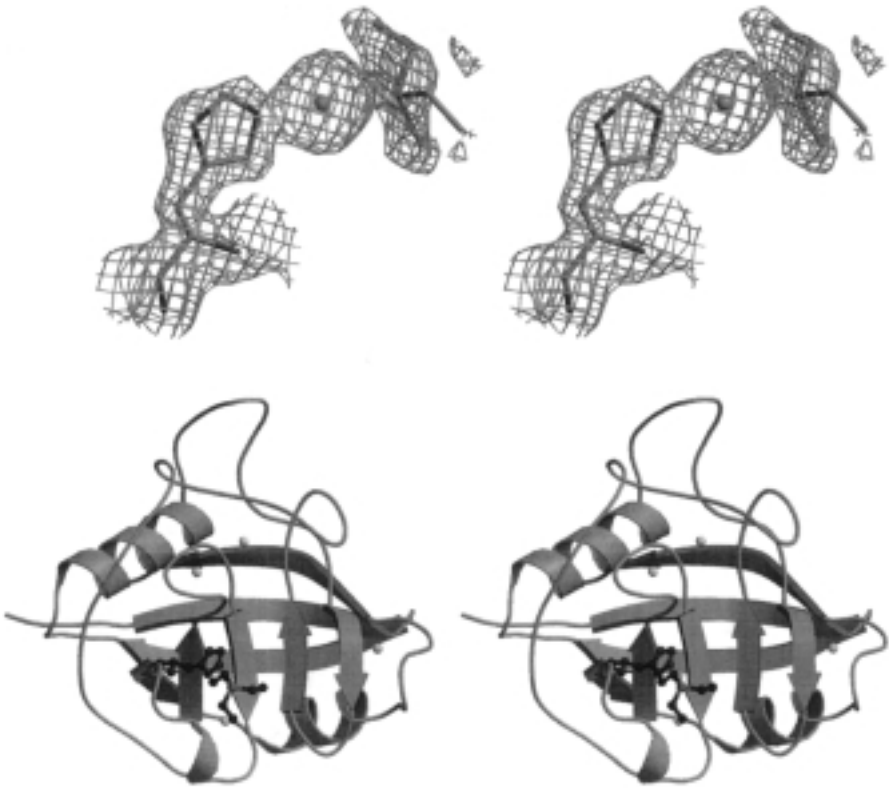

Figure 3. Top: Refined structure of the gold binding site in Cyp-3, showing His133 bound to PEt_3. The $2F_o - F_c$ electron density map is contoured at 1.75σ. The lack of definition of the PEt_3 ligand is probably due to rotational disorder. Bottom: Stereoview of Cyp-3 after reaction with **1** showing the S atoms of the four Cys residues (light spheres: Cys40 (top left), Cys168 (top right), Cys122 (bottom left), Cys163 (bottom right)) and $[AuPEt_3]^+$ bound to the Nε2 center of His133. Drawn using Bobscript,[26] MolScript,[27] and Raster3D.[28]

Au^I has a moderate affinity for N ligands in low molecular mass complexes,[19] but a much higher affinity for thiolate groups. Hence a Cys-S atom is much more effective than an imidazole-N atom in displacing the Cl^- ion from complex **1** in aqueous solution.[20] Complex **1** forms adducts with His residues of serum albumin only after the thiolate S atom at Cys34 has been saturated with Au^I, first as the mono-adduct Cys34-S-($AuPEt_3$), and then as a bisadduct Cys34-S-$(AuPEt_3)_2$.[2, 4, 21, 22] Such a bisadduct could also form with Cyp-3 in the presence of excess **1**.

This appears to be the first X-ray structural characterization of an adduct of a gold–phosphane complex with a His residue in a protein.[8] Previously it was suggested[23] that Au^I–His interactions are responsible for the ability of complex **1** to induce low-spin- to high-spin-state transitions of heme proteins such as cytochrome c, but direct structural evidence for this was not obtainable. The present results confirm that His imidazole rings can indeed be target sites for $[AuPEt_3]^+$ binding in proteins.

is visible from the protein surface, but these sites may not be able to accommodate $[AuPEt_3]^+$ without severe structural distortions and disruption of the crystal.

Gold binding at His133 should inhibit Cyp-3 since this is an active-site residue, and this was found to be the case. A chymotrypsin-coupled assay[17] of the isomerization of the tetrapeptide substrate Suc-Ala-Ala-Pro-Phe-*p*-nitroanilide (Bachem) showed that the concentration of **1** required to inhibit 50% of Cyp-3 PPIase activity was 14 nM (Figure 4), a value comparable with that for inhibition of human CypA by CsA.[18]

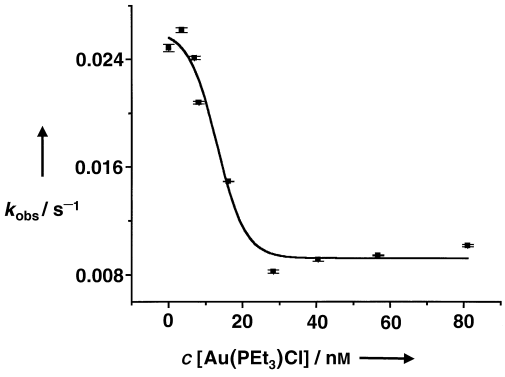

Figure 4. Variation of the observed rate constant for PPIase activity of Cyp-3 with increasing concentration of **1**. An IC_{50} value of 14 nM was calculated from this curve.

Experimental Section

$[Au(PEt_3)Cl]$ (**1**) was purchased from Strem Chemicals and Alfa-Johnson Matthey plc. Cyclophilin-3 (Cyp-3) was expressed and purified as described previously.[12]

ESI-HPLC-MS: Cyp-3 in Tris buffer (10 mM; pH 7.3), or in HEPES buffer (20 mM; pH 7.0), was treated with complex **1** (3.5 or 7 molar equivalents of a 2.5 mM stock solution in ethanol or methanol, diluted to 150 mM with buffer before use), and incubated for 1–5 days at 291 K. Prior to ESI-MS, the protein was desalted, separated from free gold complex on a Phenomenex (Jupiter) C_{18} reverse-phase column, and eluted with H_2O/acetonitrile/TFA (0.05% v/v). Positive-ion electrospray mass spectrometry was performed on a Platform II instrument (Micromass), with source temperature of 413 K and data acquisition in continuum mode, scan rate 900 u s^{-1}. The data were processed with a Mass Lynx (version 2.3) Windows NT PC data system using a maximum entropy software algorithm. Mass accuracy was within 0.1 *m/z* unit.

Determination of free SH groups: Cyp-3 (14.0 μM, concentration determined by Bradford assay) was incubated with DTNB (5 mM) in HEPES buffer (20 mM; pH 7.0) for 30 min at 291 K, and the absorption was measured at 412 nm.[24, 25]

Crystallization, Au^I soaking, and data collection: Tetragonal crystals of Cyp-3 were grown using the hanging-drop vapour diffusion method: Cyp-3 (16 mg mL^{-1}) in sodium citrate (50 mM; pH 5.6), 16–17.5% wt/v methoxypolyethylene glycol 5000 (MPEG 5000) (4 μL) suspended over sodium citrate (100 mM), 32–35% MPEG 5000 at 291 K. Average crystal dimensions: 0.5 × 0.4 × 0.4 mm. For crystal soaking, an aliquot of a stock solution of **1** in DMSO was diluted with sodium citrate (100 mM) containing 33–35% MPEG 5000, pH 5.6, giving a final Au^I concentration of 3.5 mM with 5% DMSO present. The stock solution of **1** in DMSO gave rise to only one

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$^{31}P\{^{1}H\}$ NMR peak at $\delta = 34.5$ when monitored over a period of one month, showing that the Au–P bond is stable under these conditions. Crystals were transferred from hanging drops with 0.4 – 0.5 mm cryoloops into cryoprotectant solution (3 μL, mother liquor plus 20% glycerol), and 5 s after removal from cryoprotectant were mounted in a cryoloop (Hampton Research) and flash frozen in liquid N_2. X-ray data were collected at 100 K (Oxford Cryosystems) using MAR image plates at the Daresbury synchrotron source, and processed using DENZO. The data set consists of 185907 measured reflections and provides a unique data set of 20154 reflections with an $R_{merge} = 0.068$ and an overall completeness to 1.85 Å of 0.983. R_{merge} in the 1.88 – 1.85 Å resolution shell is 0.253.

PPIase assay: Cyp-3 was incubated with **1** at 277 K at molar ratios of **1**: Cyp-3: of 0, 0.43, 0.86, 1.0, 2.0, 3.5, 5.0, 7.0, and 10.0, for 48 h in HEPES buffer (50 mM) containing NaCl (86 mM), pH 8.0, and 4 μL aliquots were then assayed at 275 K following the procedure previously described.[12]

ESI-MS spectra of Cyp-3 after reaction with Et_3PAuCl (**1**) for five days , a colour version of Figure 3, and plots of absorbance versus time for assay of the PPIase activity of Cyp-3 are available in the Supporting Information. The atomic coordinates (code 1E3B) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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DeNO*x* of Exhaust Gas from Lean-Burn Engines through Reversible Adsorption of N_2O_3 in Alkali Metal Cation Exchanged Faujasite-Type Zeolites**

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Nitrogen oxide (NO_x) adsorbents are key control components for the tailpipe emissions of transport vehicles powered with lean-burn engines. Current adsorbents are basic oxides which adsorb NO_x as nitrates, but suffer from poisoning by sulfur oxides. We discovered that NO and NO_2 can be selectively trapped as dinitrogen trioxide in alkali metal exchanged faujasite zeolites above 200 °C. The trapped N_2O_3 molecules compete with water molecules on a specific adsorption site and can be displaced by a change in partial pressure. Dinitrogen trioxide adsorption is not affected by the presence of sulfur oxides.

Modern lean-burn internal combustion engines operated with excess air show reduced fuel consumption and carbon dioxide emission but produce excessive amounts of nitrogen oxides (NO_x). The emission of NO_x at the tailpipe is the major source of pollution from transport vehicles powered by hydrocarbon fuels.[1] Since there is a trade-off between NO_x and particulate carbon formation in the lean-burn engine, an efficient NO_x-elimination system for the exhaust can offer a solution to the particulate carbon emissions at the same time.[2] In the emerging technologies that focus on reducing the NO_x emission from lean-burn engines, temporary storage

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