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**A novel platinum reagent [chloro(2,2′:6′,2″-terpyridine)platinum(II) chloride] for use in heavy-atom derivatization of protein crystals.** By DAVID M. LAWSON, *Department of Chemistry, University of York, Heslington, York YO1 5DD, England*

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## Abstract

A platinum chromophore, chloro(2,2′:6′,2″-terpyridine)platinum(II) chloride, previously used in labelling active-site histidines of serine proteases, proves to be a useful reagent in heavy-atom derivatization of protein crystals for X-ray crystallographic phase determination.


## Introduction

The multiple isomorphous replacement method remains the most powerful technique in the X-ray crystallographer's repertoire for the *ab initio* elucidation of three-dimensional protein structures (Blundell & Johnson, 1976). To this end, the search for novel compounds for use in derivatization trials continues to be a potentially fruitful field of research.

The use of chloro(2,2′:6′,2″-terpyridine)platinum(II) chloride for heavy-atom derivatization was first suggested by Ratilla, Brothers & Kostic (1987). In this paper, they described the application of this compound for non-invasive spectroscopic labelling of histidine residues in cytochromes at pH 5.0. Subsequently, the compound was found to show some affinity for cysteine residues (Brothers & Kostic, 1988), and quite high reactivity with arginine residues in addition to histidine at neutrality (Ratilla & Kostic, 1988). In later experiments, terpyridyl platinum was used to tag active-site histidines of serine proteases, in order to retard autodigestion of the stored enzymes. Furthermore, the compound could be removed later by treating the protein with nucleophilic reagents such as thiourea (Brothers & Kostic, 1990). More recently, in an independent study, terpyridyl platinum was used successfully to derivatize crystals of human tumor necrosis factor complexed with its receptor (Banner *et al.*, 1993).

## Materials and methods

Crystals of recombinant *Vibrio harveyi* acyltransferase ($M_r$ = 34 kDa) grown from 45% saturated ammonium sulfate in 100 m*M* potassium phosphate buffer pH 7.0, 1% polyethylene glycol 200 (final pH approximately 5.0), were supplied by the laboratory of Z. S. Derewenda, University of Alberta, Edmonton, Canada (Swenson *et al.*, 1992). Initial derivatization trials performed in Canada (A. M. Sharp, unpublished results) with the commonly used platinum reagent, $K_2PtCl_4$, either diminished X-ray diffraction at higher concentrations (1.0-2.5 m*M*) or failed to produce derivatives at lower concentrations (less than 1.0 m*M*).

In the present investigation, fresh crystals were transferred to an excess of stabilizing solution (same as mother liquor, but lacking dissolved protein) in glass depression plates, and left to equilibrate for 30 min. A solution of 0.1%(*v*/*v*) glutaraldehyde (Sigma Chemical Co.) was made up in stabilizing solution, into which the stabilized crystals were transferred and soaked for 12-18 h. After cross-linking, the crystals had turned pale yellow and were washed in fresh stabilizing solution. Solutions of 0.5, 1.0 and 2.5 m*M* chloro(2,2′:6′,2″-terpyridine)platinum(II) chloride (Aldrich Chemical Co.), were made up in stabilizing solution. A crystal was transferred to the 0.5 m*M* terpyridyl platinum solution, and then to the 1.0 m*M* solution after 30 min. The crystal remained intact and birefringent under a polarizing microscope, and was subsequently soaked for a total of 2 d in this solution. After soaking, the crystal had attained a pale-orange colour, and still diffracted X-rays strongly, enabling a 3.4 Å data set to be collected (a similar crystal soaked for 5 d in 2.5 m*M* terpyridyl platinum failed to diffract X-rays beyond 5.0 Å resolution). X-ray data were collected using a Siemens (Xentronics) area detector, on a Rigaku rotating anode RU-200 X-ray generator with Cu target ($\lambda$ = 1.542 Å) and graphite monochromator (operated at 50 kV and 100 mA). The crystals were monoclinic, space group $P2_1$, with cell parameters $a$ = 89.9, $b$ = 83.6, $c$ = 47.1 Å, $\beta$ = 97.3°. They were mounted in 0.7 mm diameter glass capillaries (Pantak Ltd) with $b^*$ along the rotation axis, in order to make contemporaneous measurements of Friedel equivalents. The crystal-to-detector distance was set at 10 cm, and 720 × 0.25° oscillation images were recorded at 150 s image$^{-1}$. Data were subsequently processed using the *XDS* package (Kabsch, 1988) and reduced using programs from the *CCP*4 suite (SERC Daresbury Laboratory, 1979).

## Results and discussion

Data reduction using the programs *ROTAVATA* and *AGROVATA* was satisfactory, yielding 92.4% of the available data to 3.4 Å resolution (98.4% complete to 3.8 Å), with an overall redundancy of 3.5 and final $R_{\rm merge}$† = 7.3%. This derivative data set was scaled against a native data set to 2.4 Å resolution (collected under similar conditions) using the program *SCALEIT*. The derivative data were sufficiently isomorphous to the native data in the resolution range 10.0-3.4 Å, giving a mean fractional isomorphous change‡ of 22.3% indicating a well substituted derivative.

An isomorphous difference Patterson map calculated at 4.5 Å resolution was rather complex, giving a number of significant peaks on the Harker section ($y$ = 1/2). Some of these peaks were also present in an anomalous-difference Patterson (see Fig. 1). The heavy-atom sites were located in a ($F_{\rm Pt} - F_{\rm native}$) difference Fourier map, using phase information from a mercury-derivative (mercuric chloride) data set collected previously. This method yielded a total of eight unique sites, some of which corresponded to peaks in the Patterson maps. Further analysis of these sites showed that three pairs had almost identical $y$ coordinates. These generate cross-vectors with $y$ = 1/2 in the Patterson maps [by applying symmetry operator 2 ($-x$, $y$ + 1/2, $-z$) to one site in each pair], and

† $R_{\rm merge} = (\Sigma|I - \langle I\rangle|/\Sigma I) \times 100$, where $I$ is the observed intensity.

‡ Mean fractional isomorphous change = $(\Sigma|F_P - F_{PH}|/\Sigma F_P) \times 100$, where $F_P$ and $F_{PH}$ are the structure-factor amplitudes for the native and derivative, respectively.

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Table 1. *Refined platinum coordinates and occupancies for each site*

Occupancies are absolute and temperature factors were fixed at 15.0 Å$^2$.

| Site | Refined platinum coordinates (fractional) $x$ | $y$ | $z$ | Occupancies Real | Anomalous |
|---|---|---|---|---|---|
| 1 | 0.285 | 0.501 | 0.653 | 0.836 | 0.292 |
| 2 | 0.762 | 0.449 | 0.338 | 0.423 | 0.202 |
| 3 | 0.069 | 0.308 | 0.132 | 0.233 | 0.109 |
| 4 | 0.228 | 0.496 | 0.897 | 0.253 | 0.144 |
| 5 | 0.514 | 0.475 | 0.859 | 0.351 | 0.187 |
| 6 | 0.669 | 0.450 | 0.103 | 0.163 | 0.060 |
| 7 | 0.382 | 0.145 | 0.138 | 0.193 | 0.117 |
| 8 | 0.971 | 0.477 | 0.135 | 0.347 | 0.155 |

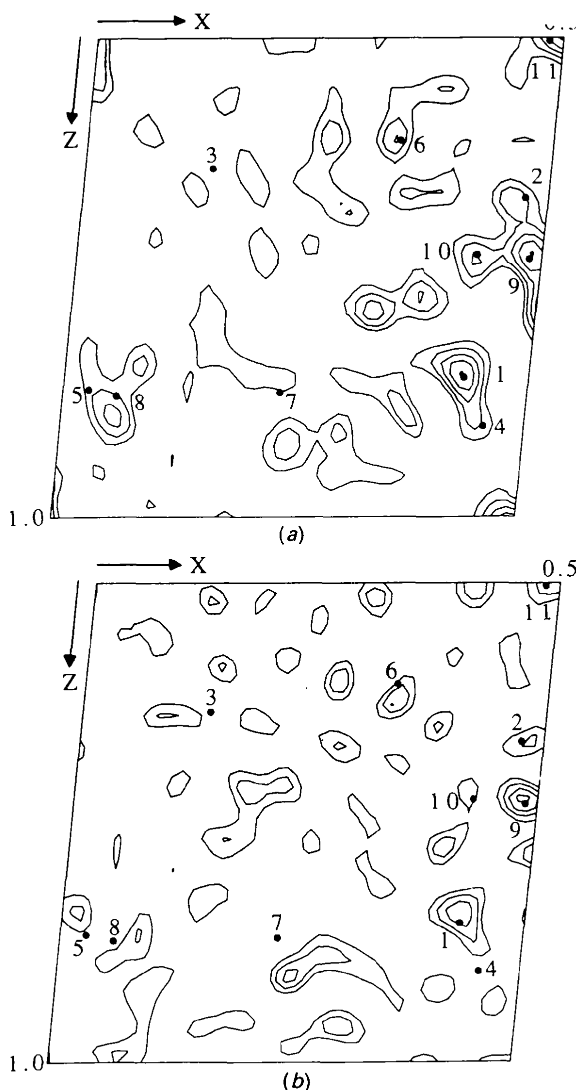


Fig. 1. The Harker sections ($y$ = 1/2) of isomorphous difference (*a*) and anomalous difference (*b*) Patterson maps for the terpyridyl platinum derivative calculated using data in the resolution range 10.0-4.5 Å. Numbers 1-8 refer to Harker vectors calculated from the coordinates of the corresponding heavy-atom sites (see Table 1). Numbers 9-11 refer to cross-vectors between different heavy-atom sites (1-4, 2-6, and 5-8, respectively).

therefore account for a further three peaks on the Harker section. The remaining peaks were not assigned.

The eight heavy-atom positions (and occupancies) were then refined in *MLPHARE*, along with those for the mercury derivative, against data in the resolution range 10.0-3.4 Å, and used to calculate heavy-atom phases (see Table 1). The platinum derivative gave a phasing power* of 1.8 and $R_{\rm Cullis}$† = 0.71 for the acentric data, and a phasing power of 1.2 and $R_{\rm Cullis}$ = 0.85 for the centric data.

Model building of the acyltransferase is well advanced using electron-density maps phased on the Pt and Hg data, and two poorer derivatives of iodine (*N*-iodosuccinimide) and uranium (uranyl acetate). The crystals display very pronounced twofold non-crystallographic symmetry, which is consistent with the 50% solvent content calculated for two molecules per asymmetric unit (Matthews, 1968). Furthermore, the Pt sites can be arranged in four pairs of non-crystallographic symmetry mates - one of each pair being associated with each molecule. This has helped to define the non-crystallographic symmetry, thereby enabling maps to be twofold averaged. To date, greater than 90% of the amino-acid sequence (Ferri & Meighen, 1991) has been fitted, and all eight of the platinum sites are adjacent to surface histidine residues in the current model (further details of the structure determination will be described elsewhere). Banner *et al.* (1993) reported that terpyridyl platinum binds to surface histidines with an adjacent hydrophobic side chain. Unfortunately, a full description of the protein-ligand interactions in the acyltransferase is not possible at 3.4 Å resolution. However, of the four pairs of equivalent Pt sites, two involve only a histidine (*e.g.* see Fig. 2), although one of these is within 4.5 Å of a second histidine. Another site involves a histidine, which forms a $\pi$ complex with an adjacent phenylalanine side chain. Moreover, this site is approximately 4 Å from an arginine side chain, but does not appear to interact with it. In the final site, the Pt has both a histidine and an arginine side chain as ligands.

Cross-linking with glutaraldehyde did not affect the diffraction quality of the acyltransferase crystals, and was therefore used routinely in heavy-atom soaking experiments. Although not specifically tested with these crystals, cross-linking is not essential for the successful derivatization of other protein crystals with terpyridyl platinum (Banner *et al.*, 1993; G. J. Davies & Z. S. Derewenda, unpublished results).

* Phasing power = $\Sigma|F_H|/\Sigma E$, where $F_H$ is the heavy-atom structure factor and $E$ is the residual lack-of-closure error ($|F_{PH} - F_P| - |F_H|$).

† $R_{\rm Cullis} = \Sigma E/\Sigma|F_{PH} - F_P|$.

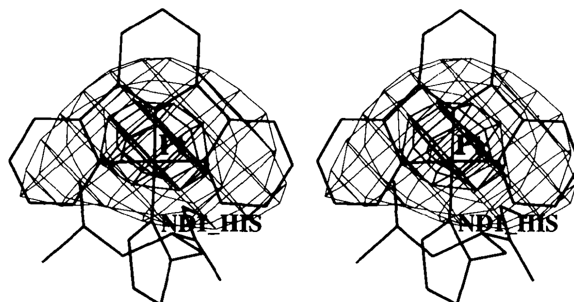


Fig. 2. Stereo figure showing terpyridyl platinum modelled into 3.4 Å resolution positive ($F_{\rm PT} - F_{\rm native}$) difference electron density adjacent to a histidine residue (site 8 in Table 1). The map is twofold averaged and contoured at three and ten times the root-mean-square density.

In conclusion, chloro(2,2′:6′,2″-terpyridine)platinum(II) chloride represents a novel compound for heavy-atom derivatization: it binds well to histidine residues in crystals of *Vibrio harveyi* acyltransferase, to give a multi-site derivative, thereby enabling isomorphous and anomalous differences to be measured. In contrast, the more commonly used platinum reagent, $K_2PtCl_4$, did not produce a useful derivative with these crystals. Furthermore, the specificity of terpyridyl platinum binding provides a useful aid to electron-density map interpretation. Nevertheless, the reactivity of this compound may cause problems in Patterson function interpretation for larger proteins in the absence of other sources of phase information, particularly when combined with a high symmetry space group. In theory, the reversibility of binding of terpyridyl platinum may enable valuable crystals to be 'desoaked' after data collection, and then resoaked in a different heavy-atom solution.

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