

ISSN 0011-1643
UDC 543.866
CCA-2018

Original Scientific Paper

Selective Labeling of the Enzyme Papain with Chloro(terpyridine)platinum(II)*

*Sharon L. Pinnow, Herb M. Brothers II, and Nenad M. Kostić***

Department of Chemistry, Iowa State University, Ames, Iowa 50011, U.S.A.

Received May 13, 1991

The complex $[\text{Pt}(\text{trpy})\text{Cl}]^+$ reacts with imidazole, histidine, and the tripeptide Gly-His-Gly to yield complexes in which the chloride ligand is replaced by the imidazole ring; $[\text{Pt}(\text{trpy})\text{Cl}]^+$ reacts with 2-mercaptoethanol, cysteine, and the tripeptide glutathione to yield complexes in which the chloride ligand is replaced by the thiolate group. When the enzyme papain is treated with an equimolar amount of $[\text{Pt}(\text{trpy})\text{Cl}]^+$, only Cys 25 at the active site is tagged with a $\text{Pt}(\text{trpy})^{2+}$ group; when papain is treated with an excess of $[\text{Pt}(\text{trpy})\text{Cl}]^+$, both Cys 25 and His 81 are so tagged. When this diplatinated enzyme is treated with a large excess of bromide anion, only the tag from His 81 is removed. The rate constants for the displacement of the chloride ligand in $[\text{Pt}(\text{trpy})\text{Cl}]^+$ with glutathione, papain, and histidine at pH 5.0 are 30.2, 21.3, and $0.274 \text{ M}^{-1} \text{ s}^{-1}$, respectively. These values indicate that the sulfhydryl group is more nucleophilic than the imidazole group and that Cys 25 at the active site of papain is somewhat shielded from external attack by the $[\text{Pt}(\text{trpy})\text{Cl}]^+$ complex. When the diplatinated papain is treated with a nucleophile stronger than histidine but weaker than cysteine, the $\text{Pt}(\text{trpy})^{2+}$ tag is selectively removed from His 81.

INTRODUCTION

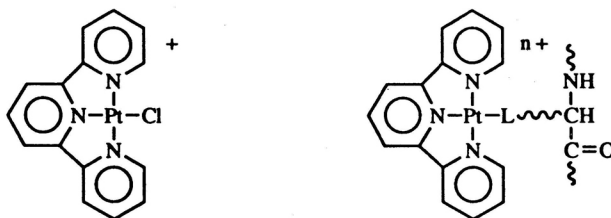
Covalent modification of amino-acid side chains has proved useful in structural, spectroscopic, and mechanistic studies of proteins.¹⁻³ The various chromophores, fluorophores, spin labels, and radioactive labels developed so far are mostly organic compounds. Except as heavy-atom scatterers for X-ray crystallography,^{4,5} metal complexes have not been widely used for covalent modification of proteins. Because substitution reactions of transition-metal complexes can be controlled precisely and because these complexes have various spectroscopic and electrochemical properties, they

* Dedicated to Professor Linus Pauling, who made seminal contributions to both enzymology and transition-metal chemistry, on the occasion of his ninetieth birthday.

** Author to whom correspondence should be addressed. Presidential Young Investigator of the National Science Foundation, 1988-92; Alfred P. Sloan Research Fellow, 1991-93.

are well-suited to many biochemical and biophysical applications. They can serve as absorption chromophores, emission fluorophores, paramagnetic spin labels, NMR-active tags, NMR relaxation agents, redox agents, and radioactive markers. Selectivity in their binding to proteins (and to other biological macromolecules) can be changed, in predictable ways, by controlling the oxidation state, hardness or softness, and coordination number of the metal and the identity and number of the ligands. Well-chosen inorganic reagents can, in principle, meet all the requirements of a good labeling reagent. They can react under mild conditions; be selective toward particular side chains or sites; be easily detected and quantitated in the modified protein; be stable yet removable under mild conditions, so that the native protein can be restored; and be non-invasive so that they do not alter the structure and function of the protein. Our goal is to exploit these advantages in practice.

Chloro(2,2':6',2''-terpyridine)platinum(II), shown below, is well-suited to covalent modification of proteins. Because only the chloride ion can be displaced by amino-acid side chains (designated L) under ordinary conditions, only one-to-one adducts can form. Such a protein adduct is schematically shown below; the charge n depends on the charge of the coordinated side chain, L. Because platinum(II) complexes are kinetically inert and thermodynamically stable, the Pt(trpy)²⁺ tag stays on the protein. Because this tag is an UV-vis chromophore, it is easily detected and quantitated in the modified proteins. Because the coordinated amino-acid side chain, L, can be displaced by an added ligand N, the tag can be removed from the protein as the complex [Pt(trpy)N]ⁿ⁺. Previous research in this laboratory, involving various cytochromes⁶⁻⁹ and enzymes,¹⁰ showed all these advantages of [Pt(trpy)Cl]⁺ and its selectivity toward cysteine and histidine residues. This reagent is now marketed and advertised by Aldrich Chemical Co.¹¹ Research in this laboratory with other platinum(II) chloro complexes of the type [Pt(tridentate)Cl] showed how the charge¹² of these reagents and their selectivity in binding¹³ can be controlled by adjusting the properties of the ancillary tridentate ligand, even though this ligand does not directly participate in the substitution reaction.



In this study we applied [Pt(trpy)Cl]⁺ to papain, a proteolytic enzyme from the papaya latex.^{14,15} In previous studies this enzyme was modified¹⁶⁻¹⁹ and chemically mutated²⁰ with organic reagents. Only three of papain's 212 amino-acid residues can potentially react with [Pt(trpy)Cl]⁺. Detailed X-ray crystallographic studies showed that Cys 25 and His 159 are members of the catalytic triad at the active site, while His 81 is distant from the active site.²¹⁻²³ Cysteine and histidine differ from each other in the nucleophilicity of their side chains (sulfhydryl *versus* imidazolium or thiolate *versus* imidazole), and we exploited this difference to achieve selective labeling in two ways.

EXPERIMENTAL PROCEDURES

Chemicals

Papain, L-cysteine, L-histidine, Gly-His-Gly, 2-mercaptoethanol, glutathione, and DL-dithiothreitol (DTT) were obtained from Sigma Chemical Co.; imidazole and $[\text{Pt}(\text{trpy})\text{Cl}]\text{Cl} \cdot 2\text{H}_2\text{O}$, from Aldrich Chemical Co.; thiourea, from J. T. Baker Chemical Co.; and NaBr and NaSCN, from Merck & Co. Distilled water was further demineralized and purified. The solvent was 50 mM acetate buffer at pH 5.0.

Instruments and Software

The UV-vis spectra were recorded with an IBM 9430 spectrophotometer, whose monochromator has two gratings. In kinetic measurements, the spectrophotometer was attached to a circulating thermostat by Fisher Scientific, model 9500. A thermostated accessory by Hi-Tech Scientific, model SFA-11, was used for rapid mixing. Kinetic results were fitted and analyzed with software produced by OLIS, Inc. and run on a Zenith 241 computer. An Acumet 805 MP pH-meter was used throughout. Dialyses were done by repeated ultrafiltrations in an Amicon 8050 cell, with Diaflo YM3 and YM5 membranes, under pressure of purified nitrogen. Platination of papain was simulated with FRODO 6.3 software on an Evans & Sutherland PS300 terminal.

Reactions of $[\text{Pt}(\text{trpy})\text{Cl}]\text{Cl}$ with Amino Acids

In one experiment, 10.00 mL of a 0.100 mM solution of $[\text{Pt}(\text{trpy})\text{Cl}]\text{Cl}$ was mixed separately with 1.00 mL of 1.00 mM solutions of cysteine and of histidine; in another experiment, this $[\text{Pt}(\text{trpy})\text{Cl}]\text{Cl}$ solution was added to a mixture of these two amino-acid solutions. In both cases the reaction mixtures were left overnight, diluted to be 25.0 μM in platinum, and examined by UV-vis spectrophotometry.

Molar Absorptivities (Extinction Coefficients) of the Model Complexes

Equimolar amounts (3.00 mL of 3.00 mM solutions) of $[\text{Pt}(\text{trpy})\text{Cl}]\text{Cl}$ and of different bioligands (imidazole, histidine, Gly-His-Gly, 2-mercaptoethanol, cysteine, and glutathione) were mixed and allowed to react overnight. The reaction mixtures were diluted 60-fold (to 25 μM concentration) and examined by UV-vis spectrophotometry.

Reaction of Papain with $[\text{Pt}(\text{trpy})\text{Cl}]\text{Cl}$

The commercial preparation of papain, which is twice crystallized and lyophilized, was further treated with a 10-fold excess of dithiothreitol for 15 min and dialyzed. The enzyme concentration was determined by UV-vis spectroscopy ($\epsilon_{280} = 58.4 \text{ mM}^{-1} \text{ cm}^{-1}$).²⁴ It was incubated overnight with equimolar amounts and with 10-fold, 20-fold, and 50-fold molar excesses of $[\text{Pt}(\text{trpy})\text{Cl}]\text{Cl}$ at room temperature. The unspent platinum reagent was dialyzed away.

Kinetics of $[\text{Pt}(\text{trpy})\text{Cl}]\text{Cl}$ Reactions with Papain and with Model Ligands

The concentration of $[\text{Pt}(\text{trpy})\text{Cl}]\text{Cl}$ was 20.0 μM , and the entering bioligands were present in excess over it. Pseudo first-order rate constants were determined by the Guggenheim method (for papain and glutathione, which react faster) or on the basis of an estimated absorbance at infinite time (for histidine, which reacts slower). Bimolecular rate constants were determined in experiments with the following concentrations of the bioligands: 270, 240, 180, and 150 μM for papain; 2.00 and 1.00 mM and 500, 250, and 150 μM glutathione; and 2.00 and 1.00 mM and 750, 500, 250, and 150 μM for histidine. The papain solutions were prepared fresh before each run. The glutathione solutions were prepared and stored under nitrogen. The histidine solutions were stored in closed flasks. The solutions of $[\text{Pt}(\text{trpy})\text{Cl}]\text{Cl}$ and of the bioligands, 1.00 mL of each, were mixed in the standard spectrophotometric cuvette with a pipette or, more correctly, with a stopped-flow accessory attached to the spectrophotometer. The reaction with the enzyme was followed at 344 nm, and the reactions with the two model ligands were followed at 275 nm. The absorbances were recorded every 30.0 s in the reactions with papain and with glutathione

and every 5.00 min in the reaction with histidine. Each run consisted of one hundred points. Each observed rate constant was an average value from one to three separate runs. The plots of the observed rate constants *versus* the ligand concentration were linear with correlation coefficients greater than 0.980. All the reactions were done at 25 ± 0.5 °C.

Removal of Pt(trpy)²⁺ Tags from the Bioligands

The following stock solutions were prepared: 3.00 mM each of [Pt(trpy)Cl]Cl, cysteine, histidine, NaBr, NaSCN, and thiourea; 1.5 mM each of [Pt(trpy)His]Cl₂ and [Pt(trpy)Cys]Cl; and 150 mM of NaBr. Compounds [Pt(trpy)Br]Cl, [Pt(trpy)SCN]Cl, and [Pt(trpy)tu]Cl₂; were prepared *in situ* by mixing 2.00 mL each of the corresponding stock solutions. The stock solutions of [Pt(trpy)His]Cl₂ and of [Pt(trpy)Cys]Cl were treated with equimolar amounts (2.00 *vs.* 1.00 mL) or with 10-fold molar excesses (0.50 *vs.* 2.5 mL) of NaBr, NaSCN, and thiourea, and with a 100-fold molar excess (1.00 *vs.* 1.00 mL) of NaBr. Solutions that were 1.50 mM in both cysteine and NaSCN, in both cysteine and thiourea, in both histidine and NaSCN, and in both histidine and thiourea were prepared by mixing 1.00 mL each of the corresponding stock solutions. To each mixture was added 1.00 mL of the [Pt(trpy)Cl]Cl stock solution. The various reaction mixtures were left overnight at room temperature. The clear solutions were diluted to be 25 μM in the platinum complex and examined by UV-vis spectrophotometry.

Papain was treated with one equivalent and with 10-fold, 20-fold, and 50-fold molar excesses of [Pt(trpy)Cl]Cl and dialyzed. It was then incubated with equimolar amounts and with 10-fold molar excesses of NaBr, NaSCN, and thiourea and also with a 100-fold molar excess of NaBr.

Computer Graphics

The van der Waals radii of hydrogen, carbon, nitrogen, oxygen, and platinum were set at 120, 170, 160, 150, and 170 pm, respectively. The platinum atom in the Pt(trpy)²⁺ group was positioned 20 pm away from the sulfur atom in Cys 25 and from the δ (the so-called pyrrole-type) nitrogen atom in His 159, exposed at the active site of papain.

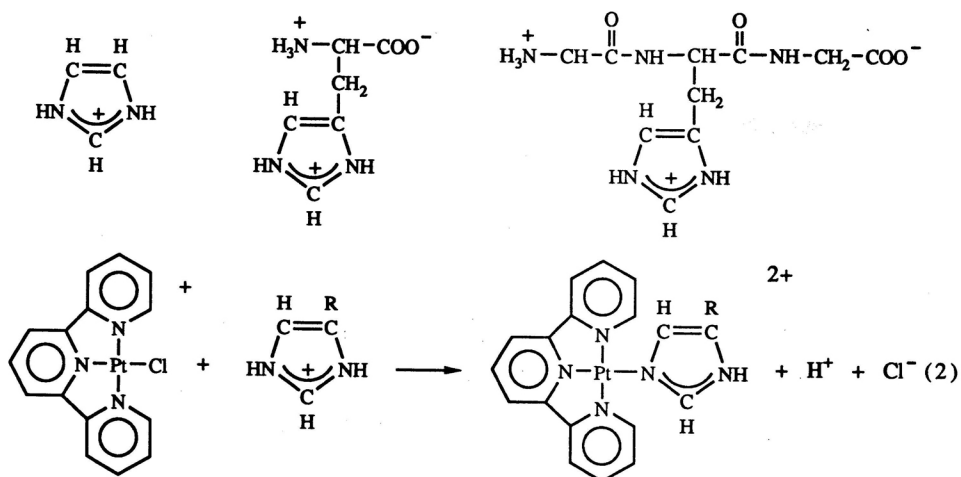
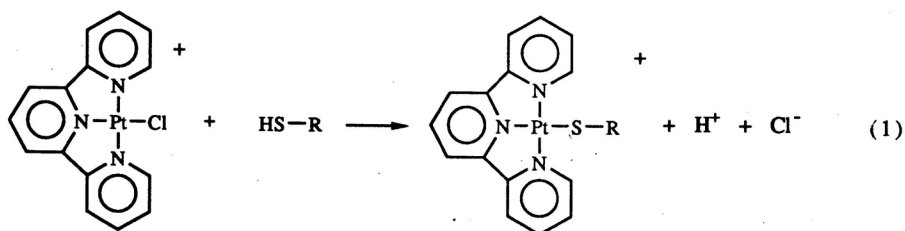
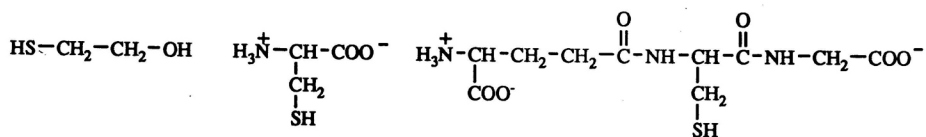
RESULTS AND DISCUSSION

Potential Binding Sites in Papain

The active site of this enzyme contains the catalytic triad composed of Cys 25, Asp 158, and His 159. There is also His 81, remote from the active site. The crystal structure of this well-characterized enzyme shows that all the three potential binding sites for [Pt(trpy)Cl]⁺, namely the cysteine and histidine residues, are accessible from the exterior.²¹⁻²³ But a sulfhydryl group is a stronger nucleophile than an imidazolium cation, and thiolate is stronger than imidazole.

The Model Complexes

We examined reactions of [Pt(trpy)Cl]⁺ with three sulfhydryl-containing ligands (2-mercaptoethanol, cysteine, and the tripeptide glutathione, γ-Glu-Cys-Gly) and with three imidazolium-containing ligands (imidazole, histidine, and the tripeptide Gly-His-Gly). The formulas (at pH 5.0) of the ligands are shown below, and their displacements of the chloride ligand in [Pt(trpy)Cl]⁺ are shown schematically in equations (1) and (2). The two nitrogen atoms are equivalent in the imidazolium cation but not in the (unprotonated) imidazole. The atom in the ε position (the so called pyridine-type nitrogen) has the pK_a of 6.0 and readily binds to metal ions; the atom in the δ position (the so-called pyrrole-type nitrogen) has the pK_a of ca. 14 and does not readily bind to metal ions.²⁵



The UV-vis absorption properties of the model complexes are summarized in Tables I and II. The bands below 300 nm are due to electronic transitions within the aromatic terpyridine ligand, and the bands in the region 300–350 nm are probably due to metal-to-ligand charge-transfer (MLCT) transitions. The absorptivities (extinction coefficients) in Table I are useful for quantitation of the $\text{Pt}(\text{trpy})^{2+}$ tags in the enzyme, while the absorption maxima and the relative intensities of the MLCT bands in Table II are diagnostic of the unidentate (fourth) ligand in the coordination sphere of platinum(II). The absorptivities depend somewhat on the concentration of the complex and on the buffer and other salts present in solution.^{6,7,26}

The kinetics of displacement reactions (1) and (2) was studied with glutathione and histidine. The respective bimolecular rate constants, 30.2 and $0.274 \text{ M}^{-1}\text{s}^{-1}$, show that the sulfhydryl group is much more nucleophilic than the imidazolium ion toward platinum(II).

Competition experiments with model complexes confirmed this conclusion. When equimolar amounts of cysteine and histidine compete for an equimolar amount of

TABLE I

Absorptivities at two wavelengths for 25 μM solutions of the model complexes $[\text{Pt}(\text{trpy})\text{L}]^{n+}$ in 50 mM acetate buffer at pH 5.0

L	n at pH 5.0	Absorptivity/ $\text{mM}^{-1} \text{cm}^{-1}$	
		278 nm	342 nm
Im	2	22.2	13.8
His	2	21.2	14.5
Gly-His-Gly	2	17.0	13.7
Average for the imidazole-containing ligands		20.1	14.0
-SCH ₂ CH ₂ OH	1	18.4	11.3
Cys	1	17.1	11.0
γ -Glu-Cys-Gly	0	16.2	10.1
Average for the thiolate-containing ligands		17.2	10.8

TABLE II

Absorption maxima^a and relative absorbances at two wavelengths for the 25 μM solutions of $[\text{Pt}(\text{trpy})\text{L}]^{n+}$ complexes in 50 mM acetate buffer at pH 5.0

L	n at pH 5.0	λ_1/nm	λ_2/nm	A_1/A_2
Cl ⁻	1	344.8	328.8	1.27
His	2	343.6	328.8	1.30
Cys	1	342.0	327.6	1.18

^a ± 0.4 nm

$[\text{Pt}(\text{trpy})\text{Cl}]^+$, the product shows MLCT absorption bands at 341.6 ± 0.4 and 327.2 ± 0.4 nm, and their absorbance quotient is 1.17. According to the values in Table II, this product is $[\text{Pt}(\text{trpy})\text{Cys}]^+$.

Papain Labeling

The labeling procedure was simple—incubation of the enzyme with $[\text{Pt}(\text{trpy})\text{Cl}]^+$ overnight, and removal of the unspent metal reagent by ultrafiltration. These experiments were done at pH 5.0 in order to minimize papain autodigestion. For the sake of consistence, all the other experiments were also done in this same buffer.

The number, identity, and locations of the $\text{Pt}(\text{trpy})^{2+}$ tags were determined by UV-vis spectrophotometry (see Figure 1) and computer graphics. On the basis of the known absorptivities of papain and of the model complexes $[\text{Pt}(\text{trpy})\text{His}]^{2+}$ and $[\text{Pt}(\text{trpy})\text{Cys}]^+$, the theoretical values in Table III have been calculated. When the reagent and the enzyme are in equimolar amounts, only one $\text{Pt}(\text{trpy})^{2+}$ tag is incorporated. The kinetic and competition experiments with the model complexes showed this tag to be attached to Cys 25 at the active site. Indeed, the bimolecular rate constant for the $[\text{Pt}(\text{trpy})\text{Cl}]^+$ reaction with papain ($21.3 \text{ M}^{-1}\text{s}^{-1}$) is similar to that with cysteine ($30.2 \text{ M}^{-1} \text{ s}^{-1}$) and much larger than that with histidine ($0.274 \text{ M}^{-1} \text{ s}^{-1}$). Although the catalytic triad has evolved in a way that enhances the nucleophilicity of Cys 25, this amino acid in the enzyme reacts a little slower than free cysteine with $[\text{Pt}(\text{trpy})\text{Cl}]^+$. This retardation may have two causes. First, the natural substrates for the enzyme are polypeptides, not a platinum(II) complex. Second, and probably more important, a protein-bound amino acid is shielded by other residues from the approaching metal complex. When a residue is exposed on the protein surface—for example, His 33 in cytochromes *c* from

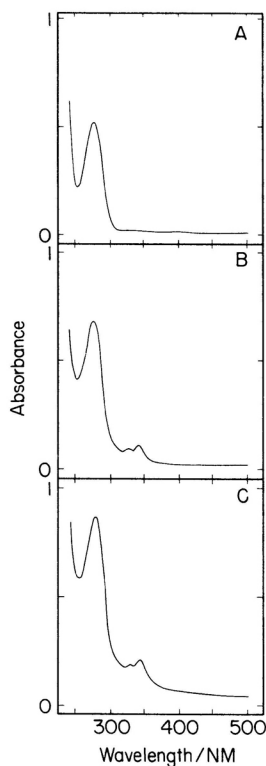


Figure 1. Ultraviolet-visible absorption spectra of $10 \mu\text{M}$ solutions in 50 mM acetate buffer at pH 5.0. (A) Papain. (B) Papain labeled with $\text{Pt}(\text{trpy})^{2+}$ at Cys 25. (C) Papain labeled with $\text{Pt}(\text{trpy})^{2+}$ at both Cys 25 and His 81.

horse heart and from baker's yeast, it reacts with $[\text{Pt}(\text{trpy})\text{Cl}]^+$ as fast as a free amino acid.⁷ The kinetics of binding of our inorganic reagent evidently reveals details of the protein topography near the binding site.

When the reagent is present in excess over the enzyme, the UV-vis spectra show two $\text{Pt}(\text{trpy})^{2+}$ tags. Even with a 50-fold molar excess of $[\text{Pt}(\text{trpy})\text{Cl}]^+$, no more than

TABLE III

Expected absorbance quotients for papain variously labeled with $\text{Pt}(\text{trpy})^{2+}$ chromophores

Labeled residues	A_{278}/A_{342}
Cys	7.00
His	5.63
Cys and His	3.88
2 His	3.57
Cys and 2 His	3.03

two tags are found in papain. This inorganic reagent is evidently selective in its reaction with the enzyme. The A_{278}/A_{342} quotient for the doubly-labeled papain is 4.0 ± 0.1 , in reasonable agreement with the value expected if Cys 25 and one of the histidine residues were labeled. Computer graphics showed the orientation of the His 159 side chain to be unfavorable for platination because the trigonal (so-called pyridine-type) nitrogen atom in the ϵ position points toward the interior of the protein.²¹⁻²³ In the case of the enzyme α -chymotrypsin, whose His 57 at the active site readily accepts a $\text{Pt}(\text{trpy})^{2+}$ tag, this nitrogen atom points toward the exterior.¹⁰ Moreover, the Cys 25 and His 159 residues are very close to each other,²⁷ so that the first $\text{Pt}(\text{trpy})^{2+}$ tag on the former probably obstructs the approach of the $[\text{Pt}(\text{trpy})\text{Cl}]^+$ complex to the latter. The second $\text{Pt}(\text{trpy})^+$ tag is most likely attached to His 81, the only other histidine residue in papain.

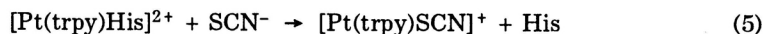
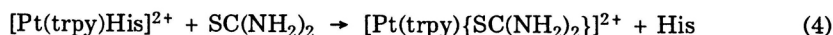
Selective Removal of $\text{Pt}(\text{trpy})^{2+}$ from Papain

Selective labeling of a biological macromolecule with smaller reagents is usually achieved by selective *attachment*. To our knowledge, this study is the first attempt at selective labeling by means of selective *removal* of some, but not of the other, metal tags from the multiply-labeled macromolecule.

We take advantage of the difference in nucleophilicities between the two binding residues, Cys 25 and His 81. Relative nucleophilicity of a ligand N toward platinum(II), designated n_{Pt} , is defined in equation (3), in which k_{N} and k_{S} are rate constants for the displacement, by the nucleophile N and by the solvent, of a given ligand in a reference complex of platinum(II). The solvent is usually methanol, and the reference complex is usually *trans*- $[\text{Pt}(\text{py})_2\text{Cl}_2]$.^{28,29} We chose three nucleophiles intermediate between imidazole (as a model for His 81) and thiophenolate (as a model for Cys 25). The n_{Pt} values are as follows: Im, 3.44; Br^- , 4.18; SCN^- , 5.75; $(\text{NH}_2)_2\text{CS}$, 7.17; and $\text{C}_6\text{H}_5\text{S}^-$, 7.17.

$$n_{\text{Pt}} = \log \frac{k_{\text{N}}}{k_{\text{S}}} \quad (3)$$

The histidine ligand in the model complex is displaced by an equimolar amount of either nucleophile according to equations (4) and (5).



It is not displaced by either an equimolar amount or a 10-fold molar excess of bromide, but is apparently displaced by a 100-fold excess of bromide. The cysteine ligand in $[\text{Pt}(\text{trpy})\text{Cys}]^+$ is not displaced by any of the three nucleophiles in the equimolar amounts, but is partially displaced by 10-fold excesses of thiourea and of thiocyanate. All of these findings are consistent with the relative nucleophilicities of the ligands.

Contrary to the expectations from these model studies, thiourea and thiocyanate that were equimolar with the doubly-labeled papain proved unsuitable for selective removal of $\text{Pt}(\text{trpy})^{2+}$ from His 81. This goal was achieved by successive treatments of the doubly-labeled enzyme with 100-fold molar excesses of bromide, followed by dialysis. At the end of this procedure, the UV-vis spectrum showed only one $\text{Pt}(\text{trpy})^{2+}$ tag per papain molecule. It is probably attached to Cys 25.

CONCLUSION

This study shows some of the advantages of transition-metal complexes as new reagents for selective modification on enzymes, especially of their active sites. Because the rate of the chloride displacement in $[\text{Pt}(\text{trpy})\text{Cl}]^+$ depends on the nucleophilicity and accessibility of the amino-acid side chains, kinetic studies can reveal important properties of the active site. Selectivity in labeling can be achieved by selective removal of labels from the protein as well as by their selective attachment to the protein.

Acknowledgements. – This research was supported by the U. S. National Science Foundation through a Presidential Young Investigator Award to N.M.K. (CHE-8858387) and by Smith Kline & French, Inc. We thank the sponsors.

REFERENCES

1. R. L. Lundblad and C. M. Noyes, *Chemical Reagents for Protein Modification*, Vols. I and II, Boca Raton, FL, CRC Press, 1984.
2. G. E. Means and R. E. Feeney, *Chemical Modification of Proteins*, San Francisco, Holden-Day, 1971.
3. A. N. Glazer, in: H. Neurath (Ed.), *The Proteins*, 3rd ed., Vol. 2, Academic Press, New York, 1977, Chapter 1 and references cited therein.
4. T. L. Blundell and L. N. Johnson, *Protein Crystallography*, Academic Press, New York, 1976, Chapter 8.
5. G. A. Petsko, *Methods Enzymol.* **114** (1985) 147.
6. E. M. A. Ratilla, H. M. Brothers II, and N. M. Kostić *J. Amer. Chem. Soc.* **109** (1987) 4592.
7. H. M. Brothers II and N. M. Kostić, *Inorg. Chem.* **27** (1988) 1761.
8. E. M. A. Ratilla and N. M. Kostić, *J. Amer. Chem. Soc.* **110** (1988) 4427.
9. N. M. Kostić, *Comments Inorg. Chem.* **8** (1988) 137.
10. H. M. Brothers II and N. M. Kostić, *Biochemistry* **29** (1990) 7468.
11. *Aldrichimica Acta* **21** (1988) 55.
12. S. C. Shah, J. S. Johnson, H. M. Brothers II, and N. M. Kostić, *J. Serb. Chem. Soc.* **53** (1988) 139.
13. X. Y. Zhou and N. M. Kostić, *Polyhedron* **90** (1990) 1975.
14. A. N. Glazer and E. L. Smith, in: P. D. Boyer (Ed.), *The Enzymes* Vol. III, 3rd Ed., Academic Press, New York, 1971, p. 501.
15. J. Drenth, J. N. Jansonius, R. Koekoek, and B. G. Wolthers, *Adv. Protein Chem.* **25** (1971) 79.
16. K. Brocklehurst, E. Salih, and T. S. Lodwig, *Biochem. J.* **220** (1984) 609.
17. J.-R. Brisson, P. R. Carey, and A. C. Storer, *J. Biol. Chem.* **261** (1986) 9087.
18. D. D. Roberts, S. D. Lewis, D. P. Ballou, S. T. Olson, and J. A. Shafer, *Biochemistry* **25** (1986) 5595.
19. N. E. Mackenzie, S. K. Grant, A. I. Scott, and J. P. G. Malthouse, *Biochemistry* **25** (1986) 2293.
20. P. I. Clark and G. Lowe, *J. Chem. Soc., Chem. Commun.* (1977) 923.
21. J. Drenth, J. N. Jansonius, R. Koekoek, and B. G. Wolthers, in: P. D. Boyer (Ed.), *The Enzymes*, Vol. III, 3rd Ed., Academic Press, New York, 1971, p. 485.
22. B. G. Wolthers, J. Drenth, J. N. Jansonius, R. R. Koekoek, and H. M. Swen, in: P. Desneulle, H. Neurath, and M. Ottesen (Eds.), *Structure-Function Relationships of Proteolytic Enzymes*, Munksgaard, Copenhagen, 1970, p. 272.
23. I. G. Kamphuis, K. H. Kalk, M. B. A. Swarte, and J. Drenth, *J. Mol. Biol.* **179** (1984) 233.
24. A. N. Glazer and E. L. Smith, *J. Biol. Chem.* **236** (1961) 2948.
25. R. J. Sundberg and R. B. Martin, *Chem. Rev.* **74** (1974) 471.
26. M. Howe-Grant and S. J. Lippard, *Inorg. Synth.* **20** (1980) 101.
27. S. D. Lewis, F. A. Johnson, and J. A. Shafer, *Biochemistry* **20** (1981) 48.
28. R. G. Pearson, H. Sobel, and J. Songstad, *J. Amer. Chem. Soc.* **90** (1968) 319.
29. M. E. Howe-Grant and S. J. Lippard, *Met. Ions Biol. Syst.* **11** (1980) 63.

SAŽETAK

Selektivno markiranje enzima papaina pomoću hlora(terpiridin)platine(II)

Sharon L. Pinnow, Herb M. Brothers II i Nenad M. Kostić

Kompleks $[\text{Pt}(\text{trpy})\text{Cl}]^+$ reaguje sa imidazolom, histidinom i tripeptidom Gly-His-Gly dajući komplekse u kojima je hlornidni ligand zamenjen imidazolnim prstenom; $[\text{Pt}(\text{trpy})\text{Cl}]^+$ reaguje sa 2-merkaptetanolom, cisteinom i tripeptidom glutationom dajući komplekse u kojima je hlornidni ligand zamenjen tiolatnom grupom. Kada se na enzim papain deluje ekvimolarnom količinom $[\text{Pt}(\text{trpy})\text{Cl}]^+$, samo Cys-25 u aktivnom mestu biva markiran grupom $\text{Pt}(\text{trpy})^{2+}$; kada se na papain deluje viškom $[\text{Pt}(\text{trpy})\text{Cl}]^+$, bivaju markirani Cys 25 i His 81. Kada se na taj dvostruko markirani papain deluje velikim viškom bromidnog anjona, grupa $\text{Pt}(\text{trpy})^{2+}$ biva uklonjena samo sa histidina 81. Konstantne brzine za supstituciju hlornidnog liganda u kompleksu $[\text{Pt}(\text{trpy})\text{Cl}]^+$ glutationom, papainom i histidinom na pH 5.0 iznose, tim redom, 30.2, 21.3 i 0.274 $\text{M}^{-1}\text{s}^{-1}$. Ove vrednosti pokazuju da je sulfhidrilna grupa nukleofilnija od imidazolijum-grupe i da je Cys-25 u aktivnom mestu papaina donekle zaklonjen od spoljašnjeg napada kompleksa $[\text{Pt}(\text{trpy})\text{Cl}]^+$. Kada se na dvostruko markirani papain deluje nukleofilom jačim od histidina, ali slabijim od cisteina, grupa $\text{Pt}(\text{trpy})^{2+}$ biva selektivno uklonjena sa histidina 81.