Discrimination of Thiolate Group in L-Cysteinate and Glutathionate by Axial Coordination Site of Five-Coordinate Trigonal-Bipyramidal Palladium(II) Complex with Tris(2-(diphenylphosphino)ethyl)phosphine

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Five-coordinate trigonal-bipyramidal palladium(II) L-cysteinato and glutathionato complexes with tris(2-(diphenylphosphino)ethyl)phosphine have been prepared. The high selectivity for the thiolato sulfur atom in the axial position has been confirmed and was applied to separation of L-cysteine from the other amino acids and selective determination of the reduced form of glutathione.

A wide range of donor atoms such as nitrogen, oxygen, phosphorus, sulfur, and halogens can form relatively stable four-coordinate square-planar palladium(II) complexes. On the other hand, five-coordinate palladium(II) complexes formed by familiar donor atoms in the second series of the periodic table such as amine nitrogen, carboxylato oxygen, or fluoride have so far not been reported. Recently, we have prepared relatively stable five-coordinate trigonal-bipyramidal palladium(II) complexes by using a tetradentate phosphine ligand, tris(2-(diphenylphosphino)-ethyl)phosphine (pp_3), and have reported thermodynamic and kinetic properties of the halo complexes $[Pd(pp_3)X]^+$ ($X = Cl^-$, Br^-, I^-).[1,2] In that study, it has been clarified that the order of stabilization of the halo complexes by X is $I^- > Br^- > Cl^-$. The above facts are well consistent with expectations from the electronic repulsion and the molecular orbital theory that reduction of the electronic repulsion is essential to give a higher coordination number than that usually observed such as five-coordinate for palladium(II) complexes and that only the donor orbitals on the relatively higher energy levels can form an effective σ bonding in such complexes compared with ordinary square-planar ones. These limitations in the coordination ability can be of great advantage to the biochemical application because five-coordinate palladium(II) complexes probably prefer sulfur donor atoms, which usually play important roles in biological systems invariably containing nitrogen and oxygen donor atoms.

In this work, we have confirmed that the selectivity of the axial coordination site in $[Pd(pp_3)X]$-type complexes can discriminate the thiolate sulfur atom in L-cysteinate (L-Hcys or L-cys)[3] from the other donor atoms in amino acids while the corresponding square-planar $[Pd(p_3)X]$-type complexes[1] (p_3 = di(2-(diphenylphosphino)ethyl)phenylphosphine) can be bound to any of amine nitrogen, carboxylate oxygen (even weakly donating sulfonate oxygen[4]), and thiolate sulfur atoms.[5] Furthermore, we have applied the selectivity to detection of the L-cysteine residue in the reduced form of glutathione (γ-L-glutamyl-L-cysteinyl-glycine) which is quite important in the biological redox and detoxification systems.[6]

The L-cysteinato complex, $[Pd(pp_3)(L\text{-Hcys})](BF_4)$,[7] was prepared by the following procedure. To a solution containing $[Pd(pp_3)](BF_4)_2$[8] (0.600 g, 0.632 mmol) in acetonitrile (70 cm^3) were added a solution containing L-cysteine (0.162 g, 1.32 mmol) and a 0.2 M NaOH aqueous solution (0.4 cm^3) in 5 cm^3 of water. The red complex was extracted with chloroform and the crystals were obtained by adding diethyl ether.[9] The red glutathionato complex, $[Pd(pp_3)(gluta)](BF_4)$ (gluta = glutathionate),[7] was similarly obtained from a mixture of an acetonitrile solution of $[Pd(pp_3)](BF_4)_2$ and a basic aqueous solution (pH = 9) of the reduced form of glutathione by adding water.[10] Electronic absorption and NMR spectra were recorded on a JASCO V-570 spectrophotometer and a JEOL JNM-A400 FT-NMR spectrometer. A 3-mm o.d. NMR tube containing a sample solution was coaxially mounted in a 5-mm o.d. NMR tube containing deuterated water as a lock solvent and phosphoric acid as a reference.

As shown in Figure 1, the ^{31}P NMR spectral pattern for the L-cysteinato and glutathionato complexes is almost the same as that for $[Pd(pp_3)(pt)](BF_4)_2$ (pt = 1-propanethiolate)[11] indicating the trigonal-bipyramidal structure with three equivalent terminal phosphorus atoms in the equatorial position (P_{eq}) and a central phosphorus atom in the axial position (P_{ax}). Furthermore, quite similar chemical shifts in Figure 1 suggest that the L-cysteinato and glutathionato ligands are coordinated with the thiolate sulfur atom as the pt ligand is, because the NMR chemical shifts for the equatorial and axial phosphorus are quite variable with σ and π bonding characteristics of the axially coordinated donor atom as previously reported.[8] In addition, the L-cysteina-

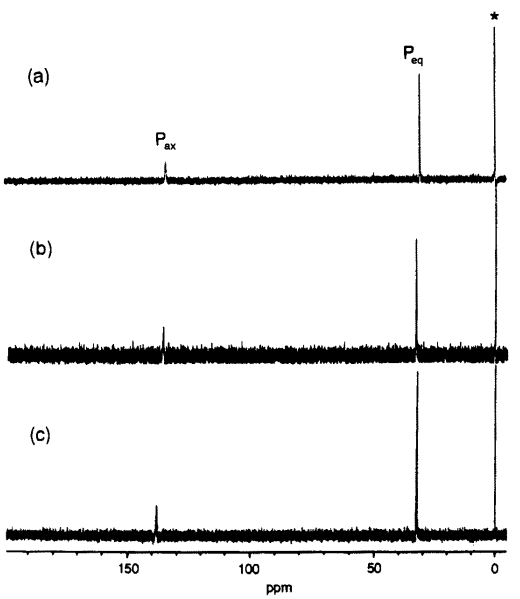

Figure 1. ^{31}P NMR spectra of the pt complex in chloroform (a), L-cysteinato complex in acetonitrile (b), and glutathionato complex in weakly basic water (pH ≈ 8) (c). Asterisk denotes the signal for phosphoric acid in the outer D_2O.

to[9] and glutathionato[10] complexes exhibit quite similar absorption spectra to that for the pt complex[11] showing two visible absorption bands which can be assigned to the transition from $^1A_1$' to ^1E' and ^1E". This is compatible with the fact that the present two complexes take the same geometry around palladium(II) as that for the pt complex. The thiolato coordination is also confirmed by the fact that the trigonal-bipyramidal complex was not formed by using L-alaninate as the axial ligand which has the structure corresponding to L-cysteinate missing a thiolate group (vide infra). The ^1H NMR signals of the peptide chain of the glutathionato complex precipitated with $Ni(NO_3)_2 \cdot 6H_2O$ collapsed in a CD_3CN/D_2O solution keeping the same ^{31}P NMR spectrum. This NMR spectral behavior is consistent with the pendant structure of the monodentate glutathionato ligand which binds a paramagnetic nickel(II) ion with free amine and carboxylato groups maintaining the trigonal-bipyramidal geometry around palladium(II).

In order to apply such a selectivity for the thiolato sulfur atom to separation of amino acids, an acetonitrile solution of $[Pd(pp_3)](BF_4)_2$ (0.02 M, 3 cm^3) was added to a 0.05 M NaOH aqueous solution (1.3 cm^3) containing four amino acids, L-alanine, L-methionine, L-penicillamine, and L-cysteine (0.08 M for each), and the resultant complex is extracted with chloroform (3 cm^3). The chloroform solution showed only the ^{31}P NMR signals for the L-cysteinato complex. When $[Pd(pp_3)](BF_4)_2$ and each amino acid were reacted separately in the same manner, the three amino acid other than L-cysteine did not form the trigonal-bipyramidal palladium(II) complex besides $[Pd(pp_3)(CH_3CN)]^{2+}$.[12] These facts indicate that the axial coordination site of the present trigonal-bipyramidal palladium(II) complex discriminates the thiolate group in L-cysteinate from the other functional groups in amino acids and that the thiolato complex is selectively extracted because the complex with the pp_3 ligand is quite soluble in nonpolar solvents and sparingly soluble in water. These results are consistent with the expectation that the five-coordinate palladium(II) prefers donor orbitals of the higher energy level compared with the four-coordinate one. The preference for the thiolate sulfur atom in L-cysteinate over the thioether one in L-methioninate is mainly due to the weak donating ability of the thioether group and steric repulsion of the methyl group on the sulfur atom with the surrounding diphenylphosphino groups, and L-penicillaminate was not coordinated exclusively due to the steric restriction caused by the two adjacent methyl groups.

The reaction solution initially containing $[Pd(pp_3)](BF_4)_2$ and the reduced form of glutathione (1:1 molar ratio) showed no ^{31}P NMR signal other than those for the glutathionato complex, showing its quantitative formation. In order to examine the applicability of this complex formation reaction to determination of glutathione, a series of absorption spectra of mixed solutions consisting of an acetonitrile solution (2 cm^3) of $[Pd(pp_3)](BF_4)_2$ (5.23×10^{-4} mol dm^{-3}) and an equivalent volume of further diluted weakly basic aqueous solutions (pH ≅ 8) of the reduced form of glutathione ($(0.00–1.95) \times 10^{-4}$ mol dm^{-3}) was recorded (Figure 2). The presence of the isosbestic point and the linearity between the absorbance and the glutathione concentration indicate that glutathionate is quantitatively coordinated to palladium(II) keeping the binary system in which the free amine and carboxylate groups in the pendant glutathionato ligand are not reacted with remaining $[Pd(pp_3)(CH_3CN)]^{2+}$.[12] Such an equilibrium behavior showing the high selectivity for the thiolato coordination enables us to determine the 10^{-6} M order of glutathione and L-cysteine residue in peptides and proteins. Furthermore, $[Pd(pp_3)(CH_3CN)]^{2+}$ did not react to form a thiolato complex with the oxidized form of glutathionate which has a disulfide bond instead of the thiolate group. Such a discriminating ability for the oxidation state is quite useful for investigation of the biological redox system where proteins oxidized by free radicals and activated oxygen are reduced back by the oxidation of the reduced form of glutathione.

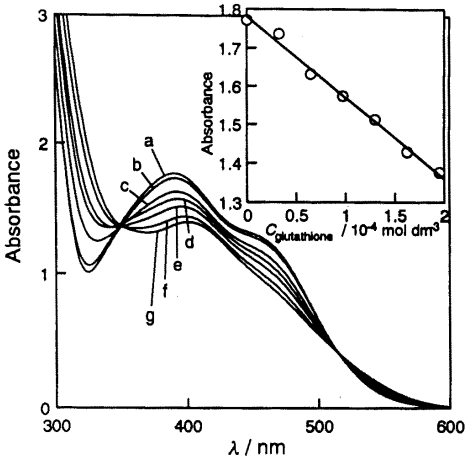

Figure 2. Absorption spectra of mixed solutions consisting of an acetonitrile solution of $[Pd(pp_3)](BF_4)_2$ (5.23×10^{-4} mol dm^{-3}) and an equivalent volume of basic solutions of glutathione (0.00 (a), 3.25 (b), 6.51 (c), 9.67 (d), 13.01 (e), 16.27 (f), and 19.52 (g) $\times 10^{-5}$ mol dm^{-3}). The inset shows the glutathione concentration dependence of absorbance at 400 nm.

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References and Notes

1 S. Aizawa, T. Iida, and S. Funahashi, *Inorg. Chem.*, **35**, 5163 (1996).
2 S. Aizawa and S. Funahashi, *Chem. Lett.*, **1994**, 2023.
3 L-Hcys and L-cys denote monovalent and bivalent anions, respectively.
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5 Unpublished data.
6 L. Flohe, H. Ch. Benohr, H. Sies, H. D. Waller, and A. Wendel, "Glutathione," Academic Press, New York (1974).
7 L-Cysteine and glutathione in the crystals can be regarded as an univalent anion or a neutral molecule because the ^{11}B NMR signal for BF_4^- as the counter anion of the cationic complex was observed in the solutions of the crystals. However, it is reasonable to consider that the neutral ligand having –COOH and $–NH_3^+$ groups is hardly formed in the basic solution or the nonpolar solvent.
8 S. Aizawa and S. Funahashi, *Anal. Sci.*, **12**, 27 (1996).
9 The elemental analysis was not successful because of a remaining inorganic residue. λ_{max}/nm (in $CHCl_3$) = 525, 405.
10 Anal. Found: C, 50.72; H, 5.49; N, 3.54%. Calcd for $[Pd(pp_3)(gluta)](BF_4) \cdot 4H_2O$: C, 50.28; H, 5.35; N, 3.38%. λ_{max}/nm (log(ε/mol^{-1} cm^{-1})) (in 0.03 M NaOH aq) = 520 (3.50), 414 (3.80).
11 Anal. Found: C, 56.88; H, 5.29; N, 0.00%. Calcd for $PdSP_4F_4C_{45}BH_{49}$: C, 57.56; H, 5.26; N, 0.00%. ^{31}P NMR (in $CDCl_3$): δ (relative to H_3PO_4 in D_2O) = 30.35 (equatorial), 134.51 (axial). λ_{max}/nm (log(ε/mol^{-1} kg cm^{-1})) (in CH_2Cl_2) = 551 (3.31), 409 (3.82), 315 (4.43). The ^{31}P NMR chemical shifts are the same as those for the dithiolato-bridged dimer with the trigonal-bipyramidal $\{Pd(pp_3)\}$ terminals, $[\{Pd(pp_3)\}_2(pdt)](BF_4)_2$ (pdt = 1,3-propanedithiolate), the structure of which was confirmed by an X-ray structure analysis (unpublished results).
12 The trigonal-bipyramidal solvated palladium(II) complex is formed in an acetonitrile solution of $[Pd(pp_3)](BF_4)_2$ as described in the Reference 8.