Research Letter

Kinetic and Mechanistic Studies on the Reaction of DL-Methionine with $[(H_2O)(tap)_2RuORu(tap)_2(H_2O)]^{2+}$ in Aqueous Medium at Physiological pH

Tandra Das,[1] A. K. Datta,[2] and A. K. Ghosh[1]

[1] *Department of Chemistry, The University of Burdwan, Burdwan, West Bengal 713104, India*
[2] *Department of Pediatrics, Burdwan Medical College, Burdwan, West Bengal 713104, India*

Correspondence should be addressed to A. K. Ghosh, alakghosh2002@yahoo.co.in

The reaction has been studied spectrophotometrically; the reaction shows two steps, both of which are dependent on ligand concentration and show a limiting nature. An associative interchange mechanism is proposed. Kinetic and activation parameters ($k_1 \sim 10^{-3}\,s^{-1}$ and $k_2 \sim 10^{-5}\,s^{-1}$) and ($\Delta H_1^{\neq} = 13.8 \pm 1.3\,kJ\,mol^{-1}$, $\Delta S_1^{\neq} = -250 \pm 4\,JK^{-1}\,mol^{-1}$, $\Delta H_2^{\neq} = 55.53 \pm 1.5\,kJ\,mol^{-1}$, and $\Delta S_2^{\neq} = -143 \pm 5\,JK^{-1}\,mol^{-1}$) have been calculated. From the temperature dependence of the outer sphere association equilibrium constant, thermodynamic parameters ($\Delta H_1^{\circ} = 16.6 \pm 2.3\,kJ\,mol^{-1}$ and $\Delta S_1^{\circ} = 95 \pm 7\,JK^{-1}\,mol^{-1}$; $\Delta H_2^{\circ} = 29.4 \pm 3.2\,kJ\,mol^{-1}$ and $\Delta S_2^{\circ} = 128 \pm 10\,JK^{-1}\,mol^{-1}$) have also been calculated.

1. Introduction

The binding of the antitumor drug cisplatin and other platinum group metal complexes, especially ruthenium(II), rhodium(III), iridium(III), platinum(II), and palladium(II) to amino acids, nucleosides, nucleotides, and particularly to DNA is still an interesting subject and has given considerable impetus to research in the area of metal ion interactions with nucleic acid constituents. Ruthenium complexes are an order of magnitude less toxic than cisplatin, and aqua complexes if used directly will be less toxic as some hydrolyzed side products are responsible for toxicity. From a literature survey [1–3], it is revealed that many potential alternative metallopharmaceuticals have been developed, ruthenium being one of the most promising, and are currently undergoing clinical trials [4–7]. Another point of interest is that DNA is not the only target. Binding to proteins, RNA [8–10] and several sulphur donor ligands, present in the blood, are available for kinetic and thermodynamic competition [11, 12].

Keeping this in mind, in this paper, we have studied the kinetic details of the interaction of our chosen complex (an aqua-amine complex of ruthenium(II)) with an S-containing amino acid DL-methionine at pH 7.4 in aqueous medium and a plausible mechanism is proposed.

The importance of the work lies in the fact that (a) the reaction has been studied in an aqueous medium, (b) the reaction has been studied at pH (7.4) which is the physiological pH of the human body, (c) the aqua-amine complex is chosen, (d) ruthenium(II) than ruthenium(III) is chosen, as ruthenium(III) is a prodrug which is reduced in the cell to ruthenium(II), and (e) the title complex maintains its +2 oxidation state even at pH 7.4 due to the presence of a strong pi-acceptor ligand tap (tap = {2-(m-tolylazo)pyridine}), where most of the other ruthenium(II) complexes are oxidized to ruthenium(III).

2. Materials and Methods

Reported method [13, 14] was used to isolate *cis*-$[Ru(tap)_2(H_2O)_2](ClO_4)_2 \cdot H_2O$. The reacting complex ion $[(H_2O)(tap)_2RuORu(tap)_2(H_2O)]^{2+}$ (**1**) was generated in situ by adjusting the pH at 7.4. The reaction product $[(tap)_2Ru(\mu\text{-O})(\mu\text{-meth})Ru(tap)_2]^{2+}$ (complex **2**) of DL-methionine, and complex **1** is shown in Figure 1. The
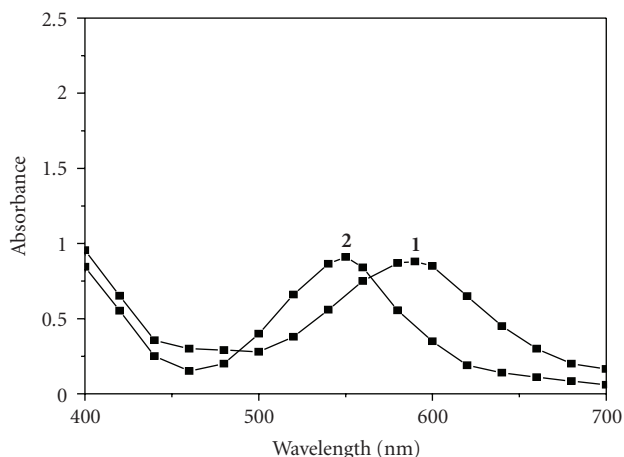
FIGURE 1: Difference in spectrum between complex **1** and product complex (**2**); [1] = 1.0×10^{-4} mol dm^{-3}, [DL-methionine] = 2.0×10^{-3} mol dm^{-3}, cell used 1 cm quartz.
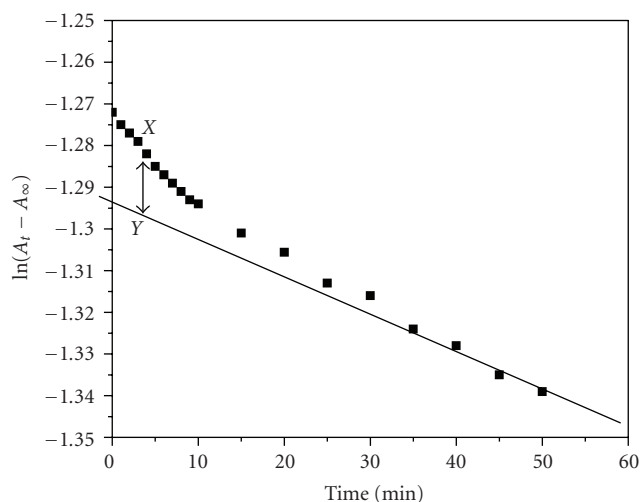

FIGURE 2: A typical plot of $\ln(A_t - A_\infty)$ versus time.

composition of **2** in solution was determined by Job's method of continuous variation and the metal: ligand ratio was found to be 2:1. The pH of the solution was adjusted by adding NaOH/HClO$_4$, and the measurements were carried out with the help of a Sartorius make digital pH meter (PB 11) with an accuracy of ± 0.01 unit. Doubly distilled water was used to prepare all the kinetic solutions. All chemicals used were of AR grade, available commercially. The reactions were carried out at constant ionic strength of (0.1 M NaClO$_4$).

3. Kinetics

The kinetic studies were done on a Shimadzu UV-2101PC spectrophotometer attached to a thermoelectric cell temperature controller (model TB 85, accuracy $\pm 0.1^\circ$C). The progress of the reaction was monitored by following the

decrease in absorbance at 600 nm using mixing technique and maintaining pseudo-first-order conditions. In Figure 2, plot of $\ln(A_t - A_\infty)$ versus time shows a consecutive nature of the reaction. Initially, it is curved and shows linear behavior in the latter stage. The rate constants were calculated using the method of Weyh and Hamm [15] as described in an earlier paper [1] using the following equation:

$$\ln \Delta = \text{constant} - k_{1(\text{obs})}t, \quad \text{when } t \text{ is small.} \quad (1)$$

The meaning of Δ is shown in Figure 2 ($\Delta = X - Y$). $k_{2(\text{obs})}$ is calculated from the latter linear portion.

4. Results and Discussion

At a fixed excess [DL-methionine] (2.0×10^{-3} mol dm^{-3}), pH 7.4, temperature 50°C, and ionic strength (0.1 mol dm^{-3} NaClO$_4$) the reaction was found to be first order in [complex **1**], that is, d [complex **2**]$/dt = k_{\text{obs}}$ [complex **1**].

The pK_a^1 and pK_a^2 values [16] of DL-methionine are 2.24 and 9.07, respectively, at 25°C. Thus, at pH 7.4, the ligand exists mainly as a neutral molecule, that is, as a zwitterion (LH$_2^+$ $\rightarrow$ LH $\rightarrow$ L$^-$). On the other hand, first acid dissociation equilibrium of the complex [Ru(tap)$_2$(H$_2$O)$_2$]$^{2+}$ is 6.6 [17] at 25°C. At pH 7.4, the complex ion exists in dimeric oxo-bridged form, [(H$_2$O)(tap)$_2$RuORu(tap)$_2$(H$_2$O)]$^{2+}$ [18–21]. At pH 7.4, the mononuclear species exists in the hydroxoaqua form. Two such species assemble to form the dinuclear oxo-bridged diaqua complex due to thermodynamic force mainly arising from pi-bonding [22] (O^{2-} donor, Ru^{II} acceptor) which is favorable for 4d ion, Ru^{II}. Now, such strong covalency reduces the acidity of the coordinated water. The oxo-bridge formation is solely dependent on pH. Electrochemical studies show that there is pH potential domain, where the μ-oxo structures stay intact. Variable temperature study does not show any effect, which is in line with the fact that oxo-bridge formation is solely pH-dependent [23, 24]. The rate constant for such process can be evaluated by assuming the following scheme

$$(\mathbf{1}) \xrightarrow{k_1} \mathbf{B} \xrightarrow{k_2} (\mathbf{2}), \quad (2)$$

where **B** is [(H$_2$O)(tap)$_2$RuORu(tap)$_2$(LH)]$^+$.

4.1. Calculation of k_1 and k_2 Values for Step (**1**) $\rightarrow$ **B** *and for* (**B**) $\rightarrow$ (**2**) *Step.* The rate constants, $k_{1(\text{obs})}$ for (**1**) $\rightarrow$ **B** and $k_{2(\text{obs})}$ for (**B**) $\rightarrow$ (**2**), were calculated following the technique described in an earlier paper [25], and the values are collected in Tables 1 and 2. The rate increases with the increase in [ligand] and reaches a limiting value for both steps. Details of the mechanism are discussed in "Mechanism and Conclusion" section. The k_1, k_2, K'_E, and K''_E for the two steps are calculated similarly and collected in Table 3.

4.2. Effect of Change in pH on the Reaction Rate. This was studied at five different pH values. $10^3 k_{1(\text{obs})}(\text{s}^{-1})$ and
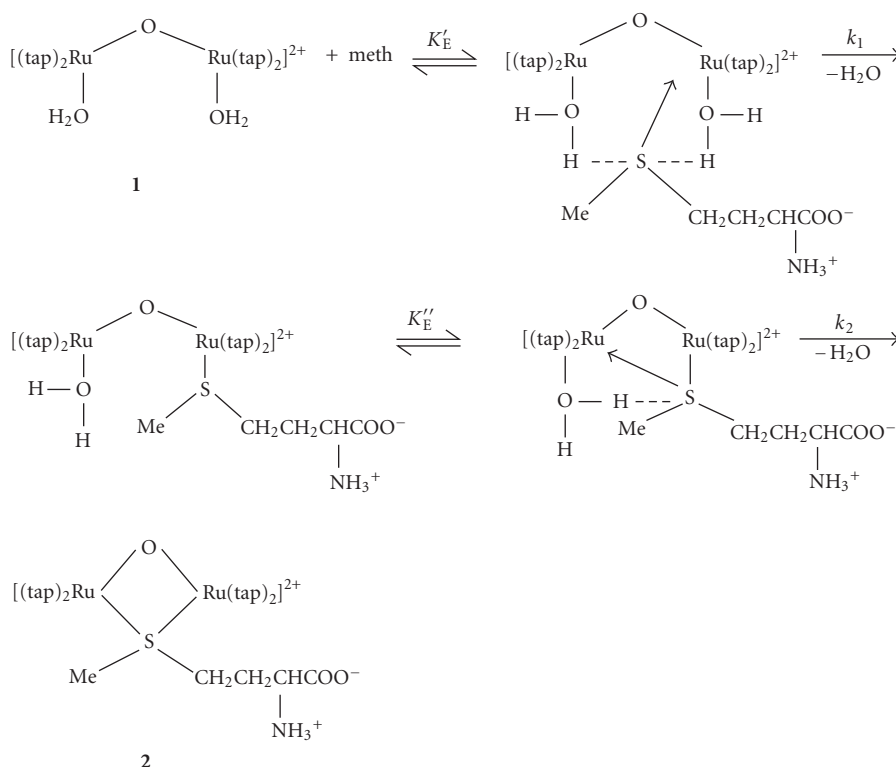
Scheme 1

Table 1: $10^3 k_{1(obs)}$ values for different ligand concentrations at different temperatures. [Complex] $= 1 \times 10^{-4}$ mol dm^{-3}, pH = 7.4, ionic strength $= 0.1$ mol dm^{-3} NaClO$_4$.

10^3 [ligand] (mol dm^{-3})	Temperature (°C)			
	45	50	55	60
2.0	0.70	0.87	1.02	1.18
3.0	1.0	1.22	1.4	1.55
4.0	1.23	1.45	1.62	1.90
5.0	1.40	1.65	2.0	2.08
10.0	1.67	2.0	2.25	2.63

Table 2: $10^5 k_{2(obs)}$ values for different ligand concentrations at different temperatures. [Complex] $= 1 \times 10^{-4}$ mol dm^{-3}, pH = 7.4, ionic strength $= 0.1$ mol dm^{-3} NaClO$_4$.

10^3 [ligand] (mol dm^{-3})	Temperature (°C)			
	45	50	55	60
2.0	2.27	3.41	5.95	8.93
3.0	2.92	4.5	8.2	11.63
4.0	3.85	5.98	10.0	15.0
5.0	5.13	7.58	12.2	18.2
10.0	7.4	10.52	17.86	25.0

$10^5 k_{2(obs)}$ values are 0.73, 0.76, 0.83, 1.04 and 1.55 (s^{-1}), and 3.3, 3.7, 4.16, 6.6, and 11.32 (s^{-1}) at pH 5.5, 6.0, 6.5, 7.0, and 7.4, respectively. In the studied pH range (pH 5.5 to 7.4), the percentage of diaqua species is reduced with the increase in pH, and the percentage of the dimer is predominant. The dimer with its two metal centers is a better center to the incoming nucleophiles. On the other hand, the pK$_1$ and pK$_2$ values of the ligand DL-methionine are 2.24 and 9.07 at 25°C. With the increase in pH from 5.0 to 7.4, the amount of the deprotonated form increases, and the zwitterionic form (LH) predominates which also partly accounts for the enhancement of the rate with increase in pH.

4.3. Effect of Temperature on the Reaction Rate. Four different temperatures with varied ligand concentrations were chosen, and the results are listed in Tables 1 and 2. The activation parameters for the steps (**1**) → **B** and (**B**) → (**2**), evaluated from the linear Eyring plots and compared with the analogous systems [1], support the proposition.

5. Mechanism and Conclusion

The low $\Delta H^{\neq}$ value, together with negative $\Delta S^{\neq}$ value, suggests ligand participation in the transition state, and an associative interchange mechanism is proposed (Scheme 1) for the interaction of DL-methionine with the title complex. The bonding mode of methionine is not fully understood, as it was not possible to isolate the solid product. In the studied reaction condition, that is, at pH 7.4, methionine exists in the deprotonated form. At first S attacks on one of the

TABLE 3: The k_1, K_E', k_2, and K_E'' values for the interaction of methionine with (**1**).

Temperature ($^\circ$C)	$10^3 k_1$ (s^{-1})	K_E' (dm^3 mol^{-1} s^{-1})	$10^5 k_2$ (s^{-1})	K_E'' (dm^3 mol^{-1} s^{-1})
45	3.06	156	18.0	70
50	3.38	179	25.0	78
55	3.70	197	36.0	98
60	4.06	207	48.0	113

two ruthenium(II), centers are assumed. This step is ligand dependent, and with increasing the ligand concentration, a limiting rate is reached. This may be due to the formation of outersphere association complex, which is possibly stabilized through hydrogen bonding. The spontaneous formation of an outersphere association complex is also supported from a negative ΔG° value calculated from the temperature dependence of the K_E values. The corresponding thermodynamic parameters are $\Delta H_1^\circ = 16.6 \pm 2.3 \, \text{kJ mol}^{-1}$ and $\Delta S_1^\circ = 95 \pm 7 \, \text{JK}^{-1} \, \text{mol}^{-1}$, $\Delta H_2^\circ = 29.4 \pm 3.2 \, \text{kJ mol}^{-1}$ and $\Delta S_2^\circ = 128 \pm 10 \, \text{JK}^{-1} \, \text{mol}^{-1}$.

The coordinated methionine in any of the ruthenium(II) centers now attacks the second ruthenium(II) center like a metalloligand, and we observe two distinct ligand dependent steps. For the ligand to behave as a bridging ligand with the oxo-bridging complex, the mono atom sulphur [26, 27] bridging has the best prospects. It is to be noted here that the second step is not a normal cyclisation step as occurs in chelation in a single central atom. Here, two metal centers are available, and after attachment of the ligand to one of the metal centers, the environment of the two centers will no longer remain the same, and when the difference in rate between two steps is larger, we observe the dependence of rate on ligand concentration carried to the second step. But when the difference between two steps is comparatively smaller as is found in a system earlier [2], the second step is found to be independent on ligand concentration. A plausible mechanism is shown here to commensurate with the experimental findings.

Acknowledgment

References

[1] A. K. Ghosh, "Kinetics and mechanism of the interaction of thioglycolic acid with $[(H_2O)(tap)_2RuORu(tap)_2(H_2O)]^{2+}$ ion at physiological pH," *Transition Metal Chemistry*, vol. 31, no. 7, pp. 912–919, 2006.

[2] A. K. Ghosh, "Kinetic studies of substitution on $[(H_2O)(tap)_2RuORu(tap)_2(H_2O)]^{2+}$ ion by DL-penicillamine at physiological pH," *Indian Journal of Chemistry A*, vol. 46, no. 4, pp. 610–614, 2007.

[3] I. Kostova, "Platinum complexes as anticancer agents," *Recent Patents on Anti-Cancer Drug Discovery*, vol. 1, no. 1, pp. 1–22, 2006.

[4] V. Brabec and O. Nováková, "DNA binding mode of ruthenium complexes and relationship to tumor cell toxicity," *Drug Resistance Updates*, vol. 9, no. 3, pp. 111–122, 2006.

[5] I. Kostova, "Ruthenium complexes as anticancer agents," *Current Medicinal Chemistry*, vol. 13, no. 9, pp. 1085–1107, 2006.

[6] C. G. Hartinger, S. Zorbas-Seifried, M. A. Jakupec, B. Kynast, H. Zorbas, and B. K. Keppler, "From bench to bedside—preclinical and early clinical development of the anticancer agent indazolium *trans*-[tetrachlorobis(1H-indazole)ruthenate(III)] (KP1019 or FFC14A)," *Journal of Inorganic Biochemistry*, vol. 100, no. 5-6, pp. 891–904, 2006.

[7] W. H. Ang and P. J. Dyson, "Classical and non-classical ruthenium-based anticancer drugs: towards targeted chemotherapy," *European Journal of Inorganic Chemistry*, no. 20, pp. 4003–4018, 2006.

[8] J. J. Roberts and A. J. Thomson, "The mechanism of action of antitumor platinum compounds," *Progress in Nucleic Acid Research and Molecular Biology*, vol. 22, pp. 71–133, 1979.

[9] A. W. Prestayko, S. T. Crooke, and S. K. Carter, Eds., *Cisplatin, Current Status and New Developments*, Academic Press, New York, NY, USA, 1980.

[10] M. P. Hacker, E. B. Douple, and L. H. Krakoff, Eds., *Platinum Coordination Compounds in Cancer Chemotherapy*, Martinus Nijhoff, Boston, Mass, USA, 1984.

[11] J. Reedijk, "Why does cisplatin reach guanine-N7 with competing S-donor ligands available in the cell?" *Chemical Reviews*, vol. 99, no. 9, pp. 2499–2510, 1999.

[12] J. Kozelka, F. Legendre, F. Reeder, and J.-C. Chottard, "Kinetic aspects of interactions between DNA and platinum complexes," *Coordination Chemistry Reviews*, vol. 190–192, pp. 61–82, 1999.

[13] S. Goswami, A. R. Chakravarty, and A. Chakravorty, "Chemistry of ruthenium. 2. Synthesis, structure, and redox properties of 2-(arylazo)pyridine complexes," *Inorganic Chemistry*, vol. 20, no. 7, pp. 2246–2250, 1981.

[14] S. Goswami, A. R. Chakravarty, and A. Chakravorty, "Chemistry of ruthenium. 7. Aqua complexes of isomeric bis[(2-arylazo)pyridine]ruthenium(II) moieties and their reactions: solvolysis, protic equilibriums, and electrochemistry," *Inorganic Chemistry*, vol. 22, no. 4, pp. 602–609, 1983.

[15] J. A. Weyh and R. E. Hamm, "Aquation of the *cis*-bis(iminodiacetato)chromate(III) and *trans*(*fac*)-bis (methyliminodiacetato)chromate(III) ions in acidic aqueous medium," *Inorganic Chemistry*, vol. 8, no. 11, pp. 2298–2302, 1969.

[16] A. E. Martell and R. M. Smith, *Critical Stability Constants*, vol. 1, Plenum Press, New York, NY, USA, 1974.

[17] B. Mahanti and G. S. De, " Kinetics and mechanism of substitution of aqua ligands from *cis*-diaqua-*bis*(bipyridyl ruthenium(II)) complex by salicylhydroxamic acid in aqueous medium," *Transition Metal Chemistry*, vol. 17, no. 6, pp. 521–524, 1992.

[18] S. J. Raven and T. J. Meyer, "Reactivity of the oxo-bridged ion $[(bpy)_2(O)Ru^{IV}ORu^V(O)(bpy)_2]^{3+}$," *Inorganic Chemistry*, vol. 27, no. 24, pp. 4478–4483, 1998.

[19] W. Kutner, J. A. Gilbert, A. Tomaszewski, T. J. Meyer, and R. W. Murray, "Stability and electrocatalytic activity of the oxo-bridged dimer $[(bpy)_2(H_2O)RuORu(OH_2)(bpy)_2]^{4+}$ in basic solutions," *Journal of Electroanalytical Chemistry*, vol. 205, no. 1-2, pp. 185–207, 1986.

[20] S. W. Gersten, G. J. Samuels, and T. J. Meyer, "Catalytic oxidation of water by an oxo-bridged ruthenium dimer," *Journal of the American Chemical Society*, vol. 104, no. 14, pp. 4029–4030, 1982.

[21] P. Ghosh and A. Chakravorty, "Hydroxamates of bis(2,2′-bipyridine)ruthenium: synthesis, protic, redox, and the electroprotic equilibria, spectra, and spectroelectrochemical correlations," *Inorganic Chemistry*, vol. 23, no. 15, pp. 2242–2248, 1984.

[22] F. A. Cotton, G. Wilkinson, C. A. Murrilo, and M. Bochman, *Advanced Inorganic Chemistry*, John Wiley & Sons, New York, NY, USA, 6th edition, 2003.

[23] J. A. Gilbert, D. S. Eggleston, W. R. Murphy Jr., et al., "Structure and redox properties of the water-oxidation catalyst $[(bpy)_2(OH_2)RuORu(OH_2)(bpy)_2]^{4+}$," *Journal of the American Chemical Society*, vol. 107, no. 13, pp. 3855–3864, 1985.

[24] J. A. Gilbert, D. Geselowitz, and T. J. Meyer, "Redox properties of the oxo-bridged osmium dimer $[(bpy)_2(OH_2)Os^{III}OOs^{IV}(OH_2)(bpy)_2]^{4+}$. Implications for the oxidation of H_2O to O_2," *Journal of the American Chemical Society*, vol. 108, no. 7, pp. 1493–1501, 1986.

[25] H. Chattopadhyay and A. K. Ghosh, "Kinetic and mechanistic studies of substitution on $[(H_2O)(tap)_2RuORu(tap)_2(H_2O)]^{2+}$ ion with uridine in aqueous medium," *Inorganic Reaction Mechanisms*, vol. 6, no. 1, pp. 9–17, 2006.

[26] L. Zhu and N. M. Kostić, "Toward artificial metallopeptidases: mechanisms by which platinum(II) and palladium(II) complexes promote selective, fast hydrolysis of unactivated amide bonds in peptides," *Inorganic Chemistry*, vol. 31, no. 19, pp. 3994–4001, 1992.

[27] L. Zhu and N. M. Kostić, "Hydrolytic cleavage of peptides by palladium(II) complexes is enhanced as coordination of peptide nitrogen to palladium(II) is suppressed," *Inorganica Chimica Acta*, vol. 217, no. 1-2, pp. 21–28, 1994.