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Complexation of Tetra-μ-carboxylato-dirhodium(II) with Imidazole

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The stepwise formation constants and the enthalpies and entropies of reactions for the formation of 1:1 and 1:2 adducts of $Rh_2(O_2CR)_4$, where R = CH_3OCH_2, CH_3, or CH_3CH_2, with imidazole have been determined by an entropy titration technique in aqueous solution at physiological pH. The thermodynamic stabilities and the negative enthalpy changes were found to be in the order propionate > acetate > methoxyacetate. This trend could be explained in terms of a desolvating effect of the R group on the bridging carboxylate ions at the axial positions of the rhodium(II) complexes. The observed variation of antitumor activity for these rhodium(II) carboxylates parallels the above order.

Introduction

In 1974 our laboratory discovered that tetra-μ-acetato-dirhodium(II) exhibited anticancer activity against Leukemia 1210 and Ehrlich ascites tumors in mice.[1] Since that time we have been investigating the anticancer activity of several rhodium(II) carboxylates and have found that the acetate, propionate, and butyrate complexes are all antineoplastic agents.[2,3] Rhodium(II) butyrate was the most potent antitumor agent followed by the propionate, acetate, and methoxyacetate complexes. All of the complexes inhibit DNA and RNA synthesis in vitro with an order of inhibition of methoxyacetate < acetate < propionate < butyrate.[2,3] These studies showed that the anticancer activity, toxicity, and enzyme inhibition increased with length of the carbon chain of the bridging acid.

In a more recent study we reported the formation constants for the complex formation reactions involving rhodium(II) methoxyacetate, acetate, and propionate and the ligands 5′-AMP, 5′-ADP, and 5′-ATP.[4] The purpose of the study was to determine if the thermodynamic stability of the rhodium(II) carboxylate adducts correlated with the disparate effects seen with respect to their biological activity. A correlation was found in that the order of stability was propionate > acetate > methoxyacetate. It was concluded that the increased stability of the rhodium(II) propionate adducts over that of the corresponding rhodium(II) acetate complex could account for at least a part of the variation in biological activity.

It is difficult to interpret the order of stability of the rhodium(II) carboxylate adducts in terms of an electronic effect. The methoxyacetate ion, being less basic than the acetate or propionate ion, should produce a lower electron density on the metal ion and thus a stronger interaction with the two axial ligands. Since rhodium(II) methoxyacetate forms weaker complexes than the rhodium(II) propionate species, some alternate interpretation seems more plausible.

In order to understand the reasons for the observed thermodynamic stabilities of the adducts we have measured the enthalpies and entropies of formation of 1:1 and 1:2 adducts of rhodium(II) methoxyacetate, acetate, and propionate with imidazole. Imidazole was chosen for the study for three reasons: (1) it is an important ligand in biologic milieu,[5] (2) it forms complexes with the rhodium(II) carboxylates at physiological pH, and (3) the system lends itself nicely to evaluation by the entropy titration technique used in this investigation.

Materials and Methods

Chemicals. Rhodium(II) acetate was obtained from Matthey Bishop, Inc., Malvern, Pa. 19355. This was further purified by recrystallization from acetone. Other carboxylates were synthesized as described previously.[6] All of the rhodium(II) carboxylates were dried before use. The purity was checked by NMR and tga and finally the molar absorptivities of the solutions were compared with the literature values: found, 224–229; lit., 230.

Imidazole was obtained from Eastman Organic Chemicals, Rochester, N.Y. It was recrystallized from an acetone–ether mixture and the purity evaluated by the standard titrimetric procedure.

Solvent. All solutions were made in a phosphate buffer prepared by dissolving potassium mono- and dihydrogen phosphates in a ratio suitable for the desired pH and ionic strength in deionized water. The concentration of total phosphate, ionic strength, and pH were 0.038 98 M, 0.1, and 7.4, respectively.

Entropy Titration. This technique allows the calculation of equilibrium constant and enthalpy and entropy of reaction from a thermometric titration curve. The procedure was popularized by Christensen et al., who successfully applied it to systems such as single-step protonation reaction[7] and multistep metal–ligand complex formation.[8] The apparatus used for continuous titration was a Tronac Model 450 calorimeter with Model 1040 temperature controller. The net heat changes Q_p's, at different points were calculated according to the standard procedure.

Correction Terms. In addition to the complexation reaction, three other factors that contribute to the measured heat and the method

Table III. Thermodynamic Parameters for the Complexation of Rhodium(II) Carboxylates with Imadazole

Rhodium(II) carboxylate	K_1	K_2	ΔH_1, kcal/mol	ΔH_2, kcal/mol	ΔS_1, eu	ΔS_2, eu
Methoxyacetate	8 776 ± 500	250 ± 22	−7.30 ± 0.03	−3.0 ± 0.3	−6.4 ± 1.5	1.0 ± 1.0
	(6 791 ± 242)[a]	(120 ± 15)[a]				
Acetate	10 500 ± 300	300 ± 64	−7.43 ± 0.03	−3.0 ± 0.3	−6.5 ± 2.0	1.2 ± 2.0
	(6 826 ± 265)[a]	(171 ± 15)[a]				
Propionate	11 660 ± 400	447 ± 52	−8.65 ± 0.02	−5.3 ± 0.2	−10.5 ± 1.0	−5.7 ± 1.0
	(7 792 ± 267)[a]	(156 ± 17)[a]				

[a] Spectrophotometric values from ref 12.

of their evaluation are listed below.

(a) The heat of dilution of the titrant, $Q_{dil,p}$, was computed from a curve obtained by carrying out a separate titration in which the sample solution was replaced by an identical volume of the solvent.

(b) The nonchemical heat, $Q_{hl,p}$, resulting from the stirrer and thermistor heating for each data point, was calculated according to the procedure described by Christensen et al.[9]

(c) Since the reactions were carried out in a phosphate buffer, some heat may result from the change, if any, of the ionization of the acid phosphate

$$H_2PO_4^- = H^+ + HPO_4^{2-} \quad \Delta H_0 = 1.20 \text{ kcal/mol}, \; pK_a = 7.1 \tag{1}$$

and of the deprotonation of the ligand (Im)

$$ImH^+ = H^+ + Im \quad \Delta H_0 = 8.79 \text{ kcal/mol}, \; pK_a = 7.1 \tag{2}$$

To account for the heat contribution from the shift of the above two ionization equilibria, it is first necessary to define the equilibrium expressions for complex formation reactions involving the rhodium(II) carboxylates (designated by M) and the ligand, L

$$M + L = ML \quad K_1 = \frac{[ML]}{[M][L]}; \Delta H_1 \tag{3}$$

$$ML + L = ML_2 \quad K_2 = \frac{[ML_2]}{[M][L]}; \Delta H_2 \tag{4}$$

In the absence of M, the free-ligand concentration, [L], is given by

$$[L] = \frac{K_a}{K_a + [H^+]} C_L \tag{5}$$

where C_L is the total concentration of the ligand. In the presence of M, the total concentration of the deprotonated ligand is

$$C_{L-LH} = [L] + [ML] + 2[ML_2] \tag{6}$$

The difference of (6) and (5) gives the extent of proton ionization of the ligand due to complexation. If the pH changes during the titration, the protonation of the conjugate base of the buffer is not equal to the ionization of the ligand. The change in $[H^+]$ should account for the extent of reaction 1 and the necessary correction should be applied for its accompanying heat change. The experiment with this end in view involved pH titrations using (a) 50 ml of rhodium(II) carboxylate and 2 ml of ligand and (b) 50 ml of buffer and 2 ml of ligand.

Corrected Heat Values. At any point p the corrected heat value, $Q_{c,p}$, is given by

$$Q_{c,p} = Q_p - Q_{hl,p} - Q_{dil,p} - \sum_{i=1}^{n} \Delta n_i \Delta H_i \tag{7}$$

where ΔH_i is the enthalpy of deprotonation of the ligand or the phosphate and Δn_i is the change in the number of moles of the deprotonated ligand or buffer. The ΔH_i's were taken from the literature[10] and the calculations of Δn_i's were carried out as described in the following section.

The total concentrations of rhodium(II) carboxylate and ligand, C_M and C_L, respectively, are

$$C_M = [M] + [ML] + [ML_2] \tag{8}$$

$$C_L = [HL^+] + [L] + [ML] + 2[ML_2] \tag{9}$$

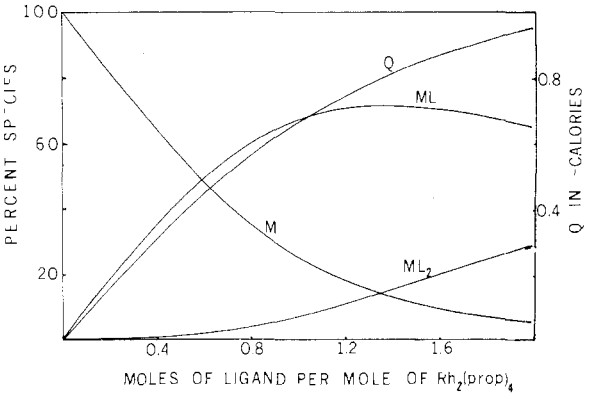

Figure 1. Species distribution and heat change for the titration of 49.98 ml of 1.96×10^{-3} M rhodium(II) propionate with 1.986 ml of 0.098 33 M imidazole.

Combining eq 3, 4, 5, 8, and 9, the following equation which is cubic in [L], can be obtained

$$C_L + [L]\{K_1C_L - K_1C_M - (K_a + [H^+])/K_a\} + [L]^2\{K_1K_2C_L - 2K_1K_2C_M - K_1(K_a + [H^+])/K_a\} - [L]^3K_1K_2(K_a + [H^+])/K_a = 0 \tag{10}$$

The values of C_M, C_L, and $[H^+]$ are known for each data point and those of K_1 and K_2 are given as first approximations. Therefore, eq 10 can be solved for [L], the free-ligand concentration for each data point. This is then used to calculate the values of [M], [ML], [ML_2], and Δn_i. Tables I and II show the corrected terms and the corrected heat for the titration of rhodium(II) propionate with imidazole.

Rigorous Least-Squares Adjustment. The calculation of the four parameters K_1, K_2, ΔH_1, and ΔH_2 and the estimation of their standard deviations were carried out according to the least-squares method of Wentworth.[11]

Results and Discussion

The residuals in the last column of Table II and the σ_{ext} (given at the bottom of Table II as "Residual") indicate that the treatment of the "entropy titration" data by the rigorous least-squares method of Wentworth describes the two-equilibrium, three-component systems quite satisfactorily. The stringent requirements for the applicability of this procedure are (a) there must be a large number of data points and (b) initial guesses for the values of the parameters to be evaluated should not vary greatly from the true values. The instrument artifacts limited the maximum number of data points to 25. The second obstacle was overcome by using values of K_1 and K_2, which were determined from preliminary spectrophotometric measurements, to obtain approximate values of ΔH_1 and ΔH_2. These were then used as the initial guesses of the four parameters.

The corrected heat values and the distribution of the three species M, ML, and ML_2 for the reaction of rhodium(II) propionate with imidazole are shown in Figure 1. All of the thermodynamic parameters for the reaction of the three carboxylates are summarized in Table III. It is apparent from Table III that, for the systems studied, the formation constants determined by the "entropy titration" technique were slightly

larger than those determined by spectrophotometric measurements; but the relative orders among the three carboxylates are the same for both the methods.[4,12] It may also be mentioned in this connection that the calculations of the enthalpy values by taking the two sets of K values did not result in any significant differences. This suggests that the F° function[4] is more sensitive to the equilibrium constants than to the enthalpies of formation, particularly in the low concentration regions at which these measurements were carried out. The use of high concentrations was not feasible in many cases because of the low solubility of the parent carboxylates and some of the adducts.

The log K values for the systems varied from 2 to 4.5, which is well within the range of the applicability of the "entropy titration" technique.[13] The residuals and errors of different parameters as shown in Table III indicate this technique is quite suitable for this system. The "entropy titration" technique, being a more direct method, gives more precise information about the thermodynamics of complex formation, in spite of some of the drawbacks mentioned earlier. The errors associated with K_1, K_2, ΔH_1, and ΔH_2 are in the usually reported range.[10,14] The ΔH_2 values have about 10 times larger errors than ΔH_1 values. This is explainable in terms of smaller concentration of ML_2 than ML_1 (Figure 1).

The equilibrium constants for the formation of complexes from rhodium(II) carboxylates and imidazole show a trend similar to those reported for the 5′-AMP complexes, i.e., propionate > acetate > methoxyacetate.[3] However, the variation in the formation constants among the three carboxylates is smaller. As expected the imidazole complexes are more stable than the corresponding 5′-AMP adducts due to the more basic donor nitrogen of the imidazole ligand. The relatively small and negative ΔS_1 values indicate that complex formation, the replacement of water by the ligand, does not involve any significant change in the cratic part of the entropy terms. As a matter of fact the stability of the adducts was found to be determined exclusively by the relatively large and negative enthalpy changes.

The variation in the thermodynamic parameters for the formation of the 1:1 and 1:2 adducts for the different rhodium(II) carboxylates is not dramatic. However ΔH_1 and ΔH_2 for the rhodium(II) propionate reactions are considerably more negative than for the other two complexes. The values for ΔS_1 and ΔS_2 are also more negative for the rhodium(II) propionate system. Apparently the rhodium(II) carboxylate–solvent interaction is pretty much confined to bonding at the two axial positions. Because of this, adduct formation with imidazole results in the loss of only the axial-bound water to the bulk solvent and thus negative ΔS_1 values. Because of the more lipophilic environment at the two axial sites on the propionate complex, the interaction with water molecules is weaker, and as a result of this the water is more easily displaced by the more basic and lipid-soluble imidazole ligand. This model nicely explains the more negative values of ΔH_1, ΔH_2, ΔS_1, and ΔS_2.

The difference in the stability of the imidazole adducts of the rhodium(II) carboxylates is certainly not large enough to account for the observed variations in the biological activity of these rhodium(II) complexes. However the thermodynamic parameters do indicate that the lipophilic nature of the rhodium(II) carboxylates is a factor in the stability of the adducts formed. Since many large biological molecules such as enzymes have lipophilic regions at or near the active sites, the more lipid-soluble rhodium(II) carboxylates would have a tendency to seek out, and react in, that portion of the molecule.

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Registry No. $Rh_2(O_2CCH_2OCH_3)_4 \cdot 2Im$, 59532-69-3; $Rh_2(O_2CCH_3)_4 \cdot 2Im$, 59532-70-6; $Rh_2(O_2CCH_2CH_3)_4 \cdot 2Im$, 59532-71-7.

Supplementary Material Available: Tables I and II, giving correction terms and corrected heat values together with the residuals for each of the 25 points for the titration of rhodium(II) propionate with imidazole (4 pages). Ordering information is given on any current masthead page.

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