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Complexes of Rhodium(II) Carboxylates with Adenosine 5'-Mono-, 5'-Di-, and 5'-Triphosphates

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Rhodium(II) acetate, propionate, and methoxyacetate form complexes with 5'-AMP, 5'-ADP, and 5'-ATP in aqueous solution at physiological pH. Apparently both the one-to-one complex Rh_2X_4·L and two-to-one complex Rh_2X_4·L_2 (where X = the acetate, propionate, or methoxyacetate ion and L = 5'-AMP, 5'-ADP, or 5'-ATP) are formed in solution. The complexation reaction is accompanied by a change from blue-green to pink. This color change is observed when an oxygen donor ligand at the two axial positions of the rhodium(II) carboxylate is replaced by a nitrogen donor ligand. The stepwise formation constants for these complexes have been determined using a spectrophotometric technique. The thermodynamic stabilities of the complexes formed by the three rhodium(II) carboxylates with 5'-AMP, 5'-ADP, and 5'-ATP were found to be in the order propionate > acetate > methoxyacetate. This correlates well with the observed trend of antitumor activity for these rhodium(II) carboxylates.

Introduction

Recently we reported the results of a study showing the effects of rhodium(II) acetate and rhodium(II) propionate (shown in Figure 1) on the Ehrlich ascites and Leukemia L1210 tumors.[1,2] Survival studies with female Swiss albino mice bearing the Ehrlich ascites tumor revealed that both of these rhodium(II) carboxylates gave rise to a significant increase in survival time. Rhodium(II) propionate was found to be a much more potent drug then the acetate.[2] It was also shown that both rhodium(II) complexes inhibit DNA and RNA polymerases in vitro with the propionate complex being the more potent inhibitor. In addition, recent work has revealed that rhodium(II) methoxyacetate lacks the potent behavior, both as an antitumor agent and as a polymerase inhibitor, that is exhibited by the rhodium(II) acetate and propionate.[3]

Equilibrium dialysis studies showed that rhodium(II) acetate-*1*-^{14}C binds to polyriboadenylate, bovine pancreatic ribonuclease A (RNase), bovine serum albumin, and denatured calf thymus DNA, polyriboguanylate, or polyribocytidylate.[2] These studies indicate that at physiological pH rhodium(II) acetate preferentially binds to compounds containing adenine and certain amino acids that contain nitrogen or sulfur atoms in their side chain.

The disparate effects seen with the rhodium(II) carboxylates with respect to their antitumor properties could be due to differences in the thermodynamic stability of the complexes they form with biologic compounds. If rhodium(II) propionate forms a more stable complex with 5'-AMP than does the acetate or methoxyacetate, it should therefore be a better inhibitor of processes involving 5'-AMP. This argument can be extrapolated to 5'-ADP and 5'-ATP, as well as nucleic acids which contain exposed adenine residues. For this reason, we have determined the formation constants of rhodium(II) acetate, propionate, and methoxyacetate complexes with 5'-AMP, 5'-ADP, and 5'-ATP. This paper reports on this study.

Materials and Methods

Chemicals. Rhodium(II) acetate was purchased from Matthey Bishop, Inc., Malvern, Pa. 19355. Rhodium(II) propionate and methoxyacetate were synthesized by a method previously described.[4] All rhodium(II) carboxylates were recrystallized before use and dried for 1 hr at 80°C in a vacuum oven to remove acetone and water.

5'-AMP, 5'-ADP, and 5'-ATP were obtained from Sigma Chemical Co. The water content of these compounds was determined by thermogravimetric analysis using a Du Pont 950 thermogravimetric analyzer. In order to test for purity the adenosine phosphates were chromatographed on a PEI-cellulose plate in 2% NH_4HCO_3.

Formation Constants from Spectral Data. A spectrophotometric method was used to determine the stepwise formation constants. Because of the blue-green to pink color change produced by the complexation reaction, the extent to which the reaction has proceeded can be monitored by scanning the visible region. A Cary Model 14 spectrophotometer and 10-cm path length cells were used in this study. All formation constants were obtained at 22°C in a 0.1 *M* potassium phosphate buffer (pH 7.5). The first step in the titration procedure was the addition of 20 ml of approximately 4.0×10^{-4} *M* rhodium(II) carboxylate into the spectrophotometer cells. This solution was then titrated with 4×10^{-4} *M* rhodium(II) carboxylate and roughly 1×10^{-2} *M* 5'-AMP, 5'-ADP, or 5'-ATP. After each addition of ligand the absorbance was measured in the region 590–530 nm. All titrations were effected using a Manostat digital pipet. The spectrophotometer cells used have a maximum volume of 27 ml. Therefore, after a volume of 27 ml was reached, the solution in the cell was poured into a clean beaker and 20 ml of this solution was pipetted back into the clean cell. The titration continued until 27 ml was reached, and the above process was repeated until termination of this experiment. Absorbances were recorded from each spectrum at 585, 570, and 540 nm and used in the calculation of the formation constants.

Rigorous Least-Squares Method of Analysis. A suitable model for the rhodium(II) carboxylate–ligand equilibria is

$$M + L \overset{K_1}{\rightleftharpoons} ML \qquad K_1 = \frac{[ML]}{[M][L]} \tag{1}$$

$$ML + L \overset{K_2}{\rightleftharpoons} ML_2 \qquad K_2 = \frac{[ML_2]}{[ML][L]} \tag{2}$$

The terms [M], [L], [ML], and [ML_2] refer to the equilibrium concentrations of the free or unbound rhodium(II) carboxylate, free ligand, 1:1 metal–ligand complex, and 1:2 metal–ligand complex. By utilizing expressions 3 and 4 with eq 1 and 2,

$$C_m = [M] + [ML] + [ML_2] \tag{3}$$

$$C_L = [L] + [ML] + 2[ML_2] \tag{4}$$

it becomes possible to group [L], C_m, C_L, K_1, and K_2 into eq 5 which is cubic in [L]. Therefore, by knowing the total

$$0 = [L]^3(K_1K_2) + [L]^2(2C_mK_1K_2 - K_1K_2C_L + K_1) + [L](C_mK_1 - K_1C_L + 1) - C_L \tag{5}$$

concentration of rhodium(II) species, C_m, and the total concentration of ligand, C_L, and given any K_1 and K_2, eq 5 becomes susceptible to numerical evaluation yielding the equilibrium concentration of free ligand. This, of course, allows calculation of the concentrations of all species at equilibrium as shown in eq 6–8.

Table I

Rhodium(II) carboxylate	Ligand	K_1	K_2	a of ML 585 nm	a of ML 570 nm	a of ML 540 nm	a of ML_2 585 nm	a of ML_2 570 nm	a of ML_2 540 nm	Residual
Methoxyacetate	AMP	1510 ± 50	158 ± 16	172.1 ± 1.7	234.6 ± 1.3	198.7 ± 2.8	103.2 ± 1.8	209.9 ± 2.0	295.6 ± 2.8	0.0033
Acetate	AMP	1893 ± 99	202 ± 14	175.2 ± 1.6	218.5 ± 0.6	205.7 ± 3.0	102.3 ± 2.0	153.0 ± 2.0	269.6 ± 2.0	0.0025
Propionate	AMP	4235 ± 267	344 ± 20	180.7 ± 1.5	221.5 ± 0.6	204.8 ± 3.0	108.9 ± 1.5	163.0 ± 1.6	275.6 ± 1.5	0.0035
Acetate	ADP	1152 ± 50	110 ± 14	166.4 ± 1.5	217.0 ± 0.6	230.8 ± 5.0	93.6 ± 6.0	139.2 ± 8.0	260.8 ± 5.0	0.0036
Acetate	ATP	1883 ± 103	128 ± 15	168.4 ± 1.5	212.2 ± 1.0	204.1 ± 3.1	92.7 ± 4.4	144.8 ± 5.0	279.0 ± 3.9	0.0032
Propionate	ATP	4488 ± 267	329 ± 24	185.3 ± 1.1	223.0 ± 1.0	203.5 ± 3.0	106.0 ± 2.2	161.2 ± 2.1	274.1 ± 2.1	0.0025

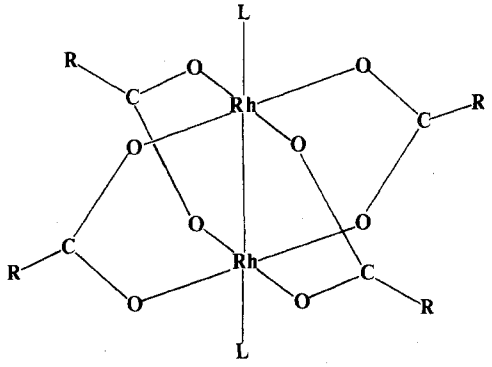

Figure 1. Structure of rhodium(II) carboxylate, where L = 5'-AMP, 5'-ADT, or 5'-ATP.

$$[M] = \frac{C_L - [L]}{K_1[L] + 2[L]^2 K_1 K_2} \quad (6)$$

$$[ML] = K_1[M][L] \quad (7)$$

$$[ML_2] = K_2[ML][L] \quad (8)$$

The complexes M, ML, and ML_2 have absorbance maxima at slightly different wavelengths. This allowed use of the Beer–Lambert relationship to calculate equilibrium concentrations at different M:L ratios. Data from the titration of rhodium(II) carboxylates with various ligands were obtained at 585, 570, and 540 nm. The formation constants and the absorptivities of ML and ML_2 were calculated using the rigorous least-squares method of Wentworth.[5]

The Wentworth method, because it provides an estimate of error for each iterated parameter, lends itself nicely to a problem of this type. The F° function was taken as

$$F^\circ_{\lambda_i} = (A_{\lambda_i}/l)_{\text{exptl}} - (A_{\lambda_i}/l)_{\text{calcd}} \quad (9)$$

$$= (A_{\lambda_i}/l)_{\text{exptl}} - \{a_{[M]\lambda_i}[M] + a_{[ML]\lambda_i}[ML] + a_{[ML_2]\lambda_i}[ML_2]\} \quad (10)$$

where A_{λ_i} is the absorbance at wavelength λ_i and l is the length of the cell. In this method it was necessary to calculate the partial of F° with respect to each parameter being evaluated. This was accomplished numerically for K°_1 and K°_2 according to eq 11. The partials of F° with respect to the absorptivities

$$\frac{\partial F^\circ}{\partial K^\circ_j} = \frac{F^\circ(K_j + \Delta K_j) - F^\circ(K_j - \Delta K_j)}{2(\Delta K_j)} \quad (11)$$

could be solved by direct differentiation of eq 10 yielding

$$\frac{\partial F^\circ}{\partial a_{[ML]\lambda_i}} = [ML] \quad (12a)$$

$$\frac{\partial F^\circ}{\partial a_{[ML_2]\lambda_i}} = [ML_2] \quad (12b)$$

As specified in the Wentworth method, manipulation of the eight partials and the F° summed over all the data points resulted in an 8 × 8 matrix and an 8 × 1 column matrix. Computing the inverse of the 8 × 8 matrix and multiplying this by the 8 × 1 matrix result in the δ matrix which contains the correction for each parameter. If convergence is detected, that is, if the sum of the squares of the residuals does not change significantly between successive iterations, the scheme stops. If convergence is not detected, each parameter is corrected by its corresponding δ from the δ matrix and the process is repeated. The criterion for convergence requires that the change in the sum of squares of residuals between successive iterations be less than $0.01\sigma_0^2$ where σ_0 is the standard deviation in the absorbance values and was calculated to be approximately 3×10^{-3}. The σ_{external} when calculated by the iteration process was found to agree closely with this number. The error on each parameter was calculated by multiplying σ_0^2 by the diagonal of the inverted 8 × 8 matrix. The error of a parameter X which was stored in the ith position of the A matrix is

$$\sigma_{X_i}^2 = \mathbf{A}^{-1}(i, i) \times \sigma_0^2 \quad (13)$$

Parameter errors reported in this paper reflect standard deviations or the square root of equation 13.

Results and Discussion

Rhodium(II) carboxylates form adducts with molecules containing electron donor atoms with bond formation occurring at the two axial positions as shown in Figure 1. The visible spectra of the adducts are very sensitive to the nature of the donor atom.[6,7] Those adducts in which an oxygen atom of the ligand is bound to rhodium are blue-green; those involving nitrogen are pink, rose red, or violet, while those containing sulfur range from burgundy to orange. When the green anhydrous rhodium(II) carboxylates are dissolved in water, the diaquo adduct is formed producing a blue-green solution. The wavelength of maximum absorbance for this complex is 585 nm. When 5'-AMP, 5'-ADP, or 5'-ATP is added to the solution, stepwise and reversible substitution for the two axial water molecules occurs causing a shift in the maximum absorbance to shorter wavelengths. This reaction is accompanied by a color change from blue-green to pink indicating one of the nitrogen atoms of adenine is involved in the bonding.

If acid is added to a solution containing rhodium(II) acetate and 5'-AMP, the color of the solution is blue below pH 4 (where the nitrogen donors are protonated) and pink above pH 4. The color change is rapid and reversible on adjustment of the pH. This indicates that the nitrogen donor atom of the ligand involved in the bonding has a pK_a of approximately 4.

The visible spectra of several solutions containing different ligand-to-rhodium(II) carboxylate ratios are shown in Figure 2. The family of curves seen in Figure 2 is characteristic of a three-component system (three absorbing species) in which the absorbance curves strongly overlap. The absorbing species are the solvated rhodium(II) carboxylate, $Rh_2X_4 \cdot 2H_2O$, the 1:1 adduct, $Rh_2X_4 \cdot (H_2O)(L)$, and the 1:2 adduct, $Rh_2X_4 \cdot 2L$. In these expressions X is acetate, propionate, or methoxyacetate and L is 5'-AMP, 5'-ADP, or 5'-ATP.

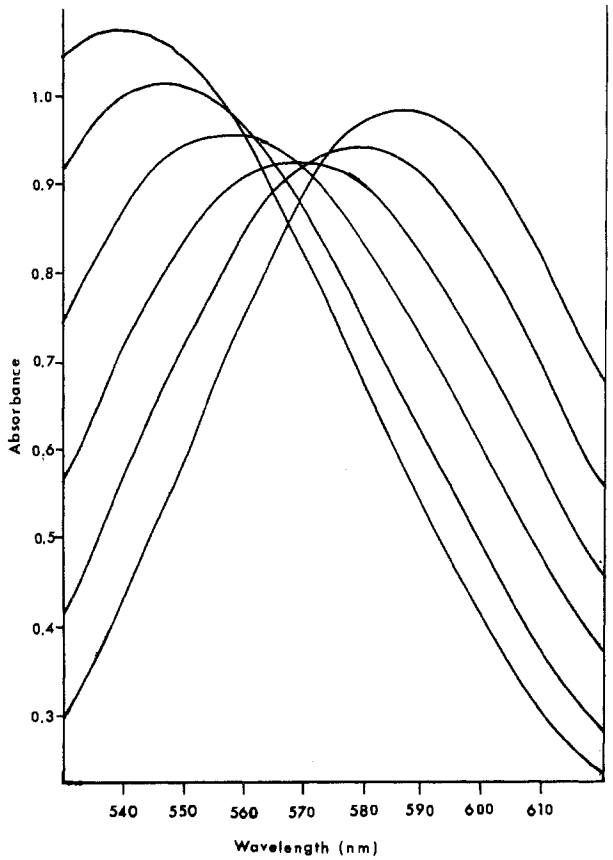

Figure 2. Absorption curves at constant Rh_2Ac_4 concentration and varied AMP concentration. $[Rh_2Ac_4] = 4.4 \times 10^{-4}$ *M*; [AMP] $= 1.5 \times 10^{-4}$–2.5×10^{-2} *M*.

The equilibrium constants shown in Table I are for the reactions

$$Rh_2X_4 \cdot 2H_2O + L \overset{K_1}{\rightleftharpoons} Rh_2X_4 \cdot (H_2O)(L) + H_2O$$

$$Rh_2X_4 \cdot (H_2O)(L) + L \overset{K_2}{\rightleftharpoons} Rh_2X_4 \cdot 2L + H_2O$$

Since the absorptivities of the 1:1 adduct, $Rh_2X_4 \cdot (H_2O)(L)$, and the 1:2 adduct, $Rh_2X_4 \cdot 2L$, were carried as variables in the calculation, the values obtained for these parameters at the three wavelengths used are also given in Table I. Each set of data is an average value from two or more experiments. The residuals, also shown in Table I, are all in the range of 0.003 which indicates that the measured absorbance values fit the model used in the calculations, namely, a three-component, two-equilibrium system.

Three important observations can be made from the data in Table I. One is that the stability of the rhodium(II) propionate complexes is about double that of the rhodium(II) acetate or methoxyacetate complexes, the second is that the number of phosphate groups has little effect on the stability of the complex formed, and the third is that K_2 is quite small considering that the second ligand is not bonding to the same rhodium as the first ligand.

It is difficult to rationalize the observed order of stability for these rhodium(II) carboxylate complexes simply on the basis of the inductive effects produced by the four carboxylate ions. Rhodium(II) methoxyacetate should induce the lowest electron density on the two rhodium ions followed by the acetate and finally the propionate. Because of this, one might expect rhodium(II) methoxyacetate to form more stable adducts with electron donor ligands. The fact that the opposite trend is observed indicates that the inductive effect is countered by other factors, possibly steric or solvation effects.

The order of thermodynamic stability for 5'-AMP, 5'-ADP, and 5'-ATP adducts of the three rhodium(II) carboxylates correlates well with the observed trend in antitumor activity. Axial bond formation with biologically significant molecules may be involved in producing the observed toxicity, anticancer activity, and enzyme inhibitions.

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Registry No. $Rh_2(CH_3OCH_2CO_2)_4 \cdot (AMP)(H_2O)$, 56437-32-2; $Rh_2(CH_3OCH_2CO_2)_4 \cdot 2AMP$, 56437-38-8; $Rh_2(CH_3CO_2)_4 \cdot (AMP)(H_2O)$, 56437-28-6; $Rh_2(CH_3CO_2)_4 \cdot 2AMP$, 56437-35-5; $Rh_2(CH_3CH_2CO_2)_4 \cdot (AMP)(H_2O)$, 56437-31-1; $Rh_2(CH_3CH_2CO_2)_4 \cdot 2AMP$, 56437-37-7; $Rh_2(CH_3CO_2)_4 \cdot (ADP)(H_2O)$, 56437-29-7; $Rh_2(CH_3CO_2)_4 \cdot 2ADP$, 56437-34-4; $Rh_2(CH_3CO_2)_4 \cdot (ATP)(H_2O)$, 56437-30-0; $Rh_2(CH_3CO_2)_4 \cdot 2ATP$, 56437-36-6; $Rh_2(CH_3CH_2CO_2)_4 \cdot (ATP)(H_2O)$, 56437-33-3; $Rh_2(CH_3CH_2CO_2)_4 \cdot 2ATP$, 56437-27-5.

References and Notes

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