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The Reactivity of Tetra-μ-acetatodirhodium(II) with Selected Di- and Tripeptides, Substituted Pyridines and Imidazole Ligands

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Introduction

We have previously demonstrated in our laboratory that several tetra-μ-carboxylato-dirhodium(II) complexes (hereafter referred to as rhodium(II) carboxylates) exhibit significant antitumor activity when tested against several animal tumor systems [1–4]. These results prompted several investigations dealing with the chemical properties and biological effects of these complexes. The carboxylato-bridged rhodium(II) dimer has been shown to form relatively stable mono and bisadducts with a wide variety of ligand types involving donor atoms such as nitrogen, sulfur, oxygen, and phosphorus [5–7]. Binding studies using equilibrium dialysis showed that rhodium(II) acetate binds to polyriboadenylate, bovine pancreatic ribonuclease A, bovine serum albumine, and denatured calf thymus DNA [3]. The rhodium(II) dimer apparently does not bind with double-stranded (native) DNA or the homopolymers of guanine, cytosine, and thymine [3]. Finally, spectral studies using aqueous solutions of rhodium(II) acetate and the common amino acids indicated that axial ligation reactions, when surveyed at physiological pH, were limited to those amino acids that contained a ring-conjugated nitrogen donor atom, such as histidine [8].

Several studies have shown the deleterious effect that rhodium(II) carboxylates exert on macromolecular DNA synthesis, both *in vivo* and *in vitro* [9]. These results are especially puzzling in light of the finding that double-stranded DNA, the usual site of inhibitor interaction, does not axially bind to rhodium(II) acetate. However, in sharp contrast to the inertness of native DNA, proteins were found to bind rhodium(II) carboxylates underscoring the possibility that the mechanism of action could involve protein binding [10]. For example, it is possible that a consequence of adduct formation may be the inhibition of any specialized functions performed by the protein; an example being the catalysis by enzymes. At the very least, this data points out the potential of proteins to serve as the immediate sites of complex deposition. In order to better understand what factors influence the interaction of the dirhodium(II) dimer with the functional moieties present in proteins, we have measured the formation constants of the 1:1 and 2:1 adducts of rhodium(II) acetate with imidazole, substituted imidazoles, as well as several dipeptides that contain both imidazole and α-amino donor functionalities. In order to compare the binding of different ring-conjugated nitrogens several carboxy-substituted pyridines were included as ligands in this study.

Experimental

Chemicals

Tetra-μ-acetatodirhodium(II) was purchased from Matthey Bishop, Inc., Malvern, PA 19355. The complex was recrystallized from an acetone–water mixture. (DL)-histidylhistidine was obtained from Nutritional Biochemical Corp., Cleveland, OH. Sigma Chemical Co. was the source of both L-histidylglycine and β-alanyl-L-histidine. The remaining compounds were of the highest purity available from Aldrich Chemical Co. and were used without further purifications. Prior to solution preparation the water content of the ligands were determined by thermogravimetric analysis.

All formation constants were measured in aqueous solution at 22 °C in either 0.1 *M* potassium phosphate buffer (pH = 7.4) or 0.1 *M* sodium borate buffer (pH = 8.3 or pH = 9.3). Depending upon the pK_a of the donor atoms involved and whether or not competition between $Rh_2(OAc)_4$ and protons for a particular site was a desirable interaction, one or the other buffer and pH was chosen.

Calculations

The numerical methods used to calculate the formation constants for mono- and bis-adduct formation have been described in earlier publications [11, 12]. Briefly, the extent of 1:1 and 2:1 adduct formation was followed by monitoring the visible absorption band centered at 585 nm. The energy of this particular transition is very sensitive to perturbations at the axial ligation site, and undergoes a shift to higher frequencies as the adduct formation reaction proceeds. Although most of the ligands used in this study contain only one nitrogen donor atom, histidylglycine and histidylhistidine have two and three possible binding sites, respectively, at pH's above the $-NH_2$ groups pK_a. The condition equations of the least-squares program were programmed to take into

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TABLE I Summary of the Formation Constants for Mono- and Bisadducts of Tetra-μ-acetatodirhodium(II).

Ligand	K_1	K_2	Donor pK_a[g,h]
3-pyridinecarboxylate[a,f] (nicotinate)	8300 ± 300	310 ± 10	4.85(7.4)
4-pyridinecarboxylate[a,f] (isonicotinate)	12300 ± 600	480 ± 10	4.96(7.4)
3,4-pyridinedicarboxylate[a] (cinchomeronate)	8820 ± 320	267 ± 11	5 20(7.4)
3,5-pyridinedicarboxylate[a]	4490 ± 150	123 ± 9	4 65(7 4)
pyridine[e]	8900 ± 400	249 ± 12	
imidazole[b]	14210 ± 610	249 ± 12	7.11(8.3, 9.3)
N-methylimidazole[b]	19620 ± 1175	377 ± 17	7.20(8.3)
histidine[b]	6880 ± 335	220 ± 15	6.05(8.3)
glycylglycine[c]	471 ± 12	16 0 ± 2 3	8.25(9.3)
glyglyglycine[c]	488 ± 13	24.0 ± 2.4	8.06(9.3)
DL-alanylalanine[c]	247 ± 9	13.0 ± 3.7	8 30(9.3)
histidylhistidine[d]	5990 ± 260	266 ± 21	5 54, 6.80, 7.82(8.3)
alanylhistidine[b]	6400 ± 300	150 ± 40	6.84(7.4)
histidylglycine[b]	7400 ± 400	220 ± 25	5.77, 7 85(8.3)

[a]Ligation occurs through a pyridyl type nitrogen [b]Ligation through an imidazole type nitrogen. [c]Ligation through a primary amine nitrogen [d]Multiple binding sites are available; the last pK_a in the list is the primary amine nitrogen, all others are imidazole nitrogens. [e]Value calculated from results obtained using rhodium(II) methoxyacetate with these ligands and extrapolated to the rhodium(II) acetate system. [f]Values taken from reference 12 [g]The pH at which the aqueous solution was buffered appears within the parenthesis. [h]pK_a values taken from references 14 and 15.

account the pH dependent competition of protonation to form $-NH_3^+$ and the additional ligation reactions. The treatment of multiple binding sites also included Hill plots to determine if the binding sites displayed cooperativity. Finally, the donor sites were assayed relative to one another according to the interaction with rhodium(II) acetate by monitoring the visible band shift at different pH values.

Results and Discussion

Stability constants of the adduct formation reactions of rhodium(II) acetate with various nitrogen donor ligands are summarized in Table I. The formation constants, K_1 and K_2, refer to the equilibrium reactions represented below.

$$Rh_2(OAc)_4(H_2O)_2 + L \overset{K_1}{\rightleftharpoons} Rh_2(OAc)_4(H_2O)L + H_2O$$

$$Rh_2(OAc)_4(H_2O)L + L \overset{K_2}{\rightleftharpoons} Rh_2(OAc)_4L_2 + H_2O$$

In order to clarify the ensuing discussion, pK_a values for each of the ligands were obtained from the available literature [14, 15] and included in Table I.

The data reveal that the acetato complex of dimeric rhodium(II) forms considerably more stable adducts with ligands that contain conjugated ring nitrogens donor atoms, as compared to ligands that contain primary amine nitrogens. Thus, if the ligands are ranked in the order of increasing monoadduct stability the trend is clearly $-NH_2 \ll$ pyridine $<$ imadazole. In the interpretation of the data with respect to the nature of the interaction between the nitrogen base and the rhodium(II) dimer we must keep in mind that differences in solvent interactions with the various nitrogen ligands will also be reflected in the stability constants. However, differences in adduct stability among the ligand types are quite large, particularly between primary amines and the nitrogen heterocycles, and should be the result of the nature of the Rh–N bond.

The stability of the adducts involving glycylglycine, glycylglycylglycine, and alanylalanine are at least an order of magnitude less stable than the dipeptides containing histidine. Among the formation constants for histidine and the dipeptides which contain the histidine moiety there is a relatively small variation, even though in the case of histidylglycine and histidylhistidine there are multiple donor sites on the ligands. With histidylglycine bonding can occur through the imidazole nitrogen or the primary amine and with histidylhistidine through two

imidazoles and the primary amine nitrogen. The visible spectrum of rhodium(II) acetate with histidylglycine and histidylhistidine changes radically as a function of pH in the vicinity of the imidazole's pK_a. If the pH is increased to the pK_a of the $-NH_2$ group no change is observed in the visible spectrum. Such behavior indicates that the $-NH_2$ group cannot compete with the imidazole moiety with respect to rhodium(II) binding. Also, Hill plots for these ligands show that there is no cooperativity displayed by these dipeptides. These results clearly show that the rhodium(II) dimer preferentially binds to the imidazole nitrogen. Another point that should be made is that in the case of histidylhistidine the formation constants could only be calculated by using the total concentration of imidazole moieties in the mathematical treatment of the data. This means that the two imidazole moieties act like independent monodentate ligands in their binding to rhodium(II) acetate. Therefore, the rhodium(II) carboxylates should bind to any exposed imidazole moieties in proteins in preference to non conjugated ring nitrogen donors that are present.

As can be seen from the data in Table I, substitution on the imidazole ring has a considerable effect on the stability of the resulting axial adducts of rhodium(II) acetate. The N-methylimidazole complex is more stable than the unsubstituted imidazole while histidine tends to form a less stable complex. Within this group of ligands, it appears that the trend in stability of the monoadducts is somewhat reflective of the basicity ordering of the imidazole nitrogen. This does not, however, preclude the existence of a significant π contribution to the bonding but does suggest that the σ interaction is the dominant factor in determining bond stability.

We recently reported the formation constants for the axial ligation reaction of rhodium(II) acetate, propionate, and methoxyacetate with pyridine, niacin, and isonicotinic acid [12]. Some of these constants are included in Table I for comparison with the results in this study. In this study the formation constants could not be experimentally determined for the pyridine adduct of rhodium(II) acetate because of the low solubility of the complex. However, using the data from reference [12] it is possible to obtain a good estimate of the K_1 value.

For all three rhodium(II) carboxylates the difference in stability of the 1:1 adducts of the *para* and *meta* substituted pyridine is essentially constant:

$$\Delta K_1 = K_1(\text{isonicotinic acid}) - K_1(\text{niacin}) \sim 4000$$

If we assume that the differences in stability between the pyridine and substituted pyridines are also constant then the K_1 value for the rhodium(II) acetate–pyridine adduct can be estimated by the following method:

$$K_1'(\text{pyr}) = K_1'(\text{nia}) + \frac{[K_1(\text{pyr}) - K_1(\text{nia})]\,[K'(\text{iso}) - K_1'(\text{nia})]}{K_1(\text{iso}) - K_1(\text{nia})}$$

where K_1' and K_1 are the formation constants for the rhodium(II) acetate and methoxyacetate adducts respectively. The value obtained by this method is given in Table I. Unlike the derivatives of imidazole, discussed in the previous paragraph, the trend in the stability of the monoadducts involving pyridine and the pyridine derivatives does not parallel the basicity of the pyridyl nitrogen. Substitution of a carboxylate group in the *para* position substantially increases the monoadduct stability over that of the more basic unsubstituted pyridine. The converse is true for the *meta* substituted pyridine; a slightly decreased stability is observed relative to pyridine. Similar results can now be reported for the adducts involving 3,5-pyridinedicarboxylate and 3,4-pyridinedicarboxylate ions. Although the disubstituted pyridines tend to form less stable complexes than the monosubstituted species, the identical trend is observed with regard to *para* and *meta* substitution. In all cases, formation constants for the *para* substituted pyridine adducts are approximately 4000 M^{-1} larger than the constants for the corresponding *meta* substituted species.

In conclusion, it is clear that the interaction between the rhodium(II) dimer and the nitrogen bases is not a simple acid–base type interaction in which the stability depends only the basicity of the nitrogen donor. It has been suggested [5–7] that the unusual Lewis acid properties of the rhodium(II) carboxylates are due to the π-backbonding capability of the rhodium(II) center. Therefore, ligands which can accept π-electron density from the filled π^* dimer orbitals form more stable complexes than those which can only σ bond. This is an attractive explanation of the observed trends in adduct stabilities reported by various researchers [7, 11–13] in the past and in this study. It is also clear that among the nitrogen bases present in proteins the imidazole nitrogen is the preferred binding site. This interaction is probably significant in the deposition of these complexes in proteins and could be a source of biologic activity.

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