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THE INHIBITION OF MALATE DEHYDROGENASE BY CHLORAMMINE–PLATINUM COMPLEXES

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SUMMARY

Equilibrium association constants have been calculated for various platinum (II) and platinum (IV) complexes.

The association constants were greatest for the dinegatively charged state, regardless of the valence state of the platinum, and the constant decreased considerably as the charge increased. There were no measurable values for positively charged complex states. The conclusion is that the electrostatic charge of the platinum complex is the most important factor causing the inhibition of the enzyme, and the steric differences have only a minor effect, while geometric variation as in the comparison of *cis* and *trans* dichlorodiammine–platinum (II) isomers yields no differences in inhibition.

INTRODUCTION

In a recent article [1] we have shown that both the *cis*- and *trans*-isomers of dichlorodiammine–platinum (II) inhibit a series of dehydrogenase enzymes. Considerable evidence has been produced to show that these inhibition reactions are reversible. These studies have now been extended to all of the possible mononuclear square planar complexes containing ammonia and/or chloride ligands in an attempt to correlate any observable trends in inhibition characteristics with structural features of the platinum complexes. In addition, trace impurities of the other complexes could have been present in the original *cis*- and *trans*-dichlorodiammine–platinum (II) preparations. Under these circumstances the relative inhibition characteristics of the various complexes was of considerable interest. For comparison purposes an octahedral complex of platinum ($K_2Pt\ Cl_6$) and two bromide complexes (Rb_2PtBr_4) and (K_2PtBr_6) were included in this study.

In the past few years, the tumor-inhibiting properties of platinum (II) complexes have been studied. For example, Rosenberg et al. [2] have reported that the electrically neutral complexes are superior in this respect. In addition Renshaw and Thomson [3] have shown that the neutral and negatively charged complexes of platinum (IV) react very differently in *Escherichia coli*. A negative complex, hexachloroplatinate (IV), became almost exclusively bound to proteins, whereas a neutral complex, tetrachlorodiammine–platinum (IV), was more likely to become associated with metabolic intermediates and nucleic acids.

In the current studies it will be shown that binding constants for a series of platinum complexes in an enzyme inhibitor reaction are strongly dependent on the charge of the complex. Consequently, it is believed that this paper should be of interest to individuals engaged in cancer chemotherapy.

PREPARATIONS

K_2PtCl_6 was obtained by dissolving platinum wire in aqua regia and neutraizing the acid with K_2CO_3 [4]. K_2PtBr_6 was prepared by refluxing K_2PtCl_6 with hydrobromic acid followed by neutralization with K_2CO_3 [5]. K_2PtCl_4 was prepared by the reduction of K_2PtCl_6 with a stoichiometric quantity of potassium oxalate [4]. Rb_2PtBr_4 was prepared in a similar manner from K_2PtBr_6 except that the final complex salt was precipitated from a concentrated solution of rubidium bromide [6]. The preparations of the isomers of $Pt(NH_3)_2Cl_2$ have been reported in a previous paper [1]. $KPt(NH_3)Cl_3$ was prepared by refluxing *cis*-$Pt(NH_3)_2Cl_2$ in ammonium acetate solution [7]. $[Pt(NH_3)_3Cl]$ was prepared by the method of Chatt et al [8]. $[Pt(NH_3)_4]Cl_2$ was prepared by refluxing *cis*-$Pt(NH_3)_2Cl_2$ in an aqueous solution of ammonia.

Pig-heart malate dehydrogenase (Lot No. 40 C-4700) was purchased from Sigma Chemical Company and was further purified on carboxymethyl cellulose [9] and recrystallized from ammonium sulfate solution. The purity, molecular weight and protein assay were determined as previously outlined [1]. The enzyme activity assay was then performed according to the procedure of Melius et al. [10], which involved the addition of an aliquot of enzyme solution to a solution containing glycine, oxidized nicotinamide adenine dinucleotide (NAD^+), and malic acid buffered at pH 9.5. The formation of NADH was followed spectrophotometrically at 340 nm and the enzyme activity was expressed in micromoles of NADH formed per $min \cdot ml^{-1}$ of solution.

ENZYME INHIBITION PROCEDURE

The inhibition studies with malate dehydrogenase by the various platinum complexes were performed as described in a previous paper by Friedman et al. [1]. For each particular platinum complex three different concentrations of complex were allowed to equilibrate for 24 h with enzyme solutions in separate experiments. Concentrations were chosen so as to produce three different degrees of inhibition in the range of 20–80% of the original enzyme activity. Every individual experiment was performed in triplicate. Each numerical value of the equilibrium association constant recorded in Table I (and defined in Eqns 1 and 2) is, therefore, the average of different complex concentrations.

$$E + I \rightleftharpoons EI \tag{1}$$

$$K_e = \frac{[EI]}{[E]\,[I]} \tag{2}$$

(where E = enzyme concentration and I = concentration of free platinum complex)

Average values of K_e and the ratio of complex to enzyme concentrations necessary to produce 50% inhibition of the enzyme are listed in Table I.

TABLE I

EQUILIBRIUM ASSOCIATION CONSTANTS FOR PLATINUM COMPLEXES

Enzyme = $1.5 \cdot 10^{-6}$ M, T = 25 °C, pH = 7.0.

Complex	K_e (M^{-1})·10^{-2}	Ratio of Pt/enzyme for 50% inhibition
$PtCl_4^{2-}$	8700	0.76
$Pt(NH_3)Cl_3^-$	290	16
cis-$Pt(NH_3)_2Cl_2$	3.0	2000
trans-$Pt(NH_3)_2Cl_2$	3.2	2100
$Pt(NH_3)_3Cl^+$	no observed enzyme inhibition	—
$Pt(NH_3)_4^{2+}$	no observed enzyme inhibition	—
$PtCl_6^{2-}$	5200	1.30
$PtBr_6^{2-}$	4300	1.54
$PtBr_4^{2-}$	9200	0.73

CONCLUSIONS

From the data in Table I, it is obvious that all of the dinegative complex ions are very efficient enzyme inhibitors. For example, 0.76 moles of $PtCl_4^{2-}$ inhibit 0.50 moles of enzyme even though the complex concentration is only 10^{-6} M. Furthermore, the square planar complexes ($PtCl_4^{2-}$ and $PtBr_4^{2-}$) and the octahedral complexes ($BrCl_6^{2-}$ and $PtBr_6^{2-}$) show very similar inhibition characteristics despite large differences in size and chemical characteristics for these complex ions. These observations would suggest that the dinegative complexes link directly to the enzyme under circumstances where steric considerations are unimportant. Both neutral dichlorodiammine–platinum (II) complexes do inhibit the enzyme, even though much higher complex concentrations are required to produce enzyme inhibition compared to experiments employing dinegative complexes. The only mononegative complex studied ($Pt(NH_3)Cl_3^-$) was intermediate in inhibiting power between the dinegative and neutral complex ions. On the other hand, no significant enzyme inhibition was observed with either of the possitively charged complex ions, even when these species were in a 1000-fold excess compared to the malate dehydrogenase concentration.

All of the data indicates that the electrostatic charge of the platinum complex is the predominant factor which influences the degree of enzyme inhibition. Because of the known stability of platinum–nitrogen bonds [11] it is very probable that any bonding between a platinum complex and the enzyme would recide at the site of a halide ligand in the coordination compound. The very similar equilibrium data for *cis*-$Pt(NH_3)_2Cl_2$ and *trans*-$Pt(NH_3)_2Cl_2$, which have Cl–Pt–Cl bond angles of 90° and 180°, respectively, suggest that steric considerations are unimportant. This implies that only one complex to enzyme bond can be formed. This hypothesis is supported by the previously mentioned observation that dinegative complex ions of different sizes produced comparable equilibrium association constants. All of the data in Table I could be explained conveniently by the formation of a single complex to enzyme bond at a site on the enzyme with net positive charge density.

It is interesting to note that Rosenberg et al. [2] has shown that *cis*-dichlorodiammine–platinum (II) is a superior tumor inhibitor compared to charged platinum

complexes. The work described in this paper suggests that a key factor in this tumor inhibition could be the ability of uncharged complexes to bind weakly and reversibly with proteins. After ingestion by an animal small equilibrium concentrations of such a complex should be distributed throughout its body. Consequently trace quantities of platinum complex should be available at the site of the tumor for considerable periods of time. Negative ions would be expected to be absorbed almost quantitatively by reaction, hence, not be available for tumor inhibition.

A similar mechanism could explain the report by Renshaw and Thomson [3], that a neutral platinum complex could pass through the cell wall and react with metabolic intermediates and nucleic acids, while almost all of the more reactive dinegative complex would be removed by a prior reaction with cytoplasmic protein.

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