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INHIBITION OF MALATE DEHYDROGENASE BY PLATINUM(II) COMPLEXES

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SUMMARY

1 The reversible inhibition of malate dehydrogenase (L-malate NAD^+ oxidoreductase, EC 1 1 1 37) by the tetrabromoplatinate(II) and tetrachloroplatinate(II) ions have been studied through a pH range 6 5–8 5 The equilibrium dissociation constants for both of those ions at low concentrations (1–4 moles of ions per mole of enzyme) were calculated over the given pH range

2 The relative rates of inhibition by these ions were studied at higher concentrations (100 moles of ions per mole of enzyme)

3 It was shown that the inhibition decreased for both ions with increasing pH in the equilibrium and rate experiments

4 Several possible mechanisms of inhibition based on these findings have been proposed The first is based upon the fact that the enzyme becomes more negative with increasing pH, and the repulsive effect with a negative inhibitor ion increases Secondly, the tetrabromoplatinate inhibits the enzyme at a rate of 6–8 times that of the tetrachloroplatinate This parallels previous evidence[23] which showed that bromide ligands are more labile than chloride ligands in platinum(II) complexes by about the same factor Thus, it has been postulated that the inhibition takes place by a replacement of the halide ligand by a nucleophilic group on the enzyme This group may be less accessable as the pH increases causing a reduction in the inhibition

INTRODUCTION

SIEGEL[1] has reported that inorganic anions, such as citrate, inhibit malate dehydrogenase by reversibly dissociating the enzyme into its subunits Both SIEGEL[1] and SILVERSTEIN AND SULEBELE[2,26] studied the inhibition of malate dehydrogenase by *p*-hydroxymercuribenzoate (PHMB) They reported that inhibition took place because a complex was formed between PHMB and free sulphydryl

Relatively high concentrations of heavy metal salts of mercury, lead, and silver are known to inhibit enzymes This was previously shown by OLSSON[3] with the inhibi-

Abbreviation PHMB, *p*-hydroxymercuribenzoate

tion of amylase with a saturated solution of AgCl, and MYRBACK[4] with the inhibition of β-fructofuranosidase by Ag^+ Myrback also suggested that the inhibition was effected by complexing a histidyl residue with the Ag^+ Few enzymes have been studied at low heavy metal ion concentrations, and no general theory has been proposed concerning their mechanisms of inhibition[5]

Recently ROSENBERG *et al* [6] reported that some platinum complexes inhibited the growth of tumors and sarcomas in rats GUTHRIE *et al* [7] then studied the inhibition of leucine amino peptidase with K_2PtBr_4 and proposed a mechanism for the inhibition

The present study deals with the inhibition of malate dehydrogenase with low concentrations of K_2PtCl_4 and Rb_2PtBr_4 at pH values between 6 0–8 5 Possible mechanisms of inhibition are also proposed

MATERIALS AND METHODS

Materials

Pig heart malate dehydrogenase (Lot No 59B 6260) was purchased from the Sigma Chemical Co and was further purified on carboxymethyl cellulose[8] and recrystallized from $(NH_4)_2SO_4$ NAD^+ (Lot No 128B-7340) and NADH (Lot No 69B-6300) were purchased from the Sigma Chemical Co The carboxymethyl cellulose, oxalacetate and L-malate were also purchased from the Sigma Chemical Co Platinum complexes were prepared from metallic platinum obtained from J Bishop and Co , Malvern, Pa All other chemicals were of reagent grade

Determination of the purity of the enzyme

The enzyme purity was determined by three different methods All three showed essentially a homogeneous material

(a) Ultracentrifugation was carried out in a Beckman-Spinco Model E Ultracentrifuge using Schlieren optics, and only a single peak was observed at 59 780 rev / min The enzyme concentration was 7 mg/ml, and the solvent was 0 15 M KCl

(b) Cellulose acetate electrophoresis was run in a temperature cooled (25°) system using a Research Specialties power supply and Sepraphore III cellulose polyacetate strips from the Gelman Instrument Co The electrophoresis was run in several buffers from pH 6–9 and in every instance only a single moving band was observed

(c) Disc gel electrophoresis was run at pH 8 5 on a polyacrylamide gel using a Canalco Analytical Disc Gel apparatus The gel was developed using a phenozine methosulfate–nitroblue tetrazolium stain for enzyme activity and an Amido black for protein concentration The enzyme and protein gels showed a major band and a very faint minor band The latter may be a multiple form of the malate dehydrogenase

Molecular weight determination

Ultracentrifugal analyses for molecular weight determination were also carried out in a Beckman-Spinco Model E Ultracentrifuge The method of YPHANTIS[9] was used, and a partial specific volume of 0 74[8,10] was used in the calculation A value of 63 000 was obtained, and this value was used in the experimental studies This value compares favorably with those obtained by various workers (68 000–70 000 (ref 11), 62 000 (ref 12), 66 000 (ref 8))

Determination of protein content

During the purification of the enzyme the measured absorption of light at wavelengths of 280 nm and 260 nm as described by WARBURG AND CHRISTIAN[13] was used to evaluate the protein concentration. For the binding studies the protein concentration was determined using the method of LOWRY *et al.*[14]

Enzyme assay

The enzyme was assayed in a Hitachi Model 139 spectrophotometer which was temperature controlled at 25°. The cuvettes had a 1-cm path length and contained 3 ml of assay solution. The absorbance increase or decrease was recorded at 340 nm, depending on whether the oxalacetate–NAD^+ or malate–NADH assay system, respectively, was used. The absorbance was converted to μM from the known molar extinction coefficient of NADH (ε_M 6.22 10^3 at 340 nm). The oxalacetate assay was run at pH 7.0 in a 0.1 M phosphate buffer while the malate assay was run at pH 9.5 in a 0.1 M glycine buffer.

Platinum preparation

Rubidium tetrabromoplatinate(II) (Rb_2PtBr_4) and potassium tetrachloroplatinate(II) (K_2PtCl_4) were prepared and analyzed as described previously[15,16].

Determination of optimum enzyme, pyridine nucleotide and substrate concentrations

Enzyme. The enzyme was diluted to various concentrations in 0.1 M phosphate at pH 7.0, and a 5- or 10-μl aliquot was added to the 3-ml assay sample. The enzyme concentration which gave the optimum change in absorbance/min using either the oxalacetate or malate system was determined to be 1.5×10^{-6} M.

Pyridine nucleotide. Using the above enzyme concentration the amount of NAD^+ or NADH was varied and it was observed that a concentration of $2 \cdot 10^{-4}$ M yielded maximum activity over the time period studied (usually no more than 3 min). This concentration was comparable to the $1.7 \cdot 10^{-4}$ M NAD^+ used in the studies of GRIMM AND DOHERTY[17].

Substrate. The enzyme activity was studied as a function of both oxalacetate and malate concentration. The activity increased with increasing malate concentration and reached a maximum at just below 0.01 M. This concentration was used in all future malate assays. The activity using oxalacetate as a substrate reached a maximum near $1 \cdot 10^{-4}$ M and then decreased with further increase in substrate suggesting inhibitory effects[18]. Therefore, the concentration of oxalacetate used in these assays was $1 \cdot 10^{-4}$ M. The influence of both the pyridine nucleotides and substrates on the activity of the enzyme has been previously discussed[12].

Equilibrium studies

Reaction mixtures were prepared by dissolving 3.1 mg of K_2PtCl_4 or 5.3 mg of $Rb_2PtBr_4 \cdot H_2O$ in a known volume of distilled water (from 12.5 to 100 ml) depending on the amount of complex to be used in the experiment. 5 μl of this solution were then pipetted into either 0.25 ml or 0.50 ml of $1.5 \cdot 10^{-6}$ M malate dehydrogenase at the desired pH. The salt concentration for most of the experiments was 0.1 M phosphate, however, in preliminary experiments the salt concentration was varied from 0.01 M to 0.2 M. These experiments were performed to observe the effect of phosphate

on the equilibrium This enzyme had been preincubated in a constant temperature bath of 25 ± 0 1° for at least 30 min From the amount of complex and enzyme the molar ratio was calculated and values ranging from 0 5 to 4 0 Pt complex malate dehydrogenase were used in these experiments If the ratios were lower the difference between the activity of the non-inhibited system and the inhibited system would not be sufficient to give accurate results If the ratios were higher the remaining activity of the inhibited system would be negligible

The enzyme–platinum system was allowed to incubate at 25° in the water bath At various times 5-μl or 10-μl aliquots were removed for assay using either malate or oxalacetate as the substrate Equilibrium was assumed when the activity of the enzyme–platinum system did not change after a given time period Equilibrium was usually attained within 24 h A control enzyme test without added inhibitor was always run along with the other samples It was assumed that the equilibrium was based on the dissociation of the 1 1 complex,

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

where E is the concentration of uncomplexed enzyme, EI is the concentration of enzyme–platinum complex and I is the concentration of uncomplexed platinum From this equation the inhibition equilibrium constant K_I can be defined as

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

If one assumes that $[EI]$ is inactive then each of the concentrations can be evaluated from the activity data and Eqns 3–5

$$[E] = \frac{a_I}{a_U}\, c_E \tag{3}$$

$$[EI] = \frac{a_U - a_I}{a_U}\, c_E \tag{4}$$

$$[I] = \left(\frac{m_R \times a_I}{a_U} - \frac{a_U - a_I}{a_U}\right) c_E \tag{5}$$

Where a_U and a_I are the activities of the uninhibited enzyme (control) and the remaining free enzyme in the enzyme-platinum complex system, respectively, c_E is concentration of enzyme and m_R is the mole ratio, platinum complex malate dehydrogenase

To determine whether the equilibrium had in any way been shifted during the 1–3 min of the assay determination, uninhibited enzyme was added to assay samples which contained inhibitor 10–1000 times the normal concentration described above The activity of the enzyme was not reduced at all suggesting that since inhibition was negligible over this time period and that the reaction was far to the left (enzyme-complex) then dissociation of this complex during the assay would be negligible

Kinetic studies

In order to study the rate of inhibition of malate dehydrogenase by the platinum complexes a high molar ratio of 100 moles of complex per mole of malate dehydrogenase had to be employed These ratios were prepared by first dissolving 3 1 mg of K_2PtCl_4 or 5 3 mg of $Rb_2PtBr_4 \cdot H_2O$ in 1 ml of distilled water which yielded 7 5 mM

solutions Then 5 μl of one of the above aqueous solutions was added to 0 25 ml of enzyme, 1 5 10^{-6} M, to give a solution which was 150 10^{-6} M in platinum complex (100 moles of complex per mole of enzyme) Then 5-μl aliquots of the complex-enzyme solution were removed at selected time intervals and assayed for malate dehydrogenase activity The rate of decrease was recorded, and complete inhibition was usually reached within an hour, however, this depended upon the nature of the complex

Sedimentation rate of platinum–enzyme complex

The $s^0_{20,w}$ value of the completely inhibited enzyme (10 moles $PtCl_4^{2-}$ per mole enzyme) was evaluated using the transport method as described by SCHACHMAN[19]

The measurement of the Schlieren pattern was made in the Spinco Model E Ultracentrifuge on a solution, 7 mg/ml in enzyme and 115 μg/ml in K_2PtCl_4, buffered to pH 7 with 0 10 M phosphate, which had been equilibrated for 24 h at 20°

RESULTS

Equilibrium studies

The initial linear portion of the ΔA *vs* time curve was used to calculate the activity of a given sample Since the platinum complexes in our experiments signifi-

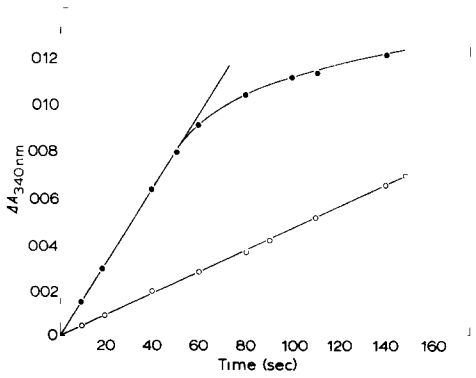

Fig 1 The time dependence rate of conversion of malate to oxalacetate by uninhibited (●) malate dehydrogenase and by K_2PtCl_4 inhibited (○) malate dehydrogenase The experimental conditions were, 1 5 10^{-6} M malate dehydrogenase, 1 5 10^{-6} M $PtCl_4^{2-}$ (previously incubated with malate dehydrogenase for 24 h before addition to substrate mixture), 0 01 M malate, 2 10^{-4} M NAD^+, 0 1 M glycine Each system (both uninhibited and inhibited) was run at 25° and pH 7 0

cantly inhibited the enzyme it was necessary to use relatively high enzyme concentrations For the noninhibited enzyme the curves deviated from linearity between 45 and 60 sec, while the inhibited enzyme maintained linearity between 1 and 3 min depending on the concentration of inhibitor (Fig 1)

The data for the inhibition of the enzyme by K_2PtCl_4 at low molar ratios is summarized in Tables I and II The data is presented for a concentration of 0 1 M phosphate, although concentrations for the range of 0 01–0 20 M phosphate yielded the same results In Table I L-malate was used as the substrate while in Table II the data was obtained by using oxalacetate as the substrate

TABLE I

INHIBITION OF MALATE DEHYDROGENASE, AT pH 7 0 AND 25°, AS A FUNCTION OF K_2PtCl_4 CONCENTRATION

The measurements were made after 24- and 48-h incubation periods The assays were run in a 0 1 M glycine buffered solution at pH 9 5 with 0 01 M malate as the substrate and 2 10^{-4} M NAD^+ as the proton acceptor

Ratio K_2PtCl_4/malate dehydrogenase	*Activity (μM ml^{-1} min^{-1}) × 10^4*		*K_I (M) × 10^7*	
	24 h	*48 h*	*24 h*	*48 h*
0 0	148 ± 3*	147 ± 5		
0 5	110 ± 6	106 ± 10	10 4 ± 3 3	8 9 ± 4 0
1 0	87 ± 5	82 ± 3	12 7 ± 2 4	11 1 ± 1 2
1 5	66 ± 3	63 ± 5	11 6 ± 1 1	10 5 ± 1 1
2 0	51 ± 2	45 ± 2	11 0 ± 0 6	8 9 ± 0 4
			11 9 ± 2 1**	9 8 ± 1 8

* Average deviation of at least 4 samples
** Average deviation of the errors

The quoted results are the average of data obtained from four separate experiments The data in Table I indicate that there is a significant decrease in the activity of the enzyme over the range of K_2PtCl_4 concentrations studies It is also observed that there was less than a 10% decrease in the activity between 24 and 48 h The K_I is calculated for both the 24 and 48 h experiments and is also tabulated in Table I The variation of K_I over a 4-fold concentration range from 0 5 to 2 0 mole ratio of complex malate dehydrogenase is very small, and the averages for both times are calculated The difference between the average K_I of 11 9 10^{-7} M for 24 h and 9 8 10^{-7} M for 48 h is about 18% This difference may be caused by errors obtained in enzyme assays at low complex concentrations An error of 10% in the activity resulted in a 50% error in the K_I at 0 5 mole ratio and about a 30% error in the K_I at 1 0 mole ratio It would have been desirable to study the inhibition at higher mole ratios, however, preliminary studies indicated that equilibrium enzyme activities were so low as to make accurate calculations for equilibrium constants impossible for mole

TABLE II

INHIBITION OF MALATE DEHYDROGENASE, AT pH 7 0 AND 25°, AS A FUNCTION OF K_2PtCl_4 CONCENTRATION

The measurements were made after a 24-h incubation period The assays were run in a 0 1 M phosphate buffered solution at pH 7 0 with 1 10^{-4} M oxalacetate as the substrate and 2 10^{-4} M NADH as the proton donator

Ratio K_2PtCl_4/malate dehydrogenase	*Activity (μM ml^{-1} min^{-1}) × 10^4*	*K_I (M) × 10^7*
0 0	259 ± 2*	
0 5	190 ± 10	11 2 ± 3 0
1 0	151 ± 5	13 0 ± 1 4
1 5	122 ± 6	13 5 ± 1 6
2 0	95 ± 5	11 6 ± 0 1
		12 3 ± 1 5**

* Average deviation from at least four experiments
** Average deviation of the errors

TABLE III

THE EFFECT OF pH ON THE INHIBITION BY K_2PtCl_4

The dissociation constant (K_I) was calculated as a function of the mole ratio K_2PtCl_4 malate dehydrogenase The mole ratio varied from 0 5 to 4 0 as indicated in the table below The measurements were made after a 24-h incubation period at 25° The assay system contained, 0 1 M glycine, 0 01 M malate and 2 10^{-4} M NAD^+ buffered at pH 9 5

pH	*K_I (M) × 10^7 at given mole ratio, K_2PtCl_4/malate dehydrogenase*					
	0 5	*1 0*	*1 5*	*2 0*	*4 0*	*Average*
6 5	7 7	8 5	6 8	5 9		7 2
7 0	10 4	12 7	11 6	11 0		11 9
8 0	23	32	30	28	29	28
8 3	38	43	31	23	31	33

ratios greater than 2 The results in Table II using the oxalacetate as substrate compare quite favorably with the results in Table I There was a 4-fold variation in K_2PtCl_4 concentrations and the inhibition was significant and measurable over this range The calculated K_I was constant within experimental error over this range, and the value of 12 3 10^{-7} M for 24 h incubation compared favorably with the 11 9 10^{-7} M using malate as the substrate Since the K_I values for both the malate and oxalacetate systems are similar it is probable that the platinum complex inhibits the enzyme by a similar mechanism in both cases Consequently, malate was employed as the substrate in all further experiments

The inhibition was then studied as a function of H^+ concentration in the pH range 6 5–8 3 The results for K_2PtCl_4 are given in Table III and for Rb_2PtBr_4 are given in TableIV Outside of this pH range rapid denaturation of the enzyme occurred For example, even at pH 6 5 there was between a 15 and 20% decrease in the initial maximum velocity (without addition of inhibitor) after 24-h equilibration, and at pH 8 3 the initial maximum velocity dropped between 1/2 and 2/3 the original activity after 24-h equilibration The results for any given pH are consistent within experimental error The average of those values, using different concentrations of platinum complex, are given in the last column The error in any given K_I value is of a similar order to the values of K_I given in Tables I and II

From both Tables III and IV it can be observed that an increase in pH in-

TABLE IV

THE EFFECT OF pH ON THE INHIBITION BY Rb_2PtBr_4

The dissociation constant (K_I) was calculated as a function of the mole ratio, Rb_2PtBr_4 malate dehydrogenase This mole ratio varied from 0 5 to 4 0 The conditions for inhibition and assay are given in Table III

pH	*K_I (M) 10^7 at given mole ratio, Rb_2PtBr_4/malate dehydrogenase*					
	0 5	*1 0*	*1 5*	*2 0*	*4 0*	*Average*
6 5	6 0	6 7	5 2	5 0		5 7
7 0		11 1	12 2	9 5		10 9
8 0	16 7	16 4	17 9	13 5	14 7	15 8

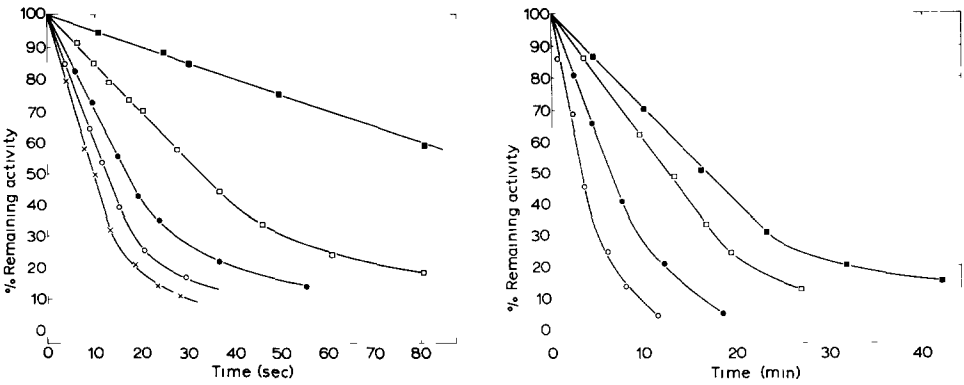

Fig 2 The time dependence rate of inhibition of malate dehydrogenase by a high concentration of K_2PtCl_4 at several pH's pH 6 0 (×), pH 6 5 (○), pH 7 0 (●), pH 7 3 (□) and pH 8 0 (■) The experimental conditions were, 1 5 10^{-6} M malate dehydrogenase, 1 5 10^{-4} M K_2PtCl_4, 2 10^{-4} M NAD^+, 0 01 M malate, 0 1 M glycine at 25°

Fig 3 The time dependence rate of inhibition of malate dehydrogenase by a high concentration of Rb_2PtBr_4 at several pH's, pH 7 0 (○), pH 7 3 (●), pH 8 0 (□) and pH 8 5 (■) The experimental conditions were, 1 5 10^{-6} M malate dehydrogenase, 1 5 10^{-4} M Rb_2PtBr_4, 2 10^{-4} M NAD^+, 0 01 M malate, 0 01 M glycine at 25°

creases the K_I and thus reduces the binding of the complex to the enzyme This effect appears to be slightly more pronounced with K_2PtCl_4 than with Rb_2PtBr_4 The $PtCl_4^{2-}$-enzyme complex is slightly more stable than the $PtBr_4^{2-}$-enzyme complex at any given pH

The apparent fluctuation in K_I is a function of K_2PtCl_4 concentration at pH 8 3 and may be due in part to the fact that only 33–50% of the uninhibited enzyme remained active at that pH, resulting in relatively large experimental errors

The rates of inhibition by Rb_2PtBr_4 and K_2PtCl_4 were studied separately in the presence of excess complex in order to determine if the kinetics of the inhibition reactions exhibited the usual characteristics for platinum(II) substitution reactions in aqueous solution In these studies high concentration of platinum complex malate dehydrogenase (100 1 mole ratio) were added in order to study the rate of inhibition over a short period of time The results are shown in Figs 2 and 3 In Fig 2 the percent of remaining activity of malate dehydrogenase was plotted against time for inhibition by K_2PtCl_4 at different pH's In Fig 3 Rb_2PtBr_4 was the inhibitor From the curves in both figures it is observed that an increase in pH decreases the rate of inhibition of the platinum complex towards the enzyme It was also possible to study the inhibitory effects at lower (pH 6 0) and higher (pH 8 5) pH's as the denaturation of the enzyme was negligible over the time period studied at the given pH of the experiment It was not possible to study the kinetics above pH 8 0 for the K_2PtCl_4 as the rate of inhibition was too slow, and below pH 7 0 for Rb_2PtBr_4 as the inhibition of the enzyme was too rapid

The initial rates of inactivation were constant for both K_2PtCl_4 and Rb_2PtBr_4 during the loss of the first 50% of enzyme activity The initial slopes of each of these lines were measured and the data is reported in Table V

The $s^0_{20,w}$ of the K_2PtCl_4 inhibited enzyme was found to be 4 1 which was identical with that of the uninhibited enzyme

TABLE V

RATE OF INITIAL INHIBITION OF MALATE DEHYDROGENASE BY K_2PtCl_4 AND Rb_2PtBr_4 AT VARIOUS pH's

The metal complexes are 100-fold mole/mole in excess of the enzyme concentration

Pt complex	*Rate of inactivation at pH**					
	6 0	*6 5*	*7 0*	*7 3*	*8 0*	*8 5*
K_2PtCl_4	5 0	3 8	2 8	1 5	0 5	<0 5
Rb_2PtBr_4	>30	30	17	8 6	4 2	3 2

* Expressed as the initial slope (% remaining activity/time in min) from Figs 1 and 2

DISCUSSION

The data presented in this paper shows that two square planar complexes of platinum, with halide ions as ligands, are inhibitors of malate dehydrogenase Since at low or moderate concentrations of platinum complex the extent of inhibition did not change during the assay procedure it was possible to determine the amount of inhibition by first externally inhibiting the enzyme and then adding it to the substrate mixture

The validity of representing K_I as a true equilibrium constant, as defined in Eqn I, depends solely on whether equilibrium had been reached during the time allotted Our studies showed that there was a slow decrease in activity up until 24 h of equilibration Between 24 and 48 h there was only a small further decrease in activity Even if it were to be assumed that equilibrium had not been reached after 24 h the approach to equilibrium must be extremely slow and the calculated K_I would still be a good measure of the inhibitory properties of the enzyme under the conditions studied Comparison of the values 11 9 10^{-7} M for the K_I of K_2PtCl_4 at pH 7 0 or 10 9 10^{-7} M for the K_I of Rb_2PtBr_4 at the same pH with the various values of K_m using malate as a substrate (8 10^{-4}–9 10^{-4} M (ref 8), 5 10^{-4}–10 10^{-4} M (ref 17), 3 10^{-4}–4 10^{-4} M (ref 20) or using oxalacetate as a substrate (2 10^{-5}–5 10^{-5} M (ref 21), 3 10^{-5}–5 10^{-5} M (ref 8)) shows the platinum halide complexes to be significant inhibitors

Both $PtCl_4^{2-}$ and $PtBr_4^{2-}$ undergo aquation reactions in aqueous solution according to the following equation

$$PtX_4^{2-} + H_2O \rightleftharpoons PtX_3(H_2O)^- + X^- \quad (6)$$

At 25° the half-line for aquation of $PtCl_4^{2-}$ is 4 6 h (ref 25) and the $PtBr_4^{2-}$ has been shown to aquate more rapidly[15] Consequently, both halide complexes were in essential equilibrium with their aquo forms at the time that the first inhibition measurement was performed after 24 h throughout the employed pH range The calculated inhibition equilibrium constants (K_I) refer to the total platinum concentrations in particular solutions The tetrahalide ions and/or their aquo forms could be the actual reactive species in solution

The fact that the inhibition using either malate or oxalacetate as the substrate in our studies yielded similar values for K_I suggests that inhibition is not affected by the substrate which is used in these experiments Also, it is implied that the deactivation mechanism is similar for both substrates The main reason for using malate as the substrate in most of our experiments was because the enzyme activity is quite

sensitive to oxalacetate concentration as shown by SIEGEL AND ENGLARD[12] These workers demonstrated, and we verified in preliminary experiments, that at high concentrations of malate the activity is independent of malate concentration while the oxalacetate-activity curve passes through a maximum The fact that the variation of phosphate ion from 0 01 M to 0 20 M did not effect the equilibrium suggests that the buffer salt did not interfere with the platinum–enzyme complex

The sedimentation rate constants ($s^0_{20,w}$ of 4 1) for both the inhibited and uninhibited enzyme were identical within experimental error These observations indicate that enzyme dissociation is not involved during inhibition by the platinum complex SIEGEL[1] reported anions such as citrate inhibited malate dehydrogenase by dissociation of the subunits while the heavy metal cation PHMB also inhibited malate dehydrogenase but not through dissociation He suggested that the probable mechanism of inhibition was through complexation of the free sulphydryl groups, however, complete inhibition did not occur until 12 equivalents of PHMB per mole of malate dehydrogenase were added

The effect of the pH on the inhibition suggests several possible mechanisms First of all, the increase in pH from 6 5 to 8 3 decreases the binding of the platinum to the enzyme, and also increases the negative charge on the enzyme for which the isoelectric pH is between 6 1–6 4 (ref 22) Because the platinum complexes are negatively charged, this suggests that electrostatic effects may influence the binding of the platinum compound to the enzyme

Previous experiments by TEGGINS and co-workers[16,23] showed that bromide ligands are more labile than chloride ligands in substitution reactions of platinum(II) complexes For example, amine molecules can displace a bromide ligand from a platinum(II) complex in aqueous solution approximately seven times faster than a chloride ligand would be displaced under comparable circumstances It is interesting to note that the data in Table V does show that the rate of inhibition of the enzyme by $PtBr_4^{2-}$ is indeed about 6–8 times faster than the rate of inhibition by $PtCl_4^{2-}$ under similar conditions Consequently, it is possible that the rate-determining step in the enzyme inhibition reaction could involve displacement of a halide ligand in the metal complex by a nucleophilic group on the enzyme Such a reaction would result in the formation of a platinum–enzyme covalent bond which could easily inhibit the enzyme activity The group may become less accessable with increasing pH leading to a reduction in inhibition This could be due to a conformational change Although the described study gives no indication of the nature of the binding group, DICKENSON *et al* [24] found that $PtCl_4^{2-}$ binds selectively to sulfur in methionine side chains in six crystalline proteins

In conclusion, it has been observed that both $PtCl_4^{2-}$ and $PtBr_4^{2-}$ are significant inhibitors for malate dehydrogenase In both instances binding constants increase with increasing acidity in the pH range 8 5–6 Indirect evidence suggests that enzyme inhibition could result from the displacement of a halide ligand in the metal complex by a nucleophilic group on the enzyme with the formation of an inert platinum–enzyme bond

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