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THE EFFECTS OF PLATINUM COMPLEXES ON SEVEN ENZYMES

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Summary

The effects of K_2PtCl_4, *cis*-$Pt(NH_3)_2Cl_2$, and *trans*-$Pt(NH_3)_2Cl_2$ on the activities of glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, dihydrofolate reductase, fructose-1,6-bisphosphate aldolase, catalase, tyrosinase, and peroxidase have been investigated. All of the enzymes which are thought to have essential sulfhydryl groups (glyceraldehyde-3-phosphate dehydrogenase, aldolase, and glucose-6-phosphate dehydrogenase) were significantly inhibited by K_2PtCl_4. The other four enzymes studied are not known to have essential sulfhydryl groups, and were not significantly affected by the Pt compounds under the conditions employed. Glyceraldehyde-3-phosphate dehydrogenase was the only enzyme inhibited by all three Pt compounds tested, with K_2PtCl_4 being the most effective and *cis*-$Pt(NH_3)_2Cl_2$ the least effective inhibitor. Semilogarithmic plots of residual activity versus inhibition time indicated that the inhibition reactions were not simple first-order processes, except for the inhibition of glucose-6-phosphate dehydrogenase by K_2PtCl_4 which appeared to be first-order with respect to enzyme concentration.

Introduction

Certain platinum ammine complexes have been shown to exhibit antitumor activity [1]. It appears that the major effect of these compounds on tumor cells is to inhibit DNA synthesis by cross-linking the complimentary strands of nucleic acids [2]. However, other researchers have reported that the *cis* and *trans* isomers of $Pt(NH_3)_2Cl_2$ are inhibitors of certain enzymes [3]. On the basis of studies with nicotinamide nucleotide dehydrogenases, it has been suggested that enzymes containing reactive sulfhydryl groups are particularly sensitive to

inhibition by Pt compounds [4]. Inhibition of thymidylate synthase by *trans*-$Pt(NH_3)_2Cl_2$ has been shown to involve an interaction of enzyme sulfhydryl groups with the Pt complex [5].

This paper describes the effects of platinum compounds on the activities of seven enzymes. The results support the earlier suggestion that inhibition of enzymes by Pt complexes results from reactions with catalytically important sulfhydryl groups.

Materials

The following enzymes were purchased from Sigma Chemical Company and used without further purification: Fructose-1,6-bisphosphate aldolase (EC 4.1.2.13) from rabbit muscle; catalase (EC 1.11.1.6) from bovine liver, glucose-6-phosphate dehydrogenase (EC 1.1.1.49) from yeast; glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) from rabbit muscle; peroxidase (EC 1.11.1.7) from horseradish. Tyrosinase (EC 1.14.18.1) was obtained from potatoes [6]. Dihydrofolate reductase (EC 1.5.1.3) was purified from a methotrexate-resistant strain of *Lactobacillus casei* [7]. The preparation of the platinum complexes has been previously described [3].

Dihydrofolate was produced by reducing folic acid with dithionite [8]. The product was precipitated, washed, lyophilized, and stored as described by Donato et al. [9].

Fructose-1,6-bisphosphate, NADPH, DL-glyceraldehyde-3-phosphate, and DL-dihydroxyphenylalanine were purchased from Sigma Chemical Company. NAD and NADP were from Calbiochem; D-glucose 6-phosphate from Nutritional Biochemicals Corporation, and guaiacol (2-methoxyphenol) from Eastman Organic Chemicals. All other chemicals were reagent grade.

Methods

Enzyme assays

Aldolase activity was determined by following hydrazone formation spectrophotometrically at 240 nm in a reaction mixture containing hydrazine [10].

Catalase activity was measured by following the disappearance of hydrogen peroxide spectrophotometrically at 240 nm [11].

Dihydrofolate reductase was assayed by following the decrease in absorbance at 340 nm resulting from the oxidation of NADPH and the reduction of dihydrofolate [7].

Glucose-6-phosphate dehydrogenase activity was followed by measuring the increase in absorbance at 340 nm resulting from the formation of NADPH.

Glyceraldehyde-3-phosphate dehydrogenase activity was measured by the increase in absorbance at 340 nm resulting from the reduction of NAD [12].

Peroxidase was assayed by measuring the change in absorbance at 470 nm that accompanies the oxidation of guaiacol by H_2O_2 [13].

Tyrosinase was assayed by measuring the change in absorbance at 420 nm resulting from the conversion of 3,4-dihydroxyphenylalanine (DOPA) to DOPA-chrome [6].

Enzyme inhibition by Pt complexes

10 mM stock solutions of the Pt complexes were prepared by dissolving the crystalline solids in 0.08 M sodium pyrophosphate buffer (pH 8.8) and were stored at 5°C in the dark. Dissolution of the *cis*- and *trans*-$Pt(NH_3)_2Cl_2$ required heating the suspensions to 60°C with vigorous shaking for about 10 min.

The inhibition reactions were carried out at 25°C and were started by mixing enzyme and inhibitor solutions to give the inhibitor concentrations shown in Table I and enzyme concentrations as follows: catalase, 173 I.U./ml; tyrosinase, 0.144 ΔA_{420}/min per ml; peroxidase, 0.812 I.U./ml; dihydrofolate reductase, 0.099 I.U./ml; glucose-6-phosphate dehydrogenase, 0.314 I.U./ml; glyceraldehyde-3-phosphate dehydrogenase, 0.455 I.U./ml; and aldolase, 2.22 I.U./ml. The progress of the inhibition reaction was followed by determining the residual enzyme activity in aliquots withdrawn at varous times and immediately added to the assay mixtures. The volumes of the aliquots were chosen to give conveniently measured reaction rates and the dilution factors of the enzymes under the reaction conditions were catalase, 151; tyrosinase, 12; peroxidase, 30; dihydrofolate reductase, 23; glucose-6-phosphate dehydrogenase, 30; glyceraldehyde-3-phosphate dehydrogenase, 60; and aldolase, 60. The activities of enzyme solutions containing no inhibitor were monitored during the course of each experiment.

Results

The results (Table I) show that only glucose-6-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and aldolase were significantly inhibited by K_2PtCl_4, and that only glyceraldehyde-3-phosphate dehydrogenase was inhibited by the *cis* and *trans* isomers of $Pt(NH_3)_2Cl_2$.

Fig. 1 depicts the progress of the inhibition that resulted when glyceraldehyde-3-phosphate dehydrogenase (Fig. 1A), aldolase (Fig. 1B), and glucose-6-phosphate dehydrogenase (Fig. 1C) were incubated in the presence of K_2PtCl_4. The inhibition experiments were carried out under pseudo-first-order conditions; however, the curved semilogarithmic plots obtained with glyceraldehyde-3-phosphate dehydrogenase and aldolase indicate that the inhibition of these two enzymes by K_2PtCl_4 is not a simple first-order process. The data in Fig. 1A suggest that the inhibition of glyceraldehyde-3-phosphate dehydrogenase by the platinum compounds may involve several processes that occur at different rates. The inhibition plots for this enzyme at three different inhibitor concentrations appear to become linear after about 1—1.5 h inhibition time, which suggests that the slowest process is first-order with respect to enzyme concentration. Extrapolation of the linear portion of the three curves fails to give a common intercept on the ordinate, suggesting that the inhibition mechanism is more complex than two first-order processes. It is also consistent with a system where the inhibitor concentration does not remain constant.

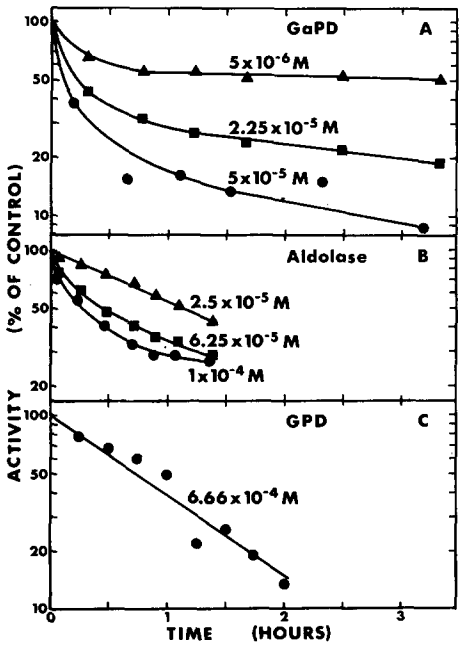
The inhibition plots for aldolase (Fig. 1B) are linear at the lowest inhibitor concentration, but become non-linear at the two higher inhibitor concentrations. This could result from conformational changes induced by the modification of sulfhydryl groups not at the active site, as suggested by other workers

TABLE I

ENZYME INHIBITION BY PLATINUM COMPOUNDS

Enzyme	K_2PtCl_4			*cis*-$Pt(NH_3)_2Cl_2$			*trans*-$Pt(NH_3)_2Cl_2$		
	Conc. (μM)	Time (min)	Act. * (%)	Conc. (μM)	Time (min)	Act. * (%)	Conc. (μM)	Time (min)	Act. * (%)
Catalase	100	75	88	100	75	100	100	75	96
Tyrosinase	640	105	96	1600	120	87	1600	120	100
Peroxidase	2000	135	98	1400	90	100	2.15	90	98
Dihydrofolate reductase	400	20	100	400	128	88	400	128	86
Glucose-6-phosphate dehydrogenase	1330	60	16	467	90	100	667	75	92
Glucose-6-phosphate dehydrogenase	666	120	14						
Glyceraldehyde-3-phosphate dehydrogenase	50	192	9	100	151	45	100	162	13
Glyceraldehyde-3-phosphate dehydrogenase	22.5	200	19						
Glyceraldehyde-3-phosphate dehydrogenase	5	200	51						
Aldolase	100	82	27	100	64	87	100	64	88
Aldolase	62.5	83	29						
Aldolase	25	83	43						

* Activity is the residual activity remaining after the inhibition times indicated, and is expressed as a percent of the enzymic activity in reaction mixtures lacking Pt compounds.



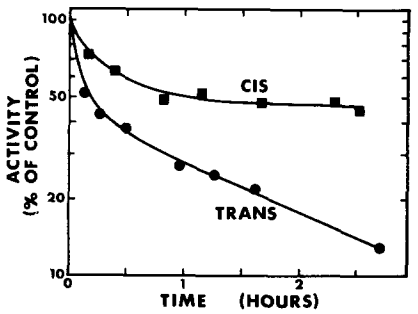


Fig. 1. Inhibition (at 25°C) of glyceraldehyde-3-phosphate dehydrogenase (GaPD) (A), aldolase (B), and glucose-6-phosphate dehydrogenase (GPD) (C) by K_2PtCl_4. The activity is plotted on a log scale, and is the residual activity remaining after the inhibition times indicated, expressed as a percent of the enzymic activity in reaction mixtures lacking the Pt complex. The concentrations of K_2PtCl_4 in the inhibition reaction mixtures are indicated in the figure.

Fig. 2. Semilogarithmic plot showing the inhibition (at 25°C) of glyceraldehyde-3-phosphate dehydrogenase by two isomers of $Pt(NH_3)_2Cl_2$. The inhibition reaction mixtures contained 100 μM *cis*-$Pt(NH_3)_2Cl_2$ (■), and 100 μM *trans*-$Pt(NH_3)_2Cl_2$ (●). Activity is the residual activity remaining after the inhibition times indicated, and is expressed as a percent of the enzymic activity in reaction mixtures lacking Pt compounds.

[14]. These data are also consistent with a system where the reaction with the platinum complex is first-order but does not completely inactivate the enzyme. In that case, it appears that aldolase retained about 25% of its original activity after the reaction with the inhibitor was complete.

The data in Fig. 1C appear to be reasonably well described by a straight line, suggesting that the inhibition of glucose-6-phosphate dehydrogenase by K_2PtCl_4 is probably a first-order process.

Fig. 2 is a semilogarithmic plot showing the progressive inhibition of glyceraldehyde-3-phosphate dehydrogenase by the *cis* and *trans* isomers of $Pt(NH_3)_2Cl_2$. The data show that the *trans* isomer was a more effective inhibitor when the two isomers were tested under identical conditions. The plots are curved for about the first hour of inhibition time, after which they both become linear. The extrapolated linear portions of both curves have nearly the same ordinate intercept. These data are consistent with a system consisting of two first-order processes where the rate of the first (fast) process is about the same with either isomer but the rate of the second (slow) process is faster with the *trans* isomer than with the *cis* isomer.

Discussion

Several enzymes have been previously shown to be inhibited by platinum compounds. Although Pt complexes react with a number of amino acid side chains in proteins including those of methionine, cysteine, histidine, arginine, glutamic acid, and aspartic acid [15], in only one case has the reacting group responsible for the inhibition of an enzyme been identified; that is thymidylate synthase, where the Pt compound apparently reacted with sulfhydryl groups [5]. The nicotinamide nucleotide dehydrogenase, malate dehydrogenase, lactate dehydrogenase, and alcohol dehydrogenases are all inhibited by platinum complexes [3], and all have catalytically important sulfhydryl groups [16—18]. The data presented here are also consistent with the concept that enzyme inhibition by Pt compounds is primarily due to reaction with essential sulfhydryl groups. Glyceraldehyde-3-phosphate dehydrogenase is known to have an essential sulfhydryl group [19], and is readily inhibited by K_2PtCl_4 and both isomers of $Pt(NH_3)_2Cl_2$. A sulfhydryl group participates in the proposed reaction mechanism of aldolase [14], one of the enzymes found to be inhibited by Pt compounds. Glucose-6-phosphate dehydrogenase from several sources is believed to have an essential sulfhydryl group [20], and in this study, the enzyme from yeast was inhibited by K_2PtCl_4. Since all of the nicotinamide nucleotide dehydrogenases examined so far have been inhibited by Pt compounds, it is interesting that dihydrofolate reductase from *Lactobacillus casei*, which contains no cysteine [7], was unaffected by the Pt complexes used in this study. The other oxidoreductases, viz., catalase, peroxidase, and tyrosinase, are not known to have essential sulfhydryl groups and were not inhibited by the Pt compounds tested. Thus, it appears that enzyme inhibition by Pt compounds may be primarily due to reaction with essential sulfhydryl groups. The sole exception is leucine aminopeptidase which is not known to contain essential sulfhydryl groups, but was inhibited by Pt complexes [21].

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