Tables III and IV and compared with the values obtained under the same experimental conditions for two of the compounds described by Cavallito and coworkers.[4]

References and Notes

(1) P. Franchetti, M. Grifantini, and S. Martelli, *J. Med. Chem.*, **18**, 839 (1975) (paper 9).
(2) J. C. Smith, C. J. Cavallito, and F. F. Foldes, *Biochem. Pharmacol.*, **16**, 2438 (1967).
(3) C. J. Cavallito, H. S. Yun, J. C. Smith, and F. F. Foldes, *J. Med. Chem.*, **12**, 134 (1969).
(4) C. J. Cavallito, H. S. Yun, T. Kaplan, J. I. Smith, and F. F. Foldes, *J. Med. Chem.*, **13**, 221 (1970).
(5) B. R. Baker and R. E. Gibson, *J. Med. Chem.*, **14**, 315 (1971).
(6) B. R. Baker and R. E. Gibson, *J. Med. Chem.*, **15**, 639 (1972).
(7) T. Karlsson, K. E. Stensiö, and K. Wahlberg, *Acta Chem. Scand.*, **27**, 2244 (1973).
(8) P. Franchetti, M. Grifantini, S. Martelli, and M. L. Stein, *Farmaco, Ed. Sci.*, **29**, 309 (1974).
(9) N. Nischimoto and T. Nakashima, *Yakugaku Zasshi*, **81**, 88 (1961); *Chem. Abstr.*, **55**, 134201 (1962).
(10) J. E. Blackwood, C. L. Gladys, K. L. Loening, A. E. Petrarca, and J. E. Rush, *J. Am. Chem. Soc.*, **90**, 509 (1968).
(11) Y. Ashani, H. Edery, J. Zahavy, W. Kunberg, and S. Cohen, *Isr. J. Chem.*, **3**, 133 (1965).
(12) G. G. Kleinspehn, J. A. Jung, and S. A. Studniarz, *J. Org. Chem.*, **32**, 460 (1967).
(13) S. E. Forman, *J. Org. Chem.*, **29**, 3323 (1964).
(14) A. P. Phillips, *J. Org. Chem.*, **12**, 333 (1947).
(15) J. L. R. Williams, S. K. Webster, and J. A. Van Allan, *J. Org. Chem.*, **26**, 4893 (1961).
(16) L. M. Jackman and S. Sternhell, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Pergamon, Oxford, 1969, p 301.
(17) F. L. Pyman, *J. Chem. Soc.*, **109**, 186 (1916).
(18) P. Fournari, P. de Cointet, and E. Laviron, *Bull. Soc. Chim. Fr.*, 2438 (1968).
(19) P. Franchetti, M. Grifantini, S. Martelli, and M. L. Stein, *J. Med. Chem.*, **17**, 18 (1974).
(20) R. E. Gibson and B. R. Baker, *J. Med. Chem.*, **17**, 1290 (1974).

Studies of Platinum Complex Inhibition of Leucine Aminopeptidase

Paul Melius*

Department of Chemistry, Auburn University, Auburn, Alabama 36830

and C. A. McAuliffe

Department of Chemistry, The University of Manchester Institute of Science and Technology, Manchester, England.

Received April 28, 1975

The inhibition of swine kidney leucine aminopeptidase by *N*-alkyl-substituted ethylenediamine liganded dihaloplatinum chelates has been investigated. The rate of this inhibition at 37° is considerably less than that for the platinum tetra- and hexahalo complexes and also the ethylenediamine dihalo chelates. Only mixed inhibition was observed in these studies. For the time studies used here little if any inhibition occurred at room temperature. L-Methionine and L-Ala-L-Met did not reverse the platinum chelate inhibition nor did they prevent it.

In earlier studies, Guthrie et al.[1] reported on the inhibition of swine kidney leucine aminopeptidase (E.C. 3.4.1.1) by halide complexes of platinum. The tetrabromoplatinate ion (5×10^{-3} *M*) inhibited the peptidase completely within 1 hr at 38° while the ethylenediamine dibromo complex required 50 hr of incubation at 38° and the same concentration of inhibitor to produce 80% inhibition of the peptidase. Melius et al.[2] presented evidence for the $PtBr_3(H_2O)^-$, aquoplatinum(II) complexes, actually acting as the enzyme inhibitor so that the rate of inhibition would depend on the rate of aquation of the Pt complexes used. Of course, if the Pt complex solutions are sufficiently aged, the rate of inhibition will depend then only on the rate of attack of nucleophilic groups in the protein on the aquoplatinum complex. The final extent of inhibition at equilibrium will depend on the K_i.

$$E + Pt(a_2NCH_2CH_2Nb_2)Cl_{x-1}\cdot H_2O \rightleftarrows E\cdot Pt(a_2NCH_2CH_2Nb_2)Cl_{x-1}\cdot(H_2O)$$

$$K_i = \frac{[E][Pt(a_2NCH_2CH_2Nb_2)Cl_{x-1}\cdot H_2O]}{[E\cdot Pt(a_2NCH_2CH_2Nb_2)Cl_{x-1}\cdot(H_2O)]}$$

Various studies by Melius,[2] Friedman et al.,[3] and Teggins and Friedman[4] indicate the haloplatinum and haloammine Pt complexes are potent general inhibitors of certain enzymes. As a continuation of these studies this report investigates the modification of the ammine ligand with various alkyl groups and the effect on inhibition of leucine aminopeptidase. Whereas many other laboratories have concentrated their efforts on the interaction of Pt complexes with nucleic acids, our emphasis has been on the Pt–protein (enzyme) interactions. Many platinum complexes have been evaluated as anticancer agents, examples of which are dichloro(1,2-diaminocyclohexane)platinum(II),[5] *cis*-dichlorodiammineplatinum(II),[6] and *cis*-dichlorobis(cyclopentylamine)platinum(II)[7] and have been found to be quite effective. Complexes **2**, **5**, and **6** reported here have been shown to be active against leukemia L1210.[8] The other complexes are currently being tested at the Chester Beattie Institute and the Imperial Cancer Research Fund.

From the various studies performed in this laboratory, it is well established that the platinum complexes react generally with proteins.[1-4,12] It is important that more specific platinum complex agents should be designed to reduce their toxic interactions with enzyme systems of the normal cells. Also the rate of reactions with proteins should be decreased so that the platinum complexes will be able to get to the appropriate tumor sites without producing toxic reactions in normal tissues.[9]

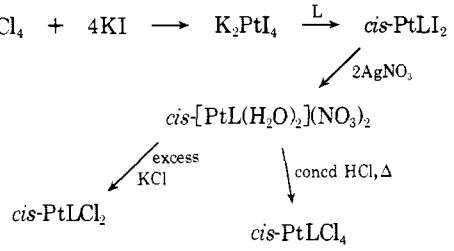
Experimental Section

The substituted ethylenediamine ligands were supplied by the Ames Chemical Co. and the platinum salts by Johnson Matthey Ltd.

Preparation of the Complexes. Platinum(II) Complexes. Potassium iodide (9.6 mmol) was added to potassium chloroplatinate(II) (2.4 mmol) in water (ca. 30 cm^3) and the solution was stirred. After 10–15 min the diamine (2.4 mmol) was added and stirring was continued for 24 hr. The product formed was filtered off and washed with water (15 cm^3). It was subsequently suspended in water (25 cm^3), silver nitrate (4.8 mmol) was added, and the mixture was stirred for 24 hr. The precipitate of AgI was filtered off after addition of 0.1 *M* HCl. Excess potassium chloride was

then added to the solution, which was gently warmed until a precipitate began to form. After cooling in ice for 24 hr, the solid was filtered, washed with water (15 cm^3) and diethyl ether (25 cm^3), and dried in vacuo. See Scheme I.

Scheme I



Platinum(IV) Complexes. These were prepared in a similar manner to the platinum(II) complexes, but in the final stage concentrated hydrochloric acid (instead of KCl) was added to the solution and boiled until chlorine gas was evolved. See Scheme I. The resulting precipitate was worked up in the usual manner.

Leucine Aminopeptidase. The method of Moseley and Melius[10] as modified by Chen[11] was used to prepare aqueous enzyme solutions. Protein concentrations of the enzyme solutions were estimated by the colorimetric procedure of Miller.[12] The enzyme assays were performed by measuring the rate of hydrolysis of leucine-*p*-nitroanilide at 405 nm at 20° in a Tris 0.10 *M*, pH 8.0, buffered solution. The maximum concentration of the leucine-*p*-nitroanilide was 1 m*M*.

The enzyme solutions were incubated with appropriate concentrations of the various platinum complexes at room temperature or 38° and the enzyme activity was then measured. The platinum complexes (Table I) dissolved very slowly in the 0.1 *M*, Tris buffer, pH 8, complexes **5** and 11 dissolved more readily, while **6**, **8**, and 3 dissolved more slowly at 25°. Complexes **10**, **4**, **7**, and **2** dissolved slowly at 60° and 1 and **9** required much more time at 70° to completely dissolve. The concentration of enzyme in the incubation mixtures was $1–5 \times 10^{-6}$ *M* and the Pt complexes ranged from 1.0 to 5.0×10^{-3} *M* which gave a $1–5.0 \times 10^3$ mole ratio of inhibitor to enzyme.

The enzyme was stable at the incubation concentration for at least a few days at room temperature; however, about 50 ± 5% of the activity was lost after 24 hr at 38–42° at 1×10^{-3} *M* concentration of the enzyme. Little, if any, inhibition of the enzyme occurred when incubated with the Pt complexes at room temperature for 24–50 hr.

A considerable number of experiments were performed in order to determine if the Pt complex inhibition was the competitive or noncompetitive type. In the range of $1–5 \times 10^{-3}$ *M* platinum complex and $1 \times 10^{-3}–1 \times 10^{-4}$ *M* substrate, only mixed inhibition rather than competitive or noncompetitive inhibition was observed.

The inhibition by the Pt complexes could not be reversed by L-methionine or L-alanyl-L-methionine added before complete inhibition occurred. Friedman and Teggins[13] found that cysteine and methionine decreased the rate of inhibition of malate dehydrogenase by K_2PtCl_4. They also determined that 38% of the enzyme activity could be regenerated by added Met to a sample of enzyme which had been completely inhibited by K_2PtCl_4. Possibly different amino acid residues are involved in Pt complex binding to leucine aminopeptidase and malate dehydrogenase.

Our results indicate a very slow rate of interaction of enzyme with the Pt complexes. Half-life of inactivations ranges from 60 to ca. 105 hr. The Pt complexes had been dissolved in the Tris, pH 8 buffer at least a couple of days in advance, so it is assumed that the complexes are in the aquated form and the interaction of enzyme is with the aquated Pt complex. The half-life for inactivation of leucine aminopeptidase by dibromo(ethylenediamine)platinum(II) was found to be ca. 15–17 hr by Guthrie et al.[1] Thus the

Table I. Inhibition of Swine Kidney Leucine Aminopeptidase by Various Platinum Complexes

	a[a]	b[a]	c[a]	% inhibn, 5×10^{-3} *M*	% inhibn, 2.5×10^{-3} *M*
1	Et_2	Me_2	Cl_4	53	31
2	MeH	Me_2	Cl_2	19	10
3	EtMe	Me_2	Cl_4	52	30
4	Et_2	MeH	Cl_2	53	35
5	EtH	H_2	Cl_2	50	40
6	*i*-Pr_2	H_2	Cl_2	63	42
7	Et_2	Me_2	Cl_2	25	12
8	EtMe	Me_2	Cl_2	40	24
9	EtMe	MeEt	Cl_4	53	24
10	Et_2	MeEt	Cl_4	35	21
11	EtH	H_2	Cl_4	60	47

[a] $Pt(a_2NCH_2CH_2Nb_2)c$. Enzyme (1×10^{-6} *M*) was incubated for 24 hr with the appropriate Pt complex and then assayed for activity. All the enzyme activities here were determined with 1 m*M* of substrate concentration at room temperature.

N-alkyl-substituted ethylenediamine liganded dihaloplatinum compounds react with the enzyme at a much slower rate. It is of interest that a single alkyl substituent is effective in decreasing the rate of inhibition of the enzyme (see **5** and 11). This property would prevent the alkyl-substituted ethylenediamine complexes from reacting rapidly with only blood proteins, for example, and never getting to tissue proteins to exert their enzyme-inhibiting effects.

Acknowledgments. This work was financially supported by the North Atlantic Treaty Organization Research Grants Program. Barbara Granett and Mitzi Maddox aided with the enzyme assays.

References and Notes

(1) R. W. Guthrie, P. Melius, and J. E. Teggins, *J. Med. Chem.*, **14**, 75 (1971).
(2) P. Melius, J. E. Teggins, M. E. Friedman, and R. W. Guthrie, *Biochim. Biophys. Acta*, **268**, 194 (1972).
(3) M. E. Friedman, B. Musgrove, K. Lee, and J. E. Teggins, *Biochim. Biophys. Acta*, **250**, 286 (1971).
(4) J. E. Teggins and M. E. Friedman, *Biochim. Biophys. Acta*, **350**, 273 (1974).
(5) G. R. Gale, E. M. Walker, L. M. Atkins, A. B. Smith, and S. J. Meischen, *Res. Commun. Chem. Pathol. Pharmacol.*, **7**, 529 (1974).
(6) B. Rosenberg, L. Van Camp, J. E. Trosko, and V. H. Mansour, *Nature (London)*, **222**, 385 (1969).
(7) T. A. Connors, M. Jones, W. C. J. Ross, P. D. Braddock, A. R. Khokhar, and M. L. Tobe, *Chem.-Biol. Interact.*, **5**, 415 (1972).
(8) K. P. Beaumont, M. J. Cleare, and C. A. McAuliffe, *Chem.-Biol. Interact.*, submitted for publication.
(9) W. T. Hardaker, R. A. Stone, and R. McCoy, *Cancer*, **34**, 1030 (1974).
(10) M. M. Moseley and P. Melius, *Can. J. Biochem.*, **45**, 1641 (1967).
(11) M. S. Chen, Ph.D. Thesis, Auburn University, 1971.
(12) G. Miller, *Anal. Chem.*, **31**, 964 (1959).
(13) M. E. Friedman and J. E. Teggins, *Biochim. Biophys. Acta*, **361**, 277 (1974).