



**Thermodynamic, structural and kinetic studies on mixed ligand
complexes of palladium(II)
propositions of PhD thesis**

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I. INTRODUCTION AND AIM OF THE WORK

As co-ordination and bioinorganic chemistry have been developing more and more complexes and metal containing compounds proved to be medicines. The vast majority of compounds are used for substitution of essential metal ions and for maintaining their appropriate concentration. More and more metals can be found at the same time, which contribute the healing process by modifying the structure of proteins and nucleic acids or by changing their function. Among the latter group can be ranked the *cis*-[Pt(NH₃)₂Cl₂] known as cisplatin that is used as an anticancer drug. According to it since the compound was discovered in 1964, intensive research work has been performed to reveal the mechanism of the antitumor effect. At the early stage of the investigations it is pointed out that the anticancer activity is based on the platination of DNA, most commonly the co-ordination to neighbouring guanine bases. Among macromolecules occurring in biological systems this is a primary binding site for the metal compound, but at the same time platinum–protein interaction occurring to a smaller extent has also got physiological importance.

These bonds are formed primarily with the participation of sulphur donor atoms in protein side chains, and they have direct role in transport processes and in the toxic side effects. To decrease the latter side effects plenty of new complexes were produced, a part of these has been successfully applied by chemotherapeutic treatments.

Revealing the mechanisms of processes taking place in the organism, and getting known the structural, thermodynamic and kinetic characteristics of the forming associates are important conditions of developing other new complexes.

However the investigation of complex forming processes is rather difficult due to the inertness of platinum(II) complexes, the determination of thermodynamic parameters are often impossible. Thus it is necessary to model of certain reactions in which platinum(II) is substituted by a metal ion that has similar co-ordination chemical properties, but it is much more labile from kinetic aspects. Palladium(II) satisfies all of the above mentioned conditions, beside the fairly same atomic size and the same square planar structure of complexes, the rate of ligand substitution reactions are higher about 4-5 order of magnitude, thus equilibrium states of the processes can be characterised. Although it should be noted, that all of the available thermodynamic data show that the stability constants of appropriate platinum(II) complexes are higher about 1-2 magnitude, therefore by comparing the equilibrium states it has to be taken into account by all means.

According to the above statements, for planning other new preparations reaction of palladium(II) complexes having different co-ordination sphere and free co-ordination site with ligands being suitable for binding metal ion and modelling the donor groups of macromolecules is to be study.

In the literature plenty of monofunctional palladium(II) complexes with different co-ordination sphere were investigated from thermodynamic and kinetic aspects till now. These complexes have different ligands formed from nitrogen donor N-acetyl amino acids and N-alkyl nucleobases. At the same time far less data are available for the co-ordination of sulphur content ligands, which is of great importance too. Therefore in the first stage of our study we investigated the mixed ligand complex formation reactions with thioether containing ligands. Besides we tried to answer, how the other donor atoms surrounding the metal ion impact the stability of mixed ligand complexes.

The metal binding ability of the different donor groups can be investigated adequately using monofuntional metal complexes, though we get more information from processes taking place in the organism if our model compounds have two free co-ordination sites similarly to the preparations applied in medicine. There are several references in literature to these types of compounds, but they focus especially on the structural description of forming complexes. Thus, the aim of our study was to characterise hydrolytic process is taking place in aqueous solutions and to describe the equilibrium features of mixed ligand complex formation reactions with N-acetyl amino acids and N-alkyl nucleobases. Based on our former results in case of these compounds we also investigated the impact of other donor atoms surrounding the metal ion.

In case of inert metal ions for the thorough description of the system knowing merely the thermodynamic constants is not enough. For the determination of a time dependent concentration distribution kinetic parameters as well as the mechanism of the process have to be revealed. In references issued till now especially the kinetic description of two-component systems can be found, whereas there is very few information about processes performing in samples that contain several kinds of ligand species at the same time. Thus in the last part of our work we studied complex formation processes versus time in case of samples containing metal complex and sulphur as well as nitrogen donor ligand. Similarly to our earlier investigations our aim was to clarify the effect of other donor functions in the co-ordination sphere.

II. EXPERIMENTAL

II.1. Determination of stability constants

The proton dissociation constants of the applied ligand and the stability constants of mixed metal complexes were determined by pH-potentiometric titrations ($I=0.2\text{ M KNO}_3$, $t=25^\circ\text{C}$). The evaluations of titration curves were performed by PSEQUAD and SUPERQUAD computational programs. In order to check the reliability of thermodynamic stability constants and characterise the binding modes of complexes ^1H NMR technique was used.

II.2. Structural characterisation of the complexes in solid phase

We have managed to crystallise two of our complexes in a single crystal form. The structures of these complexes were determined by Dr. Attila Bényei (University of Debrecen, Department of Physical Chemistry) using X-ray crystallographic method. The ORTEP and WINGX-97 SUITE computational programs were used to give the structure of the complexes from the calculated bond angles and bond lengths.

II.3. Kinetic characterisation of the complex formation processes

In order to determine the kinetic parameters of the ligand substitution processes stopped-flow technique was applied under pseudo first order conditions. The kinetic parameters and the time dependent metal ion speciations were calculated with SCIENTIST software.

III. NEW SCIENTIFIC ACHIEVEMENTS

III.1 Thermodynamic characterisation of the monofunctional palladium(II) complexes

III.1.1 Reactions with the thioether donor group

III.1.1.1. Whereas the thioether donor atom cannot be considered as a Bronsted base, the complex formation reactions do not change the pH of solutions. Since the co-ordination of various nitrogen donor ligands to the different monofunctional palladium(II) species has been well characterised we managed to perform indirect potentiometry using N(3) donor molecules (uridine, 1-methyluracile and 1-methylthimine) as a competing ligand. The stability data obtained were quite close to each other for all competing ligand, and the reliability of these parameters were also proved by ^1H NMR technique. In further studies we have used only uridine in order to determine the stability constants of mixed ligand complexes with AcMet.

III.1.1.2. The results of indirect potentiometric titrations showed that the affinity for thioether binding can be selectively modified by changing the donor atoms in the co-ordination sphere, namely the presence of aromatic nitrogen or other soft donor atom (e.g. sulphur) around the metal ion decreases the stability constants of thioether complexes and increase the tendency of metal species for hydrolysis at the same time. The differences in the stability data were quite large in case of mixed ligand complexes of $[\text{Pd}(\text{dien})]^{2+}$ and $[\text{Pd}(\text{terpy})]^{2+}$, which can be explained by the different electron density around the metal ion, the steric requirements of co-ordinated ligands and the different charges of the initial metal complexes. In order to find out which factor has the largest contribution to the stability constants we studied the co-ordination properties of $[\text{Pd}(\text{dipic})]^{2+}$ complex.

III.1.2. Mixed ligand complexes of $[\text{Pd}(\text{dipic})]^{2+}$

Similarly to dien and terpy ligands the dipicolylamine triamine molecule can also form stable monofunctional complex with palladium(II) at 1:1 metal to ligand ratio. Since this complex has not been studied before thermodynamic, structural and kinetic characterisation were also performed with this compound.

The synthesis of the complex was carried out by the method published for dien and terpy analogues in the literature. From a single crystal we determined the structure of the

complex by X-ray crystallography and compared to those of $[\text{Pd}(\text{dien})]^{2+}$ and $[\text{Pd}(\text{terpy})]^{2+}$. From the measured bond lengths and angles we can conclude that the steric requirement of $[\text{Pd}(\text{dipic})]^{2+}$ is very similar to that of $[\text{Pd}(\text{terpy})]^{2+}$, especially if we consider the H-atoms in *orto* positions. The co-ordinated aromatic nitrogen donor atoms cause a similar electron density around the metal ion to $[\text{Pd}(\text{terpy})]^{2+}$. On the other hand if we consider the *trans* labilizing effect, the $[\text{Pd}(\text{dien})]^{2+}$ complex shows analogy with $[\text{Pd}(\text{dipic})]^{2+}$.

In the thermodynamic study first we determined the affinity of $[\text{Pd}(\text{dipic})]^{2+}$ for hydrolysis and it was found, that this process is more preferred compared to the aliphatic derivatives, as in case of $[\text{Pd}(\text{terpy})]^{2+}$. In the continuation of thermodynamic studies we investigated the interaction of this complex with various nitrogen donor ligand. From the stability data obtained we can conclude that in most cases only small differences can be detected, similar metal ion speciation could be given for all triamine complexes. Only the MeC and AcLys ligands showed larger difference, namely in case of amino donor AcLys molecule no complex formation could be detected in equimolar system of $[\text{Pd}(\text{dipic})]^{2+}$ and AcLys, the hydrolysis was the preferred process. While in case of MeC ligand the deprotonation and co-ordination of exocyclic amino group was observable under neutral and basic conditions, but the stability constant of dinuclear species was smaller than that of terpy derivatives. This decreasing can be explained by the smaller stacking interaction between the co-ordinated tridentate ligands. In the last part of thermodynamic investigation we studied the co-ordination of thioether donor AcMet to $[\text{Pd}(\text{dipic})]^{2+}$. It was found that the affinity of $[\text{Pd}(\text{dipic})]^{2+}$ for thioether binding is much lower compared to that of $[\text{Pd}(\text{dien})]^{2+}$ and similar to $[\text{Pd}(\text{terpy})]^{2+}$. From all these stability data we can conclude that the electron density around the metal ion has the largest effect on the stability constants of mixed ligand complexes of thioether donor group, while the different steric conditions and charges have only a minor contribution on these parameters.

III.1.3. Formation of linkage isomers with AcHis/AcHm ligands

The imidazol side chains of peptides are the most important binding sites for metal ion binding in biological systems. Both N(1) and N(3) nitrogen atoms can bind these ions by formation of linkage isomers. Using ^1H NMR technique we proved the existence of these isomers and calculated the ratio of the species. It was found that the ratio depends on the properties of co-ordinated donor groups, on the charges for both of the complex and co-ordinating imidazole containing ligands.

III.2. Reactions of bifunctional palladium(II) complexes

III.2.1. Hydrolytic properties

We have used combined pH-potentiometric and ^1H NMR spectroscopic method to investigate the hydrolytic processes of $[\text{Pd}(\text{en})]^{2+}$ and $[\text{Pd}(\text{pic})]^{2+}$. We proved the existence of a dinuclear hydroxo bridged species for both complexes, which were not described elsewhere. Titrating the two complexes in different analytical concentration we managed to give the ratio of monohydroxo complex and its oligomers, and we found that in case of $[\text{Pd}(\text{en})]^{2+}$ a dimer-trimer, while for $[\text{Pd}(\text{pic})]^{2+}$ a monomer-dimer equilibrium can be given using $1 \times 10^{-3} - 1 \times 10^{-2}$ molar concentration range. It also should be mentioned that this hydrolysis starts in more acidic solution in case of $[\text{Pd}(\text{pic})]^{2+}$. From the calculated stability constants we can conclude that the presence of aromatic nitrogen in the co-ordination sphere (like in case of $[\text{Pd}(\text{dipic})]^{2+}$ and $[\text{Pd}(\text{terpy})]^{2+}$) increases the affinity for hydrolysis. On the other hand the bulky pyridine moiety reduces the tendency for polymerisation.

III.2.2. Reaction with N(3) donor nucleobases

We have investigated the complex formation processes with N-alkyl pyrimidine bases. The systems containing the $[\text{PdL}]^{2+}$ ($=\text{M}$) complex ($\text{L}=\text{en}$ or pic) and one of the uridine, MeUH or MeTH ligands in 1:1 or 1:2 “metal” to ligand ($=\text{A}$) ratio can be described by formation of mono (MA), bis (MA_2), mixed hydroxo (MAH_1) and hydroxo bridged dinuclear ($\text{M}_2\text{A}_2\text{H}_1$) complexes. The stability data obtained showed that similar metal ion speciation can be given for all of the coordinating N(3) ligand, but the mixed ligand complexes of $[\text{Pd}(\text{pic})]^{2+}$ always have higher thermodynamic stability constants. This latter conclusion give further support that simultaneous co-ordination of aliphatic and aromatic nitrogen donors is not favoured in the mixed ligand complexes of palladium(II). Using ^1H NMR spectroscopy we proved the existence of *cis-trans* isomers in case of $[\text{Pd}(\text{pic})]^{2+}$, which can be explained by the dissimilarity of the two free co-ordination sites.

The species formed in the system containing $[\text{PdL}]^{2+}$ ($\text{L}=\text{en}$ or pic) and MeC in 1:1 and 1:2 ratio always have higher thermodynamic stability than in case of other N(3) donor ligands, which can be explained by H-bond formation between the two co-ordinated MeC ligands and between the chelating ethylenediamine and the co-ordinated MeC ligand, too. The other difference from the previous ligands is that under neutral conditions deprotonation and co-ordination of exocyclic amino group occurred. As a result polymer structures are formed,

but these processes overlap with the hydrolysis hence, the complete thermodynamic evaluation of this systems is not possible.

III.2.2. Reactions with imidazole containing ligands

Under acidic conditions mono and bis(ligand) complexes can be detected for both bifunctional palladium(II) species. In case of AcHis protonated complexes also exist under excess of ligand, while at 1:1 ratio the formation of 7-membered (N(Im), O-) chelate can be detected, which is reflected in the lower pK value of MLH complex ($M=[Pd(en)]^{2+}$, or $[Pd(pic)]^{2+}$, $L=AcHis$). As it previously described both N(1) and N(3) donor atoms can be considered as a metal binding sites. As a consequence linkage isomers are formed, which was proved by 1H NMR spectroscopy. Increasing the pH of solutions resulted in deprotonation and co-ordination of pyrrol type NH- group (like the exocyclic amino group in case of MeC) and polymer structures are formed.

III.2.3 Co-ordination of AcMet

Combined potentiometric and spectroscopic methods were applied to investigate the complex formation processes. Comparison of the titration curves and the appropriate 1H NMR spectra revealed a significant difference in the mixed ligand complex formation processes of the two bifunctional metal species. Under acidic conditions the dissociation of $[Pd(en)]^{2+}$ occurred which can be explained by the high *trans*-effect of the co-ordinated sulphur donor atoms. Under these conditions the AcMet ligand acted as a bridging ligand, which resulted in polynuclear complex formation. Increasing of pH led to 'reco-ordination' of the ethylenediamine ligand to the free $[Pd(en)]^{2+}$, and bis(ligand) $[Pd(en)_2]^{2+}$ complexes formed, which can be detected in the NMR spectrum. Further increase of pH resulted in deprotonation and co-ordination of the acetamido -NH group of the AcMet ligand. The bidentately coordinated ((S,N') 6-membered chelate) species were quite stable, no changes were detected by addition any base to the solution. Similar dissociation process can not be observed in case of $[Pd(pic)]^{2+}$. At low pH mono and (protonated) bis(ligand) complexes were formed. Increase of pH of the solution the deprotonation and co-ordination of the acetamido -NH group occurred, which resulted in a quite stable 6-membered (S,N') chelate formation.

III.3. Kinetic characterisation of nitrogen and sulphur donor co-ordinations

By investigation of binary and ternary systems contained the $[PdL]^{2+}$ ($L=dien$ or $dipic$) complex and AcMet and/or Cyt ligand we gave the kinetic parameters of complex formation

and ligand substitution reactions. Applying the kinetic data obtained we could give a metal ion speciation as a function of time both for $[\text{Pd}(\text{dien})]^{2+}$ and $[\text{Pd}(\text{dipic})]^{2+}$.

It was found that co-ordination of AcMet ligand is kinetically preferred for both $[\text{Pd}(\text{dien})]^{2+}$ and $[\text{Pd}(\text{dipic})]^{2+}$, although mixed ligand complexes of cytidine have higher thermodynamic stability under the applied conditions. It was also proved that the presence of aromatic nitrogen in the co-ordination sphere selectively increases the rate of different coordination reactions.

IV. POSSIBILITIES HOW TO UTILITISE THE RESULTS

The aim of our work was to study the binding properties of different palladium species, that model the antitumor platinum agents.

Using the developed indirect potentiometric method the mixed ligand complexes of monofunctional palladium(II) species and thioether donor functions can be characterised thermodynamically, and estimation also can be given for platinum analogues.

The main achievement of our work that we proved the effect of coordinated donor atoms on the thermodynamic and kinetic parameters of complex formation reactions with sulphur donor ligands, namely we can selectively modify these parameters by altering the coordination sphere. Using combined thermodynamic and kinetic data of palladium(II) species, we can estimate a time dependent metal ion speciation for the biologically relevant platinum derivatives.

V. PUBLICATIONS

Articles:

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J. Chem. Soc. Dalton Trans. (2001), **2467**
5. Alexandra Myari, Gerasimos Malandrinos, Yiannis Deligiannakis, John C. Plakatouras, Nick Hadjiliadis, Zoltán Nagy and Imre Sóvágó
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2. Z. Nagy, I. Sóvágó (hungarian lecture)
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3. Z. Nagy, I. Fábián and I. Sóvágó (hungarian lecture)
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XXXIII. Komplexkémiái Kollokvium, 27-29 May, 1998, Paks, Hungary
4. Z. Nagy, I. Fábián and I. Sóvágó (poster)
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Cost D8 & ESF Workshop on Biological and Medical Aspects of Metal Ion Speciation, 22-25 August, 1998, Szeged, Hungary
5. Z. Nagy, I. Fábián and I. Sóvágó (poster)
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XXXIII. International Conference on Coordination Chemistry, 30 August – 4 September, 1998. Florence, Italy
6. Z. Nagy, I. Fábián and I. Sóvágó (poster)
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9. I. Sóvágó, Z. Nagy

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10. Z. Nagy, I. Fábián and I. Sóvágó (poster)

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