

Metal complexes in medicine: Design and mechanism of action

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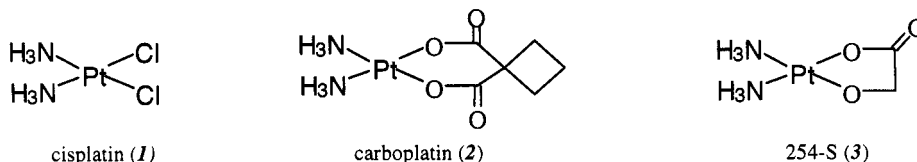
Abstract: The application of inorganic chemistry to medicine (“Elemental Medicine”) is a rapidly developing field, and novel therapeutic and diagnostic metal complexes are now having an impact on medical practice. Advances in biocoordination chemistry are crucial for improving the design of compounds to reduce toxic side-effects and understand their mechanisms of action. In this article, we focus on the chemistry of two key areas: the biotransformation of metal complexes and targeting, with particular reference to platinum anticancer, gold antiarthritic, and bismuth antiulcer drugs.

INTRODUCTION

Most of the major classes of pharmaceutical agents contain examples of metal compounds which are in current clinical use (1,2), and new areas of application are rapidly emerging. Some of these are discussed briefly below with special emphasis on the targeting of metal complexes and their biotransformation. Targeting is important because of the toxicity often associated with metal compounds. If they can be delivered only to the tissues, cells and receptors where they are required, then the toxicity may be reduced. The ease with which many metal complexes undergo ligand substitution and redox reactions is likely to mean that the active species are biotransformation products of the administered complex. Identification of these active species will lead to the more effective use of metal compounds as drugs.

PLATINUM ANTICANCER AGENTS

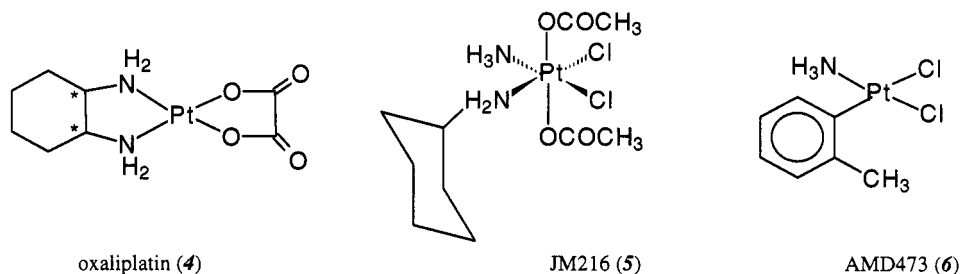
Platinum(II) complexes are now amongst the most widely used drugs for the treatment of cancer. Three injectable diammine compounds have been approved for clinical use (*I-3*), and several others are on clinical trials. There is current emphasis on reducing the toxicity of platinum anticancer complexes towards normal cells, circumventing acquired resistance to cisplatin, and increasing the spectrum of activity of platinum complexes towards a wider range of types of cancer.



The ultimate target for Pt is DNA and certain platinated DNA adducts trigger DNA degradation and apoptosis (programmed cell death). The usefulness of cisplatin in the clinic is limited by (i) the spectrum of its anticancer activity (not active enough against several types of cancer), (ii) the development of resistance after continued treatment, and (iii) its high toxicity to some normal cells.

At least three resistance mechanism have been recognised: (i) reduced transport across the cell membrane, (ii) strong binding to inactivating thiolate ligands inside the cell, e.g. glutathione and metallothionein, and (iii) repair of platinated lesions on DNA by enzymes (e.g. excision). Kidani made the important early discovery that cellular resistance to cisplatin can be overcome by changing the ammine ligands to 1,2-diaminocyclohexane (DACH), and there is much current interest in the diaminocyclohexane (DACH) complex **4** and Pt(IV) analogues which are orally active (3). It is therefore of much interest to investigate how the structures of amine ligands affect the reactivity of platinum complexes.

The orally-active Pt(IV) complex **5** (JM216), a mixed ligand complex containing ammonia and cyclohexylamine ligands, is on clinical trial. Active Pt(II) metabolites of this drug are known to be formed in blood plasma. However we cannot discount the possibility that Pt(IV) adducts are involved in the activity. Intriguingly the Pt(II) analogues of some *trans* Pt(IV) anticancer complexes have been reported to be inactive (4). The photosensitivity of many Pt(IV) complexes may limit their clinical use, although photoactivation may provide a novel strategy for activating Pt complexes *in vivo* (5).



Some of the current thinking on the chemical basis for the mechanism of action of cisplatin as an anticancer drug is summarised in Figure 1.

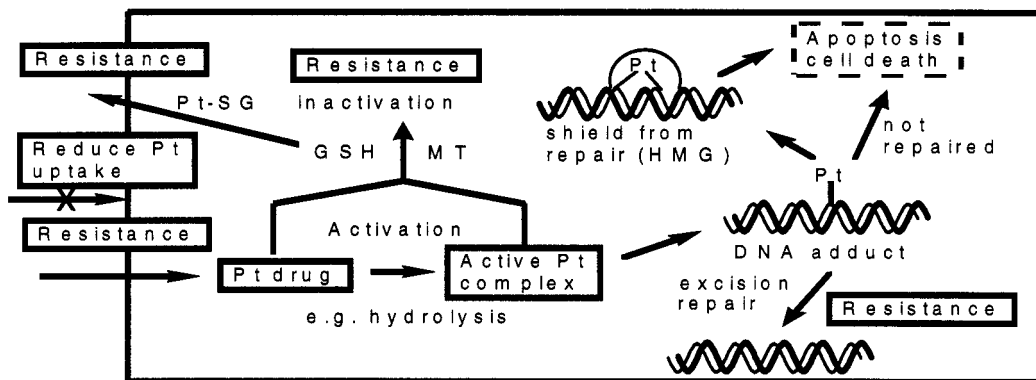


Fig. 1. A summary of some of the processes that are thought to be involved in the cytotoxicity of platinum anticancer agents.

Hydrolysis

Intracellular hydrolysis has long been thought to be an important process for the activation of chloro Pt anticancer diam(m)ine complexes. Changing the ammine ligands can markedly affect the rate of hydrolysis and the pK_a values of the resulting aqua complexes. Although aqua ligands are very reactive, e.g. towards G on DNA, hydroxo ligands are relatively inert. Our work on *cis*-[PtCl₂(NH₃)(c-C₆H₁₁NH₂)], a metabolite of the oral drug **5** (6), and on *cis*-[PtCl₂(NH₃)(2-picoline)] **6** has allowed us to compare the effects of various amines on both the hydrolysis rates and on the pK_a values. The major effect is on the hydrolysis rate.

For monoqua adducts of **5**, the pK_a values of aqua ligands *trans* to NH₃ and c-C₆H₁₁NH₂ are about the same (6.7), whereas hydrolysis of Cl⁻ *trans* to c-C₆H₁₁NH₂ is about twice as fast as *trans* to NH₃ (7). For the sterically-hindered complex **6** (AMD473), the effect of 2-picoline on the rate of hydrolysis of the Cl⁻ *trans* to NH₃ (*cis* to 2-picoline) is dramatic, being over 4x slower than the analogous Cl⁻ ligand in the non-sterically-hindered 3-picoline complex (8). The pK_a values of the aqua ligands in this complex are >0.3 units lower than those of cisplatin and a higher proportion of hydroxo species would be expected to be present under intracellular conditions. This, together with the kinetic effects, should make **6** much less reactive inside cells. The complex shows good activity against cisplatin-resistant cells, and by injection and oral administration against a cisplatin-resistant human ovarian xenograph (9), and is scheduled to enter clinical trials later this year.

Attack on DNA

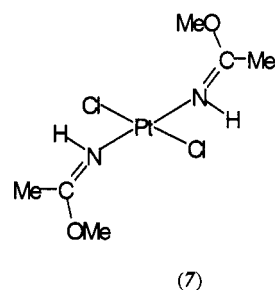
Use of ^{15}N -labelled complexes and 2D heteronuclear single quantum coherence (inverse detection) [^1H , ^{15}N] NMR spectroscopy allows the detailed pathways of reactions of cisplatin (and other ammine and amine complexes) to be followed. Intermediates at concentrations as low as $50\ \mu\text{M}$ can be detected, and the ^{15}N chemical shift is diagnostic of the *trans* ligand which makes this a powerful method for the identification of species (10). Such studies clearly demonstrate that aquation of cisplatin is the rate-limiting step in the attack on GG sequences of oligonucleotides. The formation of monofunctional adducts with Pt bound to N7 is followed by ring closure to form the stable intrastrand GG chelate.

The GG chelate is known to be an important adduct in cells. The selectivity of Pt for GG sequences is related to the high electron density at such sites (most easily oxidized sites of DNA) although AG (but not GA) chelates are also formed. Insight into the nature of GG chelates can be gained from the recent X-ray structure of $\text{d}(\text{CCTCTG}^*\text{G}^*\text{TCTCC})\cdot\text{d}(\text{GGAGACCAGAGG})$ reported by Takahara et al. (11). The duplex is bent (by about 45°) and there is some destacking of bases near the platination site. Intriguingly the square-planar coordination sphere is distorted with Pt lying ca. $1\ \text{\AA}$ out of the plane of the G bases (12). The two ends of the duplex are folded differently into A- and B-DNA, but this may be a consequence of crystal packing. The strain associated with a GG-Pt chelate on duplex DNA may account for the observed equilibrium in solution between a folded, bent, form and a partially denatured, distorted, form of the duplex $\text{d}(\text{ATACATG}^*\text{G}^*\text{TACATA})\cdot\text{d}(\text{TATGTACCATGTAT})$, which is dependent on the pH, ionic strength and temperature (13).

Monofunctional adducts

Unexpected was the finding that one of the two monofunctional adducts formed during the reaction of cisplatin with the 14mer DNA duplex $\text{d}(\text{ATACATGGTACATA})\cdot\text{d}(\text{TATGTACCATGTAT})$ was very long lived with a half-life of 80 h at 298 K (14). This has been identified as the 5'G adduct *via* enzymatic digestion studies (15). The life-times of the two monofunctional G adducts with the 14mer GG single strand were similar, suggesting that the 3D structure of DNA plays a role in stabilising the long-lived adduct either by shielding the Cl ligand from hydrolysis (compare the reactions of complex **6** described above), or by hindering the approach of the incoming 3'-G N7 ligand. Molecular modelling studies demonstrate that H-bonding between the NH_3 ligands and carbonyl groups on DNA play a major role in determining the orientation of the Pt-Cl bonds and their accessibility. Molecular mechanics calculations show that although the chloride ligand in the monofunctional adduct faces outward, away from the helix, the aqua ligand which replaces it after hydrolysis faces inwards on account of its strong H-bonding properties. Modelling of transition states is now required. Chottard et al. have made elegant use of HPLC methods to trap monofunctional intermediates and show that GG platination and chelation rates for cisplatin diaqua complex $[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})_2]^{2+}$ depend on the DNA base sequence (16).

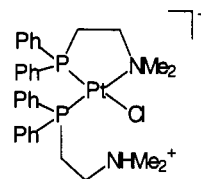
The biological significance of long-lived monofunctional adducts on DNA remains to be determined but these alone may be sufficient to kill cells if they are not repaired, which seems to be the case for the active *trans* iminoether complex **7** (17). Long-lived monofunctional adducts may also promote the formation of DNA-protein cross-links. Platinated lesions on DNA which would normally be repaired by enzymes may be protected from repair by high mobility group (HMG) proteins (18), Fig. 1. Our NMR studies of the GG chelate of the 14mer with cisplatin described above suggest that the kinked duplex binds in the elbow region of HMG1 box A (13).



DNA base recognition by Pt complexes

Under physiological conditions cisplatin does not attack the DNA base thymine (T), but changing the ligands on Pt(II) to amino phosphines allows this to be achieved. Aminophosphine ligands bind strongly to Pt(II) but in bischelated *cis* complexes the Pt-N bond is relatively labile on account of the high *trans* influence of P and steric

interactions between the substituents on the N atoms. Thus chelate ring-opening in these complexes can be controlled by the substituents on N, by the size of the chelate ring, by the pH (protonation of the displaced N ligand), and concentration of competing ligands such as Cl⁻. Despite the presence of four phenyl groups in complexes such as **8**, they are usually soluble in water.



(8)

Reaction between complex **8** and 5'-guanosine monophosphate (5'-GMP) occurs rapidly with opening of one of the chelate rings and binding to N7.

However, GMP can be displaced by Cl⁻ at high concentrations. This complex does not bind to A, but does bind to T (and U) at N3 (19). Deprotonation of N3 may be assisted by the displaced amino group. Protonated amine dangling arms can take part in secondary interactions with DNA phosphate groups.

Complex **8** exhibits activity against cisplatin-resistant tumour cells *in vitro* although there is as yet no evidence for activity *in vivo*. Two mechanisms may be responsible for the cytotoxicity. Firstly the complex may act as a positively-charged lipophilic antimetabolic agent similar to [Au(dppe)₂]⁺. Secondly, in the ring-opened form, it can bind to DNA bases and form lesions different from cisplatin. Preliminary DNA work supports the latter hypothesis (20).

Binding to methionine

For cisplatin, binding to methionine (Met) would normally be considered as an inactivation step. The metabolite [Pt(Met)₂] has been detected in the urine of patients treated with cisplatin and it is a relatively unreactive complex, existing in solution as a mixture of 3 diastereoisomers of each of the 2 geometrical isomers (21). The *cis* isomer predominates over the *trans* isomer by 87:13, and interconversion between the two is slow (half-lives for conversion of *cis* to *trans* 22.4 h, and *trans* to *cis* 3.2 h at 310 K). However S-bound L-Met, as opposed to S,N-chelated L-Met, is more reactive as a ligand on Pt(II) and can be slowly replaced by N7 of G (22,23). Transfer of Pt onto DNA via Met-containing peptides or proteins may therefore be possible. Monofunctional adducts of the type [Pt(en)(G)(L-Met-S)] appear to be very stable (24) and so methionine may play a role in trapping these adducts. Also the high *trans* influence of S as a Pt(II) ligand can lead to the facile labilization of N ligands and this allows cisplatin to react with GMP faster in the presence of L-Met than in its absence (25) which introduces another route to DNA platination.

Since carboplatin hydrolyses and reacts with chloride too slowly to account for its biological activity (26), activation by reaction with Met derivatives could provide an important pathway. Reaction of carboplatin with L-Met leads to a surprisingly stable ring-opened intermediate with a half-life of 28 h at 310 K. This intermediate may be stabilised by intramolecular H-bonding, and related complexes appear to be present in urine after carboplatin administration (27).

Metallothionein and glutathione

The binding of Pt(II) to thiolate S tends to be irreversible, in contrast to thioether S. Reactions between cisplatin and intracellular thiols such as glutathione (the tripeptide γ -Glu-Cys-Gly) may therefore inactivate the drug and be part of cellular resistance mechanisms (28). Moreover, there is over-expression of the pump for glutathione conjugates in cisplatin-resistant cells suggesting that Pt-glutathione complexes are pumped out of the cell (29).

Cisplatin resistance in some cell types involves the low-molecular-mass cysteine-rich protein metallothionein (MT). Cisplatin administration leads to the induction of MT in e.g. the liver, and may bind and inactivate Pt, but MT may also be involved in scavenging free radicals. Reactions between MT and cisplatin lead to displacement of the ammine ligands and give rise to Pt₄ clusters containing 7-10 Pt per mol (30). Pt binding is ca. 10-30x stronger than Zn(II) and Cd(II) (31). Transplatin reacts with MT faster than cisplatin (32).

TARGETING VIA TRANSFERRIN RECEPTORS: RUTHENIUM AND TITANIUM

The Fe(III) serum transport protein transferrin (MW 80 kDa) can be used in targeting strategies, either for blocking iron uptake or for release of a cytotoxic metal complex inside cells if the adduct is labile under the acidic conditions in endosomes (pH ca. 5.5). Transferrin receptors are known to be over-expressed on cancer cells and ^{67}Ga is known to be transported in this way and to localise in tumour tissues. $\text{Ga}(\text{NO}_3)_3$ has been used as an anticancer agent (2). Ru(III) is also taken up by cells as a transferrin complex but in the case of the anticancer complex *trans*- $[\text{RuCl}_2(\text{indazole})_2](\text{InH})$, a complex which hydrolyses at about the same rate as cisplatin, binds to only one of the ligands in the Fe(III) binding site (His), without the concomitant binding of the usual synergistic anion carbonate (33). The binding can be reversed by citrate and the Ru-transferrin complex has a high antiproliferative activity (34). The Ru(III) complex $\text{Na}[\text{trans-RuCl}_4(\text{DMSO})(\text{imidazole})]$ is more active against secondary than against primary tumours and this antimetastatic activity may be of clinical value. It also binds reversibly to transferrin (35). Two Ti(IV) complexes are currently on clinical trial, budotitane and titanocene dichloride. These compounds show a strong tendency towards hydrolysis which presents challenges for their formulation prior to administration. The reactive ligands acetylacetonate and cyclopentadiene may play a major role in the activity of the complexes, however we have recently demonstrated (36) that Ti can bind strongly to transferrin and so this may provide a further route for Ti transport and delivery to cells.

GOLD ANTIARTHRITIC DRUGS

Injectable Au(I) thiolate drugs such as aurothiomalate (Myocrisin[®]), aurothioglucose (Solganol[®]), and aurothiopropanol sulfonate (Allochrysin[®]), and the oral drug auranofin (Ridaura[®]), are widely used for the treatment of difficult cases of rheumatoid arthritis (37). EXAFS (38) and WAXS (39) studies have shown that the injectable thiolate complexes are polymers containing linear Au(I) and bridging thiolate sulfurs, in chain or cyclic structures. Formulated aurothiomalate usually contains a slight excess of thiolate over gold. None of the injectable thiolate drugs have been crystallized, but the X-ray crystal structure of a related cyclic hexameric 1:1 Au(I) thiolate complex has been reported (40). Gold(I) has a much higher affinity for thiolate S compared to thioether S, and a much lower affinity for N and O ligands. Therefore Au(I) binds to DNA very weakly and is not usually carcinogenic or mutagenic. Thiolate exchange reactions on Au(I) are facile (41) and therefore the administered drugs are probably not the pharmacologically-active species. A summary of some of the current knowledge of the metabolism of gold drugs is illustrated in Fig. 2.

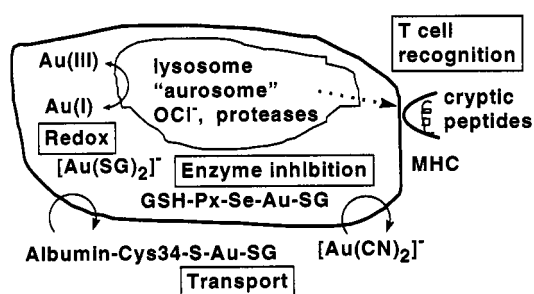


Fig. 2 Biotransformations of gold antiarthritic drugs.

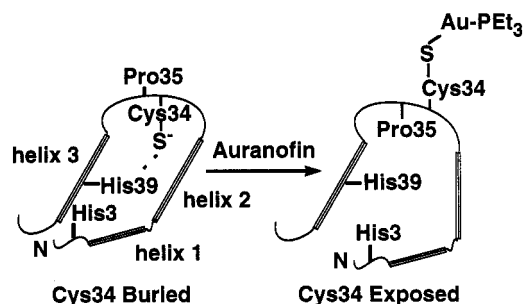
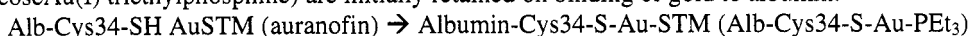


Fig. 3. Reaction of Au(I) drug with Cys34 of serum albumin.

Gold(I) has the highest affinity for thiols with the lowest pK_a values. Consequently, in blood, most of the circulating Au(I) (ca 10-15 μM) is bound to cysteine-34 of serum albumin which has a pK_a of ca. 5. The rate of binding of gold drugs to Cys34 appears to be determined by the rate of opening of the cleft in which this residue is situated (42,43). Gold binding causes a 'flip-out' of Cys34. Auranofin binds relatively rapidly but the negatively-charged aurothiomalate polymer binds slowly especially at low ionic strength. Domain I of albumin in which Cys34 is situated has a high net negative charge (ca. -10).

The thiomalate and PEt_3 ligands of aurothiomalate (AuSTM) and auranofin (tetraacetyl- β -D-thioglucoseAu(I) triethylphosphine) are initially retained on binding of gold to albumin:



The phosphine ligand is liberated slowly with the formation of OPEt_3 . Albumin can transfer Au(I) into cells. An SH shuttle mechanism is probably responsible for the transport across cell membranes, and in the red cells this involves hexose and anion transporter proteins (44). Specific metal transport proteins, perhaps those intended for Cu(I) transport (45), may transport Au(I) in the cytoplasm but nothing is known about them. Glutathione is known to bind to intracellular Au(I) (46) and the gold is mobile within the cell due to dynamic exchange reactions. This complex can inhibit the Se enzyme glutathione peroxidase by binding to the active site selenocysteine residue forming GSH-Px-Se-Au-SG (47). Also $[\text{Au}(\text{SG})_2]^-$ can be excreted from cells and the Au(I) transferred back to albumin (48). Therefore $[\text{Au}(\text{SG})_2]^-$ is an important metabolite of gold drugs.

Another important metabolite is $[\text{Au}(\text{CN})_2]^-$. The urine and blood plasma of most patients treated with gold drugs contain $[\text{Au}(\text{CN})_2]^-$ at levels as high as 100 ppb (49), and the enhanced uptake of gold into the red cells of smokers is due to the formation of this species after inhalation of HCN in smoke (50). Certain white blood cells contain the enzyme myeloperoxidase which can produce CN^- from SCN^- . $[\text{Au}(\text{CN})_2]^-$ may be a key metabolite since it can inhibit oxidative burst in white cells, and itself has antiarthritic, anticancer and anti-HIV activity. Interestingly aurothioglucose also has anti-HIV activity, which is thought to be due to binding of Au(I) to Cys532 of protein gp41 on the surface of the virus (51).

Under the oxidative conditions that exist in inflamed joints, oxidation of Au(I) to Au(III) is likely and $[\text{Au}(\text{CN})_4]^-$ or $[\text{Au}(\text{CN})_2\text{Cl}_2]^-$ may be formed. The formation of Au(III) may be responsible for some of the side-effects of gold therapy (52). Antigen processing by proteases takes place in lysosomes where Au(III) may be formed. This could lead to modification of 'self proteins' which are degraded and transported to the cell surface. The presentation of unnatural ('cryptic') peptides at the cell surface could lead to T cell recognition and triggering of the immune response (53). Gold(III) has a remarkable ability to deprotonate peptide amide bonds even at highly acidic pH values. The tripeptide Gly-Gly-His, for example, readily forms a square-planar complex with Au(III) at pH 2 via binding to the terminal amino group, two deprotonated amide nitrogens and imidazole N of His (54). Ultimately much gold is deposited in lysosomes ('aurosomes') of cells but the chemical form of it is not known. It is not metallic Au, but probably a protein complex. Here gold may inhibit lysosomal enzymes which are responsible for destruction of joint tissue.

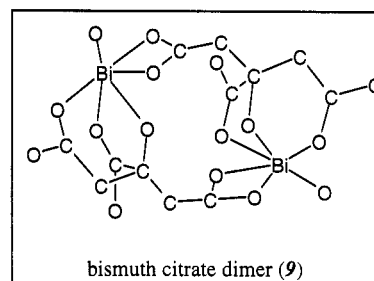
The hypothesis that the active species of gold drugs are metabolites and not the drugs themselves could explain the variability of the clinical response to gold therapy both in terms of side-effects and efficacy (50). Further investigations into ways of controlling the metabolism of gold complexes e.g. through the simultaneous administration of other agents or through redesign should lead to their more effective use.

BISMUTH ANTIULCER DRUGS

Bismuth(III) compounds such as bismuth subcitrate and subsalicylate are widely used for the treatment of diarrhoea, dyspepsia and gastric and duodenal ulcers (55). Bi(III) is active against the bacterium *Helicobacter pylori* which is associated with the mucus layer of ulcers. This bacterium relies on the activity of the Ni enzyme urease to protect it from highly acidic conditions via the production of ammonia. Our chemical studies suggest a parallel between the chemistry of Bi(III) and Fe(III) (56) and interference with the uptake and metabolism of Fe(III) may partly account for the antibacterial activity. Domenico et al. (57) have found that strains of bacteria which produce siderophores are more resistant to Bi(III), and that such resistance is predictive of bacterial virulence.

Bi(III) is a highly acidic metal ion, with a first pK_a for hydrolysis as low as 1.5, and its aqueous chemistry is dominated by hydroxide and oxide complexes. The hydrolysis of Bi(III) and formation of polycationic species such as $[\text{Bi}_6\text{O}_4(\text{OH})_4]^{6+}$ even at acidic pH, may allow it to block Ca^{2+} channels and to disrupt the glycocalyx/cell wall of *H. pylori* (58). Coordination numbers for Bi(III) are highly variable, ranging from 3 to 10, and the coordination geometries are often irregular. In some structures there is a clear stereochemical role for the $6s^2$ lone pair of electrons.

The structures of Bi(III) citrate complexes are complicated and contain chains, sheets and other networks (55,59,60). Hence many are referred to as colloids. They are usually based on the dimer $[\text{Bi}_2(\text{cit})_2]^{2-}$ **9** which contains strong Bi(III)-alkoxide bonds and a double carboxylate bridge (61). Vacant terminal coordination sites allow this unit to aggregate. The nature of the aggregates depends on the pH, ionic strength and nature of the counter cation. Thus the solution chemistry of colloidal bismuth subcitrate (CBS) which contains potassium and ammonium as cations appears to differ from that of ranitidine bismuth citrate (62).



Bi(III) binds strongly to both O and S ligands. The thiol glutathione is probably involved in the transport of Bi(III) *in vivo*. By competition reactions with citrate and EDTA (63), it was possible to establish the formation of the complex $[\text{Bi}(\text{SG})_3]$ and to measure its stability constant ($\log K$ 29.6, 298 K, $I=0.1$ M). This complex is kinetically labile exchanging GSH at a rate of ca. 1500 s^{-1} at biological pH. ^1H NMR studies show that Bi(III) binds to GSH inside red blood cells and the rate of uptake of Bi(III) seems to be limited by passage through the cell membrane.

Despite the large ionic radius of Bi(III) (1.03 \AA), it binds strongly (56) to both the N- and C-lobe binding sites of the Fe(III) transport protein transferrin ($\log K$ 19.42 and 18.58, 5 mM bicarbonate, pH 7.4, 310 K) which is present in blood serum at a concentration of ca. $30 \mu\text{M}$. The strong binding arises from the presence of two tyrosinate ligands in the N- and C-lobe Fe(III) sites, the other ligands being His, Asp and a didentate carbonate anion. The presence of the latter in the Bi complex was verified by ^{13}C NMR. The strength of metal binding to transferrin correlates with metal ion acidity and allows prediction of the strength of metal binding for other metal ions (64).

CONCLUSION

We have summarized some of the progress which is being made in understanding the mechanisms of action of three metals widely used in therapy: Pt, Au and Bi. For Pt(II) there is good evidence that DNA is the target. Pt-N bonds (trans to Cl and O) are strong and relatively inert, whereas both Bi(III) and Au(I) are more labile metal ions, and for these there is little evidence for DNA binding. Instead their targets appear to be proteins. Despite the widespread use of these metal ions in drugs, their biocoordination chemistries are still poorly understood and exciting research work lies ahead. Future studies are likely to be focused on the selective delivery of metal complexes to particular types of cell and to specific targets. In this way it will be possible to increase their effectiveness and control side-effects. The study of elemental medicine is not only likely to lead to novel therapeutic and diagnostic approaches, but is also likely to make major contributions to our understanding of natural biological processes.

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