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Coordination chemistry of metals in medicine: target sites for bismuth

Peter J Sadler *, Hongyan Li, Hongzhe Sun [1]

Department of Chemistry, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JJ, UK

Contents

Abstract

Bismuth compounds are used for the treatment of gastrointestinal disorders and may also be useful for the treatment of other diseases. Bi(III) exhibits a highly variable coordination number (3–10) and often an irregular coordination geometry. The coordination chemistry of

* Corresponding author. Tel.: +44-131-6504729; fax: +44-131-6506452.
E-mail address: p.j.sadler@ed.ac.uk (P.J. Sadler)
[1] Present address: Department of Chemistry, University of Hong Kong, Hong Kong SAR.

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Bi(III) with carboxylates and aminocarboxylates is dominated by intermolecular interactions which leads to polymeric structures. Bi(III) binds strongly to the thiolate sulfur of the tripeptide glutathione, however these adducts are also kinetically labile which allows rapid translocation of Bi(III) inside cells. The major biological target for Bi(III) appears to be proteins and enzymes. Bi(III) binds to both Zn(II) sites (e.g. metallothionein) and Fe(III) sites (e.g. transferrin and lactoferrin) in proteins and enzymes and inhibits the bacterial Ni enzyme urease. © 1999 Elsevier Science S.A. All rights reserved.

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1. The chemistry of bismuth

1.1. Properties of the element

Bismuth, atomic number 83, the heaviest stable element in the periodic table, was first discovered as early as the 15th century, and was established as an element in 1739 by Potts and Bergmann [1]. It is most commonly found in nature as the oxide (Bi_2O_3), carbonate ($(BiO)_2CO_3$) and sulfide (Bi_2S_3), and is also produced as a byproduct of lead, zinc and copper mining. Many Bi isotopes are known, but only ${}_{83}{}^{209}Bi$ ($I = 9/2$) occurs naturally. Artificial isotopes are known with masses between 187 and 216 with half lives of milliseconds to millions of years.

Of the two major oxidation states of bismuth (III and V, but II and IV are also known), the $3+$ state is the most common and most stable form, in contrast to arsenic and antimony. The electronic configurations are:

Bi(III): $(Xe)4f^{14}5d^{10}6s^2$ and Bi(V): $(Xe)4f^{14}5d^{10}$

Bi(V) is a powerful oxidant in aqueous solution, with a Bi(V) /Bi(III) potential $E^\circ = 2.03$ V, although recently two Bi(V) complexes with benzenoid and non-benzenoid arenes as ligands were reported to be stable even in aqueous media [2]. Little redox data for either Bi(III) or Bi(V) complexes appears to have been reported.

1.2. Bi(III) compounds

According to Pearson's hard-soft acid-base (HSAB) theory [3], Bi(III) is a borderline metal ion [4]. Recently Bi(III) was found to have a high affinity for both oxygen and nitrogen ligands in aqueous solution. It binds to nitrogen donor macrocycles even in strongly acidic solutions (pH ca. 0) [5]. The stability constants of Bi(III) with a series of nitrogen donor ligands have been determined via differential pulse polarography. Separate peaks can be observed in differential pulse polarograms for free Bi(III) and complexed Bi(III) which greatly simplifies the determinations. Bi(III) binding to ligands is usually fast, with equilibration within a few minutes, an exception being the macrocycle 1,4,8,12-tetraazacyclopentadecane ([15]aneN$_4$), for which equilibration required two to three weeks.

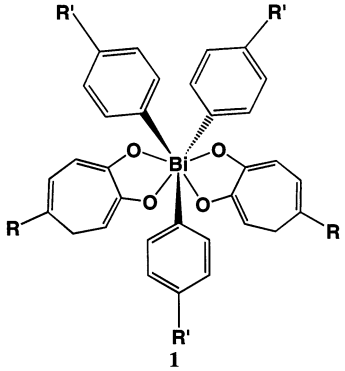
In general, the structures of Bi(III) compounds are similar to those of As and Sb compounds, but more complicated. The coordination number of Bi varies from 3 to 10 [6,7]. Compared to As and Sb, more structures of Bi complexes are known. The structure of the aqua complex $[Bi(H_2O)_9]^{3+}$, is the same as those of the lanthanide

ions $[Ln(H_2O)_9](SO_3CF_3)_3]$ [7]. Bismuth coordinates to nine water molecules with a tricapped trigonal prismatic structure (CN:9) without recognizable stereochemical activity for the lone pair of electrons. The oxide Bi_2O_3 is strictly basic, whereas As_2O_3 and Sb_2O_3 are amphoteric. Bi_2O_3 dissolves in mineral acids to give salts. The hydroxide, $Bi(OH)_3$, can be precipitated by the addition of base. The first pK_a for a Bi(III) aqua ligand is 1.51 [8]. At high pH $[Bi(O)]^+$ forms, and partial hydrolysis of Bi(III) leads to the formation of polymeric cations such as $[Bi_6O_4(OH)_4]^{6+}$. The X-ray crystal structure of this cation shows six Bi atoms at the apices of an octahedron at non-bonding separations (Fig. 1). The octahedron is face-capped by eight oxo or hydroxide functions, forming Bi–O–Bi bridges [9]. The lone pair of electrons on Bi is directed away from the cage [10]. Bi(III) also shows a high affinity for other oxygen donor ligands and complexation with polyethylene glycols and crown ethers has been reported recently [11]. Most of the Bi(III) polyethylene glycol complexes are dimeric, with two bridging alkoxide oxygen atoms. Coordination of each Bi(III) is completed by a nitrate anion. The Bi–alkoxide bonds are short (2.24 Å) indicative of strong covalent character, and this strong bond appears to give rise to a stereochemical role for the lone-pair of electrons. The crown ether complexes do not exhibit such strong covalent interactions, and do not display lone-pair effects. Bismuth alkoxide complexes (e.g. $[Bi(OC_2H_5)_3]$ and $[Bi(Oi\text{-}C_3H_7)_3]$) show potential as precursors for new superconductor formulations [12].

The X-ray crystal structures of several Bi(III) thiolate complexes have been determined recently [13–17]. In these complexes, bismuth exhibits coordination numbers of 3, 4, 5 and up to 7 with thiolate sulfurs. In the examples shown in Fig. 2, Bi has pyramidal, trigonal pyramidal, square-based pyramidal and octahedral geometry. The Bi–S bond distances are ca. 2.5–2.7 Å for intra-molecular interactions, and ca. 3.1–3.4 Å for intermolecular (long-range) interactions.

1.3. Bi(V) compounds

Most of the Bi(V) complexes prepared to date are five-coordinate, although Bi(V) in triarylbismuthate complexes such as $Ar_3Bi(HCO_2)_2$ (where Ar = Ph, *p*-Tol) [18], is six-coordinate. Almost all of these Bi(V) complexes are very unstable in aqueous media. However seven-coordinate Bi(V) tropolonato complexes, tri(aryl)tropolonato-bismuth(V) (**1**), (where R = H or NO_2; R′ = H or CH_3), have been recently reported to be unusually stable, probably due to the steric shielding of the bismuth ion [2].



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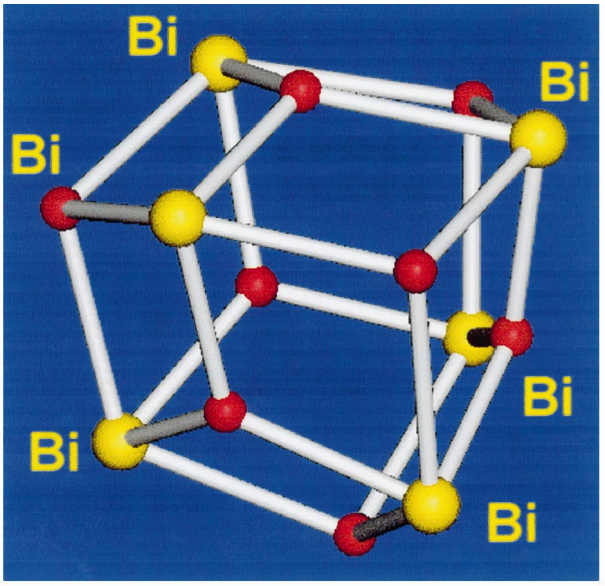


Fig. 1

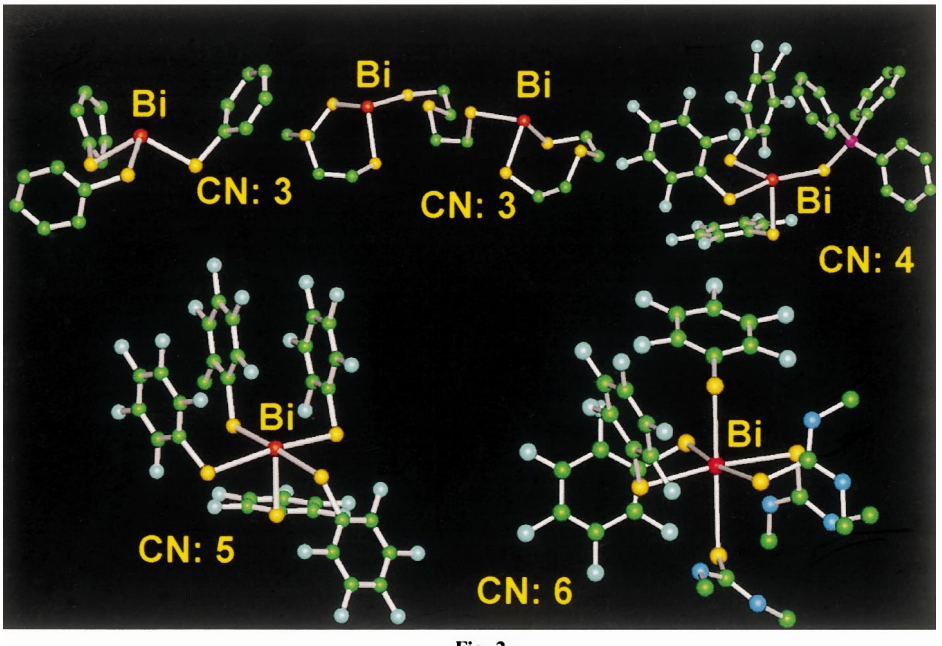


Fig. 2

Fig. 1. Crystal structure of the cluster $[Bi_6O_4(OH)_4]^{6+}$. The hydrogen atoms are omitted.
Fig. 2. Crystal structures of bismuth thiolate complexes showing various coordination numbers of Bi and irregular geometry: $Bi(SAr)_3$ (CN:3, where Ar = 2,4,6-*t*-$Bu_3C_6H_2$), Bu groups omitted for clarity [14]; bis((1,3,6-trithia-2-bismocan-2-yl)thio)ethyl)sufide (CN:3) [17]; $[Bi(SC_6F_5)_3(SPPh_3)]$ (CN:4) [13]; $[Bi(SC_6F_5)_5]^{2-}$; $[Bi(SC_6F_5)_3\{S=C(NHMe)_2\}_3]$ [13]. Color code: Bi, red; C, green; F, pale blue; N, blue; P, purple; S, yellow.

2. Bismuth in medicine

Bismuth has long been associated with medicine. The earliest use of bismuth compounds (bismuth subnitrate) in medicine appears to have been in the Middle Ages. The first full account of the internal administration of a bismuth compound was in 1786 by Louis Odier for the treatment of dyspepsia [19]. In Britain, bismuth seems to have entered firmly into medicinal use as early as the 19th century. The compounds used included derivatives of phenol, bromophenol, pyrogallol, naphthol and salicylate.

In 1889, Felix Balzer first discovered that bismuth might be useful as an antisyphilitic agent [20]. Even after the introduction of penicillin as a safe and rapid treatment for treponemal infections, bismuth was still found to be useful. Its gradual action was thought to be particularly valuable in the tertiary and quaternary stages of syphilis. In addition, bismuth nitrate in combination with morphine was a constituent of Ferrier's snuff, an inhalation for nasal catarrh. Bismuth and iodoform were even widely advocated as a surgical wound dressing due to their antimicrobial and antibacterial effects [21].

During this century, various bismuth compounds (subnitrate, subgallate, subcitrate, tartrate, subcarbonate and subsalicylate) have been used to treat syphilis, hypertension, infections, skin conditions and gastrointestinal disorders [22,23]. Since the 1970s, two bismuth compounds have been most commonly used worldwide—bismuth subsalicylate (BSS, Pepto-Bismol®; the Procter & Gamble Company, Cincinnati, Ohio, USA) for the prevention and treatment of diarrhoea and dyspepsia, and colloidal bismuth subcitrate (CBS, De-Nol®; Gist Brocades, Delft, The Netherlands, launched in 1976) for the treatment of peptic ulcers. The latter (CBS) has been used successfully in the treatment of both gastric and duodenal ulcer disease. It is said to be as effective as the histamine H_2-antagonists such as cimetidine. CBS differs from previous bismuth compounds in that it is highly water soluble, giving a colloidal solution, in contrast to bismuth subnitrate and subsalicylate. Attempts to determine the solid-structure of CBS have been reported [24,25]. Recently the effectiveness of bismuth has been attributed to its bactericidal action against *Helicobacter pylori* (an organism which was first discovered in 1983 named as *Campylobacter pyloridis*, later amended to *H. pylori*). Several bismuth compounds alone or in combination with antibioties have been found to be effective against *H. pylori* in vitro, and the longer remission times achieved with bismuth therapy are probably due to the elimination of the organism by bismuth [26]. Clinical studies with CBS and BSS show that patients treated with bismuth alone experience a slower relapse than patients treated with other ulcer-healing agents [27], due to bactericidal action of these two complexes against *H. pylori*.

There was an outbreak of bismuth-induced neurotoxicity (encephalopathy) in France and Australia in the 1970s due to careless use (over 10 grams per day) [28]. The mechanism of action for bismuth drugs needs to be investigated, as toxic side-effects may be related to bismuth interactions with key molecules in the body.

New bismuth-containing drugs are currently being developed. A ranitidine bismuth citrate compound (made by GlaxoWellcome, Tritec® and Pylorid®) has

recently been approved for marketing and is on sale in a number of countries around the world. It combines the antisecretory action of ranitidine with the mucosal protectant and the bactericidal properties of bismuth [29,30].

Another use of bismuth in medicine is in radio-therapy. ^{212}Bi, is a strong alpha-particle emitter, has a short half-life (1 h) [31], and can be obtained in large quantities from a ^{224}Ra generator. This isotope can be used as a targeted radiotherapeutic agent for cancer therapy when attached via complexing ligands such as dtpa (diethylenetriaminepentaacetate) and dota (1,4,7,10-tetra-azacylododecane *N*,*N*′,*N*″,*N*‴-tetraacetate) to monoclonal antibodies [32]. Bismuth complexes such as $\{Na_2[BiO(mp)_3]\cdot 3H_2O\}$ and $\{Bi(tgn)_3(H_2O)]\cdot 3.5H_2O\}$ (where mp = 6-mercaptopurine; tgn = thioguanine) have been shown to exhibit anti-cancer activity [33]. Skinner et al. have found that $\{Na_2[BiO(mp)_3]\cdot 3H_2O\}$ is effective in treating Dunning ascitic leukemia in rats [34], and recently it has been reported that some organometallic bismuth(III) thiolate complexes exhibit an optimum cure rate of 100% against fluid Ehrlich ascites tumor with a therapeutic index of 3.2 to 5.0 [35]. Some bismuth complexes have been shown to inhibit HIV-1 virus production from chronically-infected H9 cells with selectivity indices of ca. 5 [36].

3. *Helicobacter pylori* bacterium

H. pylori was discovered about a hundred years ago by German pathologists, and was isolated by two Australian doctors Warren and Marshall in 1983 [37]. The presence of this bacterium in the gastric mucosa is associated with chronic active gastritis, and has been implicated in more severe gastric mucosal conditions, including gastric atrophy, peptic ulceration, mucosa-associated lymphoid tissue lymphomas, and even gastric cancer [38]. Bismuth is known to inhibit growth of this bacterium although the mechanism of action is unknown. *H. pylori* is a micro-aerophilic, slow-growing, Gram-negative spiral-shaped bacterium. It has a smooth outer coat, with several whip-like tails (flagellae) at one end. Due to the presence of the flagellae, it can penetrate the mucus layer of the mucosa. The curved structure of *H. pylori*, as well as specific adhesions and its flagellae, enable it to travel within the sanctuary zone. It produces the multisubunit nickel-containing enzyme urease [39]. This enzyme catalyzes the hydrolysis of urea to form ammonia and carbon dioxide (Eq. (1)). It is commonly thought that the ammonia produced from urea in the stomach and catalyzed by urease gives rise to severe cytotoxic effects on mammalian cells within the gastric epithelium. The ammonia neutralizes the micro- and macro-environment of the bacterium and therefore aids survival in the acidic conditions of the gastric lumen and mucosa (pH ca. 2).

$$H_2NCONH_2 + H_2O \xrightarrow{\text{urease}} [H_2NCOO^- + NH_4^+] \rightarrow 2NH_3 + CO_2 \quad (1)$$

Another important enzyme in *H. pylori* is catalase, a manganese-containing enzyme, which protects *H. pylori* from being destroyed by neutrophils, part of the natural defense (immune) system of the body.

4. Methods for the study of Bi

Similar to isotopes of arsenic and antimony, ^{209}Bi NMR has not been widely used because of its large electric quadrupole moment (Table 1) [40], thus leading to broad resonances. Resonances for ^{209}Bi can be detected only in highly symmetrical environments, and only two ^{209}Bi NMR spectra have been reported, those of $Bi(NO_3)_3$ (dissolved in nitric acid) with a line-width of 3.2 kHz (chemical shift of -24 ppm), and $[BiF_6]^-$, with a line-width of 44 Hz, chemical shift 0.0 ppm, and J_{Bi-F} 3823 $\pm$ 3 Hz [41], but no useful chemical studies have been carried out. NMR studies of bismuth complexes have therefore relied on observations of ligand nuclei (e.g. ^{1}H, ^{13}C). Nuclear quadrupole resonance has also not been useful for studies of bismuth. Single crystal X-ray diffraction gives useful structural information if suitable crystals can be obtained, and since bismuth is a very heavy element, very small crystals are used to avoid absorption effects. X-ray absorption near edge structure (XANES), and extended X-ray absorption fine structure (EXAFS, e.g. Bi L_{III} edge) are likely to be useful in the study of bismuth coordination spheres but there appear to be no previous reports of this. Using EXAFS we have been able to determine the coordination spheres of Bi in glutathione, metallothionein and transferrin complexes [42].

The most common method of determining the total bismuth content of a sample is by atomic absorption spectroscopy (AAS), and more recently by inductively-coupled-plasma-mass spectrometry (ICP-MS). The former requires digestion of the sample, and large interferences from high concentrations of salts retard the application to biological samples. ICP-MS seems to be a suitable method for determining bismuth in all kinds of materials with a detection limit as low as 0.01 ppb ($\mu g\ l^{-1}$, 4.8×10^{-9} M) [43]. Using direct inject nebulization (DIN) instead of pneumatic nebulization (PN), memory effects can be dramatically suppressed and less sample volume is required [43]. By combining HPLC (FPLC) and ICP-MS, it is possible to study the speciation of Bi in biological systems. Recently the Bi content of blood plasma was determined by ICP-MS before, during and after the intake of the bismuth anti-ulcer drug CBS [44].

5. Bismuth citrate complexes

Although bismuth citrate complexes are currently used as antiulcer drugs, their structures have only recently been investigated. The empirical formula of colloidal bismuth subcitrate (CBS), one of the clinically-used antiulcer drugs, is often given as: $K_3(NH_4)_2[Bi_6O_3(OH)_5(Hcit)_4]$ [45]. By variation of the pH and the ratios of bismuth and citrate, nine different bismuth citrate adducts have been isolated and characterized by X-ray crystallography. Most of them contain the stable dinuclear unit $[Bi(cit)_2Bi]^{2-}$ with additional O^{2-}, OH^- and H_2O ligands. This dinuclear unit can aggregate further via citrate bridging to form channels and also form sheets via H-bonding; the high solubility of CBS is probably due to the formation of such channels and sheets. The coordination number of bismuth in these complexes is

Table 1
NMR properties of group 15[a]

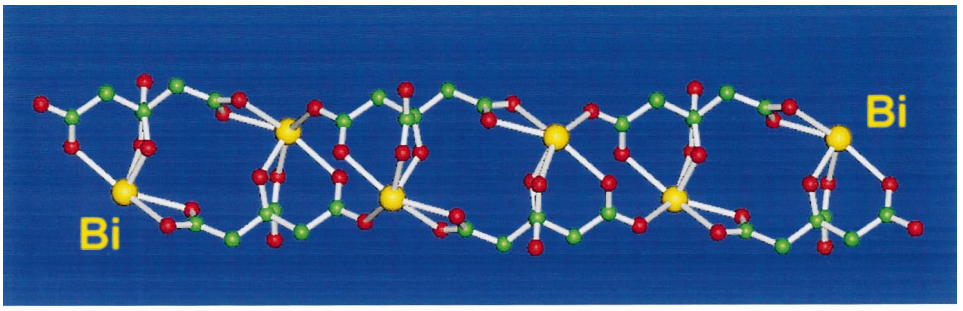
Nucleus	Spin	Abundance (%)	Resonance frequency (100 MHz, ^{1}H)	Quadrapole moment (10^{-28} m^2)	Sensitivity (^{13}C)
^{14}N	1	99.63	7.23	0.02	5.7
^{15}N	1/2	0.37	10.14	0	0.022
^{31}P	1/2	100	40.48	0	377
^{75}As	3/2	100	17.18	0.31	144
^{121}Sb	5/2	57.36	24.09	−0.40	527
^{123}Sb	7/2	42.64	13.04	−0.50	113
^{209}Bi	9/2	100	16.35	−0.37	819

[a] See reference [40].

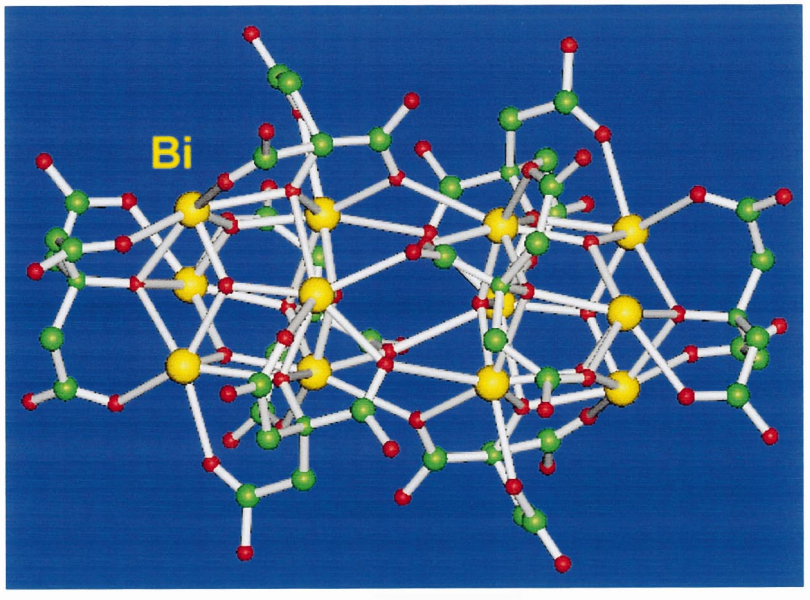
usually high, from 6 to 10, and there are short Bi–alkoxide (C–O$^-$ of citrate) bonds of ca. 2.13 Å. The stereochemical role played by the lone pair of electrons of Bi(III) in these compounds is particularly notable. All of the bound atoms lie on one side of the Bi coordination sphere, and the vacant axial site is occupied by the lone pair of electrons. However none of these complexes has exactly the same composition as the drug itself (CBS), and therefore the solid-state and solution structures of CBS are still unknown, although it seems likely that the dominant feature is the dimeric unit $[Bi(cit)_2Bi]^{2-}$ aggregated through citrate bridges and H-bonding. It is clear that bismuth citrate complexes can have complicated structures, being dependent on pH, concentration, [Bi:cit] ratio and the counter cations. At neutral pH, multinuclear clusters such as $[Bi_{12}O_8(cit^{4-})_8]^{12-}$ (Fig. 3b) are also formed by citrate and oxo-bridging [46]. The latter cluster can further aggregate to form Bi_{24} in concentrated solutions, and dissociates into smaller clusters such as $[Bi_6O_4(cit^{4-})_4]^{6-}$, with some release of citrate when diluted [46].

The new antiulcer drug, ranitidine bismuth citrate (RBC, Tritec®, Pylorid®) is highly soluble in water (ca. 1.0 g ml^{-1}), giving a pH of 4.6. The solution and solid-state structures of RBC have been investigated [47,48]. In aqueous solution, different bismuth citrate species are in fast exchange on NMR time-scale; at pH values greater than 6.2 the exchange rate decreases and different species can be observed [47,49]. By means of diffusion-ordered 2D [^{1}H, ^{13}C] HSQC-NMR spectroscopy together with isotope-labelling of citrate ($^{13}C2/^{13}C4$), a wide range of types of bound citrate in aqueous solutions of ranitidine bismuth citrate at pH 7.4 can readily be detected at low Bi concentrations (5 mM, Fig. 4) [50]. There appears to be an equilibrium between free citrate, $[Bi(cit)_2Bi]_n^{2n-}$ and multinuclear bismuth citrate clusters at physiological pH values.

In RBC, ranitidine is not coordinated to Bi, and appears to be involved in specific second-coordination sphere interactions with bismuth citrate via its $HNMe^+$ group. Complexation of Bi(III) to both citrate and ranitidine in acidic solutions (pH 2.5–3.0) has been detected by differential pulse polarography [47].



(a)



(b)

Fig. 3. Polymeric structures of Bi(III) citrate complexes. (a) Polymeric anionic chain of $[\{Bi(cit)_2Bi\}_n]^{2n-}$, and (b) hexanuclear cluster $[Bi_{12}O_8(cit)_8]^{12-}$. Color code: Bi, yellow; C, green; and O, red.

There is a rapid deprotonation equilibrium between at least two species in solutions containing Bi(III): citrate in a 1:10 mol ratio. At pH > 5.8, a second polarographic peak appears suggesting that at least two types of Bi(III) citrate complex exist under these conditions, in agreement with NMR data. The solid-state structure of ranitidine bismuth citrate may be closely related to that of $Na_2[Bi_2(citrate)_2]\cdot 7H_2O$ in view of the similarity in their chemical compositions (Bi:cit = 1:1), crystallization

conditions (ca. pH 4), and solid-state ^{13}C-NMR spectra [48,49]. $Na_2[Bi_2(citrate)_2]\cdot 7H_2O$ also contains the highly stable dimeric unit $[Bi(cit)_2Bi]^{2-}$ in which two Bi(III) ions are doubly bridged by citrate carboxylate oxygens. These dimeric units are assembled into infinite polymeric chains (Fig. 3a) and sheets via carboxylate bridging and electrostatic cross-links. Such polymers, formed into chains and sheets, could be deposited on the ulcer crater forming a protective coating, or on bacteria in ulcers. Cleavage of the double bridges between dimeric units by citrate or water would give singly-bridged polyanionic species. Various tiny crystalline species have been reported to be present in ulcer craters of patients after treatment with colloidal bismuth subcitrate [51].

6. Bismuth complexes with biomolecules

6.1. Bismuth binding to oxygen-containing molecules

The crystal structures of several bismuth complexes with organic acids have been characterized recently. These include D-lactate, L-(−)-malate, oxalate and L-(+)-tartrate complexes [52,53] in which bismuth exhibits a high coordination number (e.g. 8 and 9). All the complexes are polymeric via carboxylate oxygen bridging. In

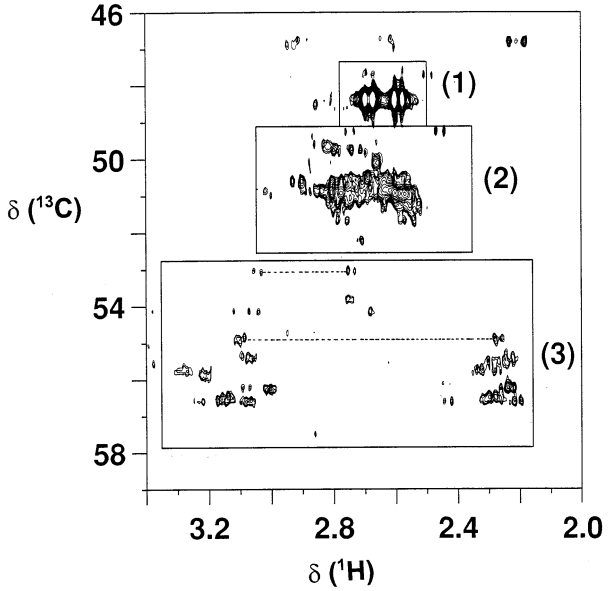


Fig. 4. 2D [^{1}H, ^{13}C] HSQC-NMR spectrum of 5 mM ranitidine Bi(III)$^{13}C_2$/$^{13}C_4$-citrate at pH 7.4 [50]. The volume integrals of peaks in areas labelled (**1**)–(**3**) were used to measure relative self-diffusion coefficients. The peaks in region **2** have similar shifts to those of ranitidine bismuth citrate at pH 4.5. The dotted line illustrates the connectivity for two non-equivalent H atoms attached to the same ^{13}C atom of citrate.

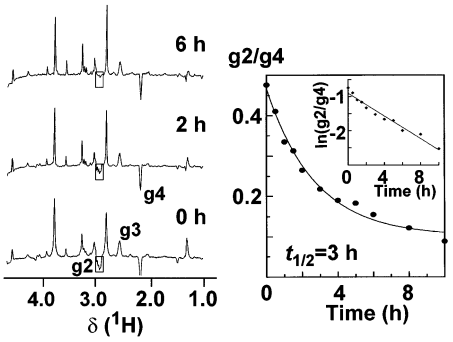


Fig. 5. Hahn spin-echo ^{1}H-NMR spectra ($\tau = 60$ ms) of human red blood cells before and at various times after addition of rantidine bismuth citrate [59]. The gradual decrease in intensity of peaks for the β protons of Cys residue of gsh indicates a slow passage of Bi(III) across the cell membrane ($t_{1/2}$ 3 h) with formation of an intracellular Bi gsh complex.

the bismuth oxalate complex, the bismuth is also bridged by chloride ions. Five-membered chelate rings are preferentially formed in these complexes probably due to the large size of Bi(III) [54]. It is not clear if these ligands are targets for bismuth in biological systems.

6.2. Bismuth complexes with thiolate ligands

As Bi(III) is a borderline metal ion, it is not surprising that it binds strongly to thiolate sulfur. The amino acid cysteine and tripeptide glutathione (γ-L-Glu-L-Cys-Gly, gsh) can prevent the precipitation of CBS at pH 2.0, and animal studies have shown that simultaneous oral administration of bismuth salts and thiolates produces a significant rise in the bismuth concentration in blood plasma [55,56]. Infra-red spectroscopic studies suggest that Bi(III) coordinates only to the thiolate group of cysteine [57], while the X-ray crystal structure of the complex $[(SC(CH_3)_2CH(NH_2)CO_2]BiCl$ shows chelation by sulphur, nitrogen and oxygen of tridentate D-penicillamine [58]. The interaction of bismuth with oxygen atoms of the carboxylate groups leads to a polymeric two-dimensional structure.

Bismuth also forms stable complexes with the tripeptide glutathione (gsh) and N-acetyl-L-cysteine (nac) with a stoichiometry $[Bi(H_{-1}gsh)_3]$ or $[Bi(H_{-1}nac)_3]$ [59]. Glutathione is widely used as a model system for binding of Bi by larger peptides and proteins. It is present in many cells at relatively high concentrations (ca. 0.5–10 mM) and may play a role in the transport and delivery of Bi(III) in cells and biofluids. It has been shown that a gsh-dependent hepatobiliary transport mechanism exists for Bi. Transport of each Bi(III) ion results in the co-transport of three molecules of glutathione [60]. The binding constants of Bi with gsh and nac have been determined from studies of competition reactions between ethylenediamine tetraacetate (edta) and gsh (or nac) giving $\log K$ 29.6 and 31.4 ($I = 0.1$ M $NaNO_3$, 298 K) for $[Bi(H_{-1}gsh)_3]$ and $[Bi(H_{-1}nac)_3]$, respectively. Both NMR and EXAFS [49] suggest that the deprotonated thiolate

group is the only strong binding site for Bi(III) in these ligands, and Bi(III) induces unusually large chemical shift changes of 1.4 ppm for the two β-CH_2 Cys protons of gsh and nac. In spite of the extremely high thermodynamic stability of $[Bi(H_{-1}gsh)_3]$, bound gsh is kinetically labile and bound gs^- exchanges with free gsh only slowly at pH 4 (3 s^{-1}, 298 K) but faster at biological pH (ca. 1500 s^{-1}, 298 K). Spin-echo ^{1}H-NMR studies show that Bi appears to pass through red cell membranes slowly ($t_{1/2}$ ca. 3 h, Fig. 5), probably via a shuttle mechanism involving membrane proteins, and forms an intracellular complex with gsh, probably $[Bi(H_{-1}gsh)_3]$.

6.3. Bismuth (III) complexation with metallothionein and transferrin

Systematic studies of the binding of proteins and enzymes to Bi(III) have not been reported [61,62]. In the stomach, bismuth has been shown to bind strongly to connective tissue proteins, mucus glycoproteins and enzymes [63], but little is known about the binding mode or kinetic behavior. Detailed studies on two proteins, metallothionein and transferrin, are described below.

6.3.1. Metallothionein

Metallothionein is a small protein with only ca. 60 amino residues, one third of which are cysteine residues. The protein appears to be involved in the normal

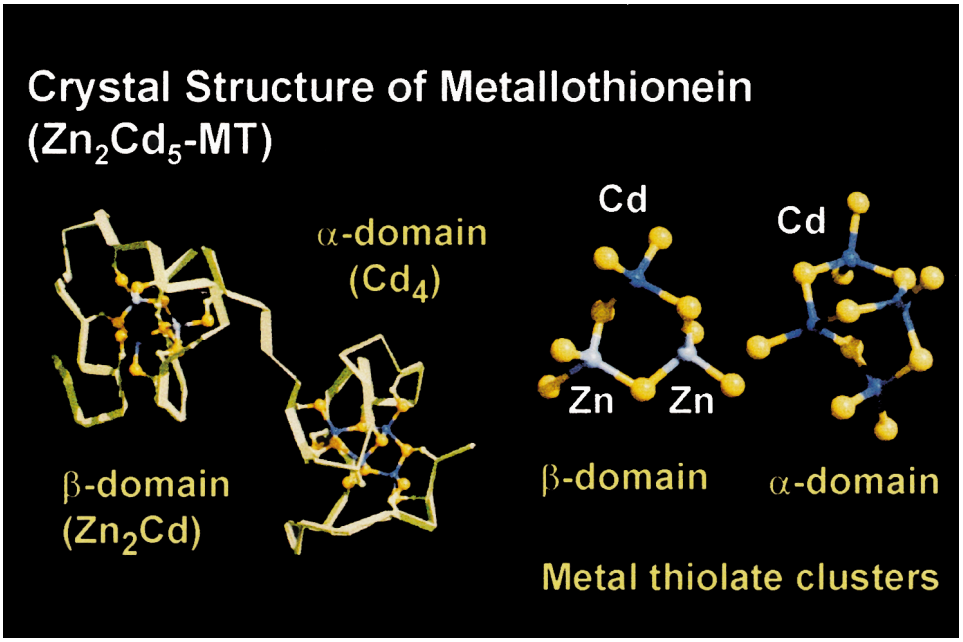


Fig. 6. X-ray crystal structure of rabbit liver metallothionein (Zn_2Cd_5–MT II) [adapted from reference [69]. The metal thiolate clusters are shown in more detail on the right.

storage of up to seven Zn(II) ions or ten Cu(I) ions in vivo. The physiological functions of this protein include donation of Zn(II) to apo-enzymes, control of cell differentiation and proliferation, and detoxification of metal ions such as Cd(II), Hg(II), and Au(I) [64]. Recently it has been shown that there is a brain-specific metallothionein (MT-III) which may have important neurophysiological and neuromodulatory functions [65] and may be a target for Bi(III) in the brain. The structures of rat and human liver metallothionein both in the solid-state and solution have been solved by X-ray crystallography and NMR spectroscopy [66–69]. It consists of two domains (α and β), which enfold two metal–thiolate clusters (Fig. 6). The β-domain (N-terminal) contains nine cysteines and forms a cluster of three Cd or Zn ions liganded by three bridging and six terminal Cys thiolates. The α-domain (C-terminal) contains 11 Cys and forms a cluster of four Cd or Zn ions bound to five bridging and six terminal Cys thiolates. Each of the seven metal ions has tetrahedral coordination geometry.

Bismuth is known to be a potent inducer of renal metallothionein (MT) synthesis, and preadministration with bismuth can protect from some of the toxic side-effects induced by the anticancer drug cisplatin, without affecting anti-tumour activity in human patients [70–72]. The protection probably involves Bi(III) induction of metallothionein synthesis. However there appear to be few reported studies of the chemistry of Bi–metallothionein complexes. Strong binding of Bi(III) to metallothionein is expected, since bismuth has a higher affinity for thiolate ligands than for oxygen and nitrogen donor ligands.

Our recent work [73] has shown that Bi(III) binds very strongly to metallothionein with a stoichiometry Bi:MT = 7:1 and can readily displace both Zn(II) and Cd(II) in biphasic processes. In contrast to Zn(II) and Cd(II), bismuth is still bound to the protein even in strongly acidic solutions (pH ca. 1). ^{1}H-NMR studies have shown that both Zn(II) and Cd(II) in the β-domain (three metal cluster) of MT are displaced by Bi(III) much faster than from in the α-domain (four metal cluster). Extended X-ray absorption fine structure (EXAFS) spectroscopy indicates that Bi(III) is coordinated to three sulfur atoms with average Bi–S distances of 2.55 Å [73].

6.3.2. Transferrin

Transferrin is an 80 kDa glycoprotein which transports Fe(III) in blood, and is recognised by cell surface receptors (proteins) when fully loaded with Fe(III) in both its binding sites. The diferric protein is internalized by cells, placed in vesicles (endosomes) where the pH is lowered to 5.5 and the Fe(III) is released [74]. In human blood, transferrin is only ca. 30% saturated with Fe(III), and hence there is capacity (ca. 50 μM) for binding to other metal ions that enter the human body. Similar proteins have been found in mucous (lactoferrin) and recently in several Gram-negative bacteria (ferric-ion-binding protein, FBP) such as *Haemophilus influenzae*, *Neisseria meningitidis* (nFBP), *Serratia marcescens* (sFBP) and *Yersinia enterocolitica* (yFBP) [75]. Interaction of Bi(III) with the latter protein may be relevant to its antimicrobial activity.

Bismuth binds strongly to both N- and C-lobe iron binding sites of human serum transferrin and recombinant N-lobe transferrin [76,77]. The uptake of Bi(III) by

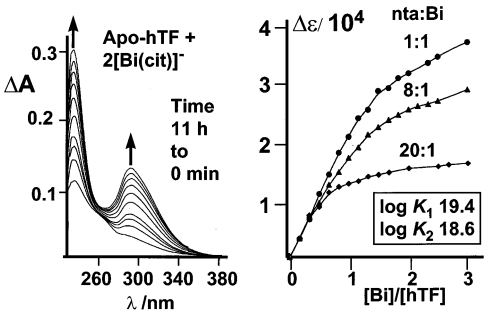


Fig. 7. Slow uptake of Bi(III) citrate complex by apo-transferrin in the presence of 5 mM bicarbonate at pH 7.4, 310 K (left) and determination of Bi–hTF binding constants via titration with various mol ratios of [Bi(III)(nta)$_x$] [76].

apo-transferrin from bismuth citrate complexes is very slow (hours at 310 K, Fig. 7) and occurs in at least two steps, whereas transfer from bismuth nitrilotriacetate is rapid (minutes). ^{13}C-NMR suggests that bismuth binds to transferrin along with carbonate (CO_3^{2-}) as a synergistic anion (Fig. 8), which is similar to Fe(III). This bound form of the anion cannot readily be removed even after extensive dialysis. Binding of Bi(III) occurs preferentially to the C-lobe of transferrin. This order of lobe-loading has been confirmed by 2D [^{1}H, ^{13}C] heteronuclear multiple-quantum coherence (HMQC) NMR studies using recombinant ε-[^{13}C]Met-hTF (Fig. 9) in which all nine Met residues are enriched with ^{13}C at the SCH_3 group. The most dramatic change is for Met464, a residue situated in the hydrophobic patch (V454-W460-M464) of helix five, which backs onto the metal binding site in the

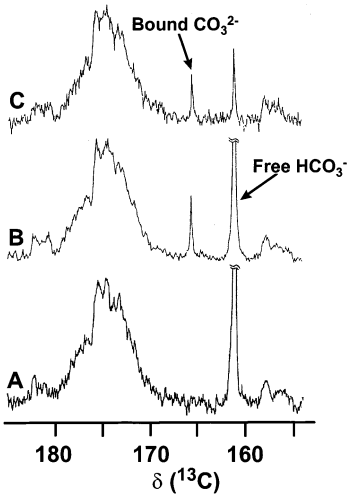


Fig. 8. ^{13}C-NMR spectra of hTF in the presence of 10 mM carbonate (pH 7.25) confirming binding of carbonate to Bi to form a ternary complex. (A) Apo-hTF in the presence of 10 mM bicarbonate. (B) After addition of 2 mol equivalent Bi(III) (as [Bi(nta)]), and (C) after dialysis.

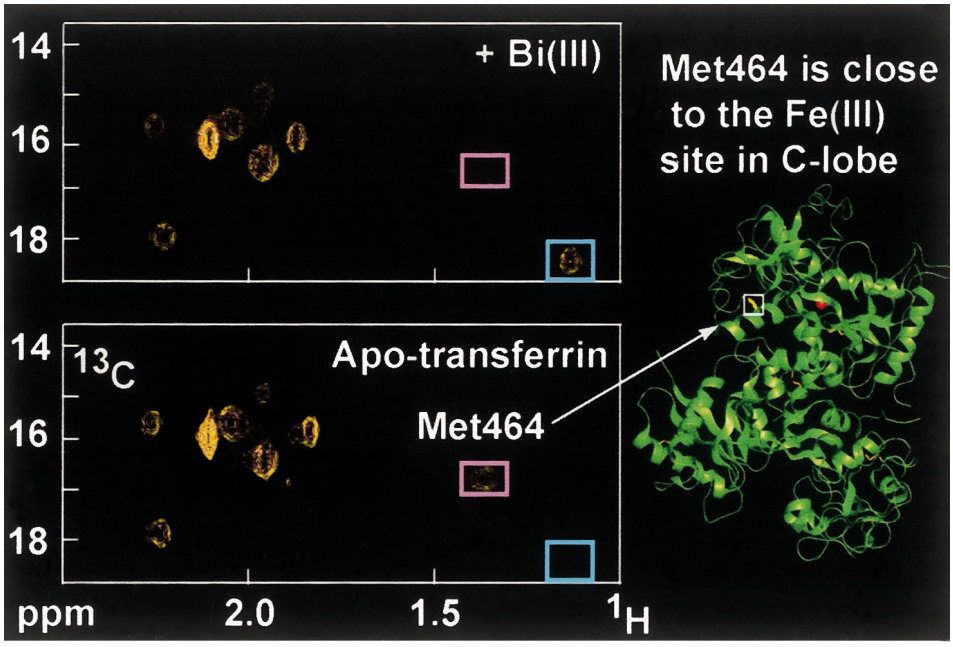


Fig. 9

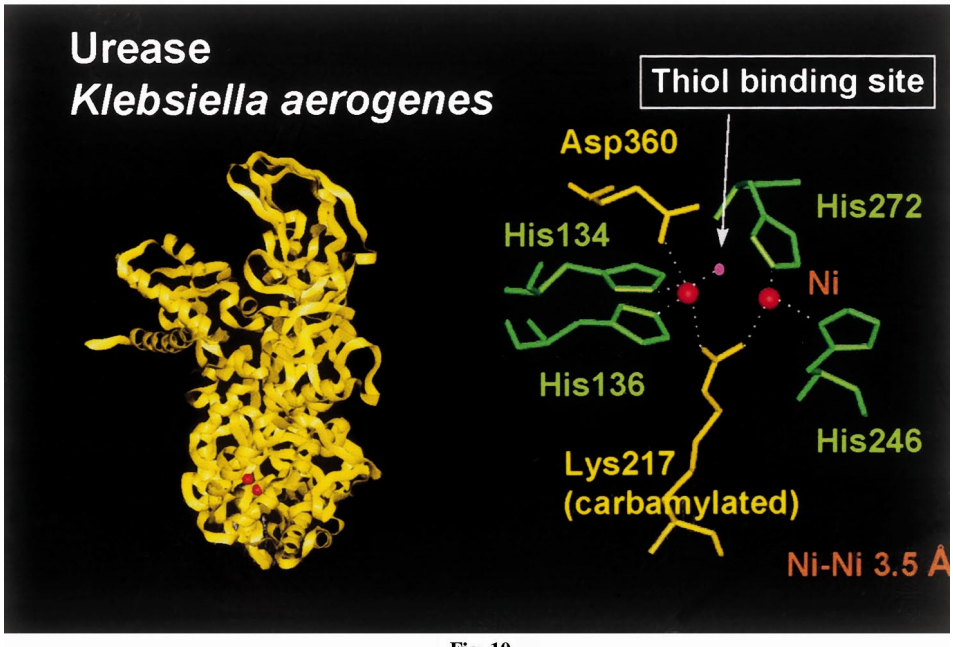


Fig. 10

Fig. 9. NMR determination of the order of lobe-loading (N- and C-lobe metal-binding sites) of human serum transferrin with Bi. 2D [^{1}H, ^{13}C] HMQC-spectra of ε-[^{13}C]Met-apo-hTF in the presence of 10 mM bicarbonate, pH 7.4, after addition of Bi complexes. The shift for the peak of Met464, which is in a hydrophobic patch of helix five and backs into the iron binding site in the C-lobe, indicates binding of Bi(III) preferentially to the C-lobe.

Fig. 10. X-ray crystal structure of urease from *Klebsiella aerogenes* (PDBid:1KRC) [88], showing the dinuclear Ni_2 site and the carbamylated lysine residue (Lys217).

C-lobe. The changes in shift of the ^{1}H-, ^{13}C-NMR resonances of Met can be used not only as a probe for determining the order of lobe-loading of transferrin with metal ions, but also as fingerprints of conformational changes induced by Bi(III) and other metal ions. Bi(III), Fe(III), Ga(III) and Al(III) probably induce similar conformational changes in hTF, since the changes in shifts of Met SCH_3 resonances are almost identical [78].

Although Bi(III) binds to human serum transferrin relatively strongly ($\log K_1 = 19.42$ and $\log K_2 = 18.58$, respectively, at 310 K, 5 mM bicarbonate, pH 7.4, Fig. 7), it can be displaced by the natural ion, Fe(III). Competition reactions between transferrin and citrate indicate that even in the presence of 100-fold excess of citrate, most of the Bi(III) can still bind to transferrin. The strong binding of Bi(III) to transferrin can be correlated with its high acidity [79,80]. This correlation allows prediction of the strong binding of other highly acidic metal ions such as Ti(IV) (used in anticancer agents) which has now been verified [81], and also suggests that the two tyrosine ligands play a dominant role in the strength of binding.

6.3.3. Enzyme inhibition

Enzyme inhibition is thought to play a role in the action mechanism of bismuth-containing drugs. The mode of action of CBS in the treatment of gastroduodenal disorders may involve the prevention of adhesion of *H. pylori* to epithelial cells and inhibition of enzymes secreted by *H. pylori*, such as proteases, lipases, glycosidases, and phospholipases [82]. It has also been found that CBS can induce a dose-dependent inhibition of phospholipases A (PLA2) and C in both *H. pylori* lysates and filtrates, and it has been suggested that bismuth binds to the calcium site of PLA2 [83]. Bismuth subcitrate inhibits F_1-ATPase, an enzyme involved in bacterial energy metabolism. The inhibition can be prevented and reversed by the thiol glutathione. Bi(III) can also inhibit the enzyme pepsin at pH 1.0–2.0 [84].

Various bismuth drugs can inhibit alcohol dehydrogenase (ADH) from *H. pylori* and consequently suppress acetaldehyde (toxic to mucosal cells) production from endogenous or exogenous ethanol (Eq. (2), where NAD^+/NADH is the dehydrogenase coenzyme) [85,86]. ADH contains two Zn(II) ions, which occupy two separate domains. One of them resides in the catalytic domain and is coordinated to two Cys thiolate groups, nitrogen from a His imidazole and to one water molecule. The other Zn(II) (structural) is bound to four thiolates of Cys residues [87]. The inhibition is probably due to the displacement of zinc(II) (structural) by Bi(III), since Bi(III) has a higher affinity for thiolate groups than Zn(II).

$$R - CH_2 - OH + NAD^+ \xrightarrow{ADH} R - CHO + NADH + H^+ \quad (2)$$

The unusual feature of *H. pylori* is its growth under highly acidic conditions. For this, it relies on the activity of the nickel-containing enzyme, urease. The recent X-ray crystal structure of urease from *Klebsiella aerogenes* shows the presence of a binuclear Ni(II) active site (Fig. 10). The two Ni(II) ions are 3.5 Å apart and

bridged by a carbamylated Lys residue [88]. One of the Ni(II) ions coordinates pesudo-tetrahedrally to two His nitrogens, one oxygen from bridging carbamylated Lys and one water oxygen. The second Ni(II) ion is five-coordinate and approximately trigonal-bipyramidal via one oxygen of the bridging carbamylated Lys, two His nitrogen, one carboxylate oxygen of Asp and one water oxygen. Bismuth complexes inhibit *H. pylori*-produced urease, and a Bi mercaptoethanol complex has ca. 1000 times higher activity than mercaptoethanol alone against urease [89]. Mercaptoethanol inhibits the enzyme both directly by bridging the two Ni(II) ions in the active site through the sulfur atom and indirectly by forming a disulfide bond with a Cys, hence sealing the entrance to the active site cavity [90]. Bismuth(III) inhibition of urease may play an important role in the antibactericidal activity of bismuth-containing drugs [91].

7. Conclusion and perspectives

Despite the widespread use of bismuth compounds in medicine, its chemistry and biochemistry are currently poorly understood. Recent work has begun to elucidate the structures of Bi(III) thiolates, carboxylates and aminocarboxylate complexes in particular. The occurrence of a highly variable coordination number (3–10) and irregular coordination geometry, together with the apparent activation of a strong lone-pair effect in certain complexes (e.g. those with alkoxide ligands) is highly characteristic of Bi(III), as is the strong acidity of Bi(III) aqua complexes. Complexes crystallized from aqueous solution commonly contain bridging carboxylate or oxo/alkoxide ligands and the chemistry of Bi(III) citrate antiulcer drugs appears to be dominated by polymeric species. The interaction of these polymers with membrane surfaces may be important to their bioactivity. The rates of ligand exchange on Bi(III) are highly variable and pH-dependent.

Little is known about the biocoordination chemistry of Bi(III) with proteins, enzymes and cell membranes although this could be very important to the biological activity. It appears that the target sites of Bi(III) in proteins and enzymes are both Fe(III) sites (e.g. N and O ligands in transferrin) and Zn(II) sites (e.g. S ligands in metallothionein). Glutathione forms a strong complex $[Bi(H_{-1}gsh)_3]$ which may be involved in transport of Bi(III) in cells and bacteria. Further exploration of the thermodynamics and kinetics of chemical and biochemical reactions of bismuth under biologically-relevant conditions is now warranted particularly in view of current interest Bi(III) antiulcer drugs, in the use of ^{212}Bi in radiotherapy, and the discovery of bismuth complexes with anticancer and anti-viral activity.

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Appendix A. Abbreviations

ADH	alcohol dehydrogenase
BSS	bismuth subsalicylate
CBS	colloidal bismuth subcitrate
dien	diethylenetriamine
edta	ethylenediamine tetraacetate
gsh	glutathione (γ-L-Glu-L-Cys-Gly)
H. pylori	*Helicobacter pylori*
hTF	human serum transferrin
ida	iminodiethanoate
IL2	interleukin2
MIC	minimum inhibitory concentration
MT	metallothionein
nac	*N*-acetyl-L-cysteine
nta	nitrilotriacetate
RBC	ranitidine bismuth citrate
trien	triethylenetetramine

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