12.1.3.7. *Effect of concentration, time of soak and temperature on heavy-atom binding*

Most heavy-atom derivatives are prepared by diffusing or dialysing the compound into the crystals. Concentrations have typically ranged from 0.1–100.0 m*M*. Occasionally, concentrations as low as 0.001 m*M* have been employed to maintain crystal integrity. Low concentrations favour sites where the interactions between the heavy atom and the protein ligands are strongest. Decreasing the number of non-specific interactions minimizes the amount of heavy-atom reagent in the lattice. The latter absorbs X-rays without contributing to the diffraction pattern except at low angles. Increasing the concentration may give rise to other binding site(s). Usually, the higher the concentration employed, the shorter the soak time required for equivalent substitution. Short soak times at high concentrations tend to denature the crystals more often than long soaks at low concentrations. At very high concentrations (*i.e.* >100 m*M*), the heavy-atom compound perturbs the protein crystal–mother liquor equilibrium by withdrawing water molecules from the hydration shell around the periphery of the crystal. Disorder of the crystals can sometimes be avoided by the application of a cross-linking reagent (*e.g.* glutaraldehyde). The optimal concentration is the lowest concentration that consistently reproduces intensity differences in the diffraction pattern of 15–25% without cracking and disordering the crystals.

Length of soak may be important. The heavy-atom data bank shows that, typically, soak times range from one day to one week. Useful derivatives have been prepared with a soak time of an hour to over a year. If no binding is apparent after several days, extending the soak time to over a week may produce some binding, but this is rare. Soaks of 24 hours for simple inorganic salts and up to one week for other types of heavy-atom compounds will normally suffice when screening for binding. The concentration of the heavy-metal compound that can be achieved will depend on its solubility in the crystal stabilization solution. Normally, the longer the soak, the greater the occupancy. Exceptions can arise due to undesirable chemical reactions between components present in the derivatization solution.

For covalent-bond formation, the length of soak and the concentration can be short (*e.g.* 1 h, 0.01 m*M*). This is especially true for mercury derivatives of proteins that have reactive sulfhydryls (Ringe *et al.*, 1983).

Variations in the temperature can also alter the rate of reaction. The UO_2 acetate derivative of rhombohedral insulin binds twenty times more slowly at 4 °C than at ambient temperature (Blundell, 1968). A lower temperature allows greater control over the rate of substitution. Conversely, heavy-atom derivatives that do not appear to bind may do so upon elevation of the temperature.

12.1.4. Amino acids as ligands

The reactivity of the heavy-atom reagent will also depend on the state of the amino-acid residues in the protein.

The thiolate anion of *cysteine*, a potent nucleophile, reacts almost irreversibly with mercuric complexes or organomercurials. It also acts as a fast-entering attacking group in S_{N2} ligand substitution reactions with other class B metals (*e.g.* Ag, Ir, Rh, Pt, Pd, Au), forming stable complexes. Below pH 6, the thiolate anion becomes protonated. As covalent reactions are less sensitive to hydrogen-ion concentration than ligand substitution reactions, cysteines still bind rapidly with mercurials, but there is negligible reaction with other class B metals (Petsko *et al.*, 1978).

Cystines are very weakly reactive in ligand substitution reactions. However, $PtCl_4^{2-}$ binds to disulfides in some proteins with displacement of a chloride ion (Lipscomb *et al.*, 1970; Sigler *et al.*, 1968). Mercurials rarely insert spontaneously into disulfide linkages. However, substitution of mercury can be achieved either by the prior application of a reducing agent such as dithiothreitol (Ely *et al.*, 1973; Sperling *et al.*, 1969), or by direct application of reducing mercurous ions (Sperling & Steinberg, 1974).

The non-ionizable thioether group of *methionine* is unreactive towards mercurials, but the lone pair of electrons on sulfur allows nucleophilic S_{N2} ligand substitution. Methionine will displace Cl, I, Br and NO_2 ligands from platinum complexes to form a stable bond. The reaction of methionine with platinum compounds is not pH sensitive within the normal range. The residue may become unreactive through oxidation, first to the sulfoxide and then to the sulfone; only the sulfoxide can be reduced readily by thiols or other reducing agents.

Below pH 6, *histidine* exists mainly as an imidazolium cation. Although this is not reactive as a nucleophile, it can interact electrostatically with anionic complexes. At pH 7 and above, the unprotonated imidazole is a good nucleophile, being able to displace Cl, Br, I and NO_2 ligands from platinum, silver, mercury and gold complexes. Electrophilic substitution of iodine in the imidazole ring is feasible, but the conditions are severe and it has not proved very useful in preparing derivatives.

At pH < 8.5, the ε-amino group of *lysine* is protonated, allowing it to form weak electrostatic interactions with anionic heavy-atom complexes, but not to participate in S_{N2} substitution reactions. Above pH 9, the free amino group can displace Cl but not Br, I or NO_2 ligands from platinum and gold complexes. The pK_a of the guanidinium group of *arginine* is very high (>12 in proteins), so it interacts electrostatically as a cation with heavy-atom anionic complexes.

The indole ring of *tryptophan* is relatively inert to electrophilic substitution by iodine, but the ring nitrogen can be mercurated (Tsernoglou & Petsko, 1976). The reaction is not pH dependent, but there should be no competing nucleophiles in the mother liquor. Tryptophan does not usually participate as a ligand in substitution of heavy-atom complexes.

The phenolate oxygen anion of *tyrosine* is a good nucleophile and has the potential to bind a substantial number of heavy-atom complexes *via* S_{N2} ligand substitution reactions. However, it has a very high pK_a value of 10.5. Below pH 10, the protonated oxygen predominates, making electrophilic aromatic substitution by iodine the principal reaction.

Aspartic and glutamic acids have side-chain pK_a values in the range 3 to 4. At low pH, they will be protonated and unreactive. Above pH 5, the side chains will be anionic, making them good ligands for class A cations such as uranyl and rare earths. *Glutamine and asparagine* take part in metal coordination but rarely bind strongly enough to metal ligands on their own.

Hydroxyl groups of *serines and threonines* are fully protonated at normal pH values and are consequently not reactive nucleophiles. Abnormally reactive serines, usually at the active site as in serine proteases and β-lactamases, can react with heavy-atom reagents to give useful derivatives.

12.1.5. Protein chemistry of heavy-atom reagents

The heavy-atom data bank (Islam *et al.*, 1998) can be used to analyse the most commonly used heavy-atom reagents: these are given in Table 12.1.5.1. This shows that platinum, gold, mercury and uranyl have provided the most useful reagents.

The heavy-atom data bank can be used as a source of information about the reactivity of proteins to different heavy-atom reagents. This provides the basis for the following analysis.

Copyright © 2006 International Union of Crystallography

Table 12.1.5.1. *The 23 most commonly used heavy-atom reagents*

The first column gives the number of times the reagent has been used in the analyses included in the heavy-atom data bank.

No.	Compound
287	Potassium tetrachloroplatinum(II)
111	Potassium dicyanoaurate(I)
103	Uranyl acetate
101	Mercury(II) acetate
98	Mercury(II) chloride
85	Ethylmercurythiosalicylate (EMTS)
82	Potassium tetraiodomercurate(II)
81	*para*-Chloromercuriobenzenesulfonate (PCMBS)
75	Trimethyllead(IV) acetate
73	Potassium pentafluorooxyuranate(VI)
73	Phosphatotris(ethylmercury)
61	Potassium tetranitritoplatinum(II)
60	Uranyl nitrate
58	Potassium tetracyanoplatinum(II)
57	Dichlorodiammineplatinum(II)
51	Potassium hexachloroplatinum(IV)
51	Methylmercury chloride
44	Potassium tetrachloroaurate(III)
42	*para*-Chloromercurybenzoate (PCMB)
39	Lead(II) acetate

12.1.5.1. *Hard cations*

Uranyl-ion complexes have proved the most popular A-group metal reagents for preparing heavy-atom derivatives of protein crystals (see Table 12.1.5.1). UO_2^{2+} is a linear, covalent group based on U(VI), the most stable oxidation state of uranium. Table 12.1.5.2 lists the most commonly used uranyl derivatives. Uranyl compounds may show $2+4$, $2+5$, or $2+6$ coordination, with ligands lying in or near a plane normal to the O═U═O^{2+} axis. These equatorial ligands may be neutral (*e.g.* H_2O) or anionic (*e.g.* NO_3^-, CH_3COO^-, oxalate^{2-}, F^-, Cl^- or O_2^-); the nitrate and acetate are bidentate ligands. An example is given in Fig. 12.1.5.1. Anionic complexes, such as $UO_2F_5^{3-}$, have been found near negatively charged amino-acid residues (*e.g.* Glu and Asp), suggesting that the equatorial ligands have been displaced. At low pH, uranyl groups have been located near the hydroxyl groups of threonine and serine residues.

The fifteen lanthanides have similar chemical properties and are generally used as nitrates, acetates or chlorides (Blundell & Johnson, 1976; Carvin, 1986). The lanthanide contraction, a steady decrease in size with increasing atomic number, allows selection of an ion with a radius that will give high occupancy and isomorphism. Gadolinium and samarium salts have the added advantage that the number of anomalous electrons is high.

Lanthanide ions have greater selectivity than the uranyl ion, which often forms clusters on the protein surface. Uranyl complexes and lanthanide ions are not very soluble above pH 7 and pH 9, respectively, due to the formation of hydroxides. Phosphate buffers should be avoided since they will compete for the heavy atom, often giving insoluble phosphates. In the presence of citrate, samarium is chelated and, since the citrate is difficult to replace, reaction may be inhibited. However, exchanging the buffer for Tris or acetate may enable a useful derivative to be obtained.

12.1.5.2. *Thallium and lead ions*

Thallium and lead can provide useful derivatives, especially in their lower oxidation states, Tl(I) and Pb(II), when they resemble class A metals. Owing to the non-group valence and presence of an inert pair of electrons, the ionic radii of Tl^+ (1.44 Å) and Pb^{2+} (1.21 Å) are greater than most class A metals. Thallous and plumbous cations prefer carboxylate rather than imidazole or sulfur ligands, although Pb^{2+} occasionally manifests its intermediate character by interacting with imidazole groups. Thallic (Tl^{3+}) and plumbic (Pb^{4+}) ions are similar to class B metals, showing preferential binding to soft ligands, but they are easily reduced in protein solutions.

12.1.5.3. *B-metal reagents*

The most useful members of the B-metal group, platinum, gold and mercury, give rise to an extensive range of heavy-atom compounds which form covalent, electrostatic and van der Waals complexes with proteins. Some compounds can bind to the protein molecule in different ways; for example, $PtCl_4^{2-}$ can bind either covalently to the thioether group of methionine or electrostatically with positively charged residues.

Mercury compounds have proved very successful for preparing heavy-atom derivatives of protein crystals (Table 12.1.5.1), mainly due to the ease of formation of covalent bonds with cysteine residues. An example is given in Fig. 12.1.5.2 in which mercuric chloride has been used to replace zinc in thermolysin. Hg^{2+} complexes are commonly two-coordinate linear and four-coordinate tetrahedral. The most popular mercury reagents are given in Table 12.1.5.3. The covalent character in Hg—*L* bonds, especially in the two-coordinate complexes, can cause solubility problems in aqueous solutions. However, an excess of an alkali metal salt (*e.g.* $HgI_2 + 2KI \rightarrow K_2HgI_4$) will often convert the compound to a more soluble anionic complex of the type HgX_4^{2-}, where $X = Cl^-$, Br^-, I^-, SCN^-, NCS^-, CN^-, SO_4^{2-}, oxalate^{2-}, NO_3^- or NO_2^-. In the presence of ammonium salts at high pH values, the cationic tetraammine complex, $Hg(NH_3)_4^{2+}$, tends to form. Variation in the

Table 12.1.5.2. *The five most popular uranium derivatives*

The first column gives the number of times the reagent has been used in the analyses included in the heavy-atom data bank.

No.	Compound
103	Uranyl acetate
73	Potassium pentafluorodioxyuranate(VI)
60	Uranyl nitrate
8	Uranium(VI) oxysulfate
4	Sodium triacetatedioxyuranate(VI)

Table 12.1.5.3. *The five most popular mercury derivatives*

The first column gives the number of times the reagent has been used in the analyses included in the heavy-atom data bank.

No.	Compound
101	Mercury(II) acetate
98	Mercury(II) chloride
85	Ethylmercurythiosalicylate (EMTS)
82	Potassium tetraiodomercurate(II)
81	*para*-Chloromercuriobenzenesulfonate (PCMBS)

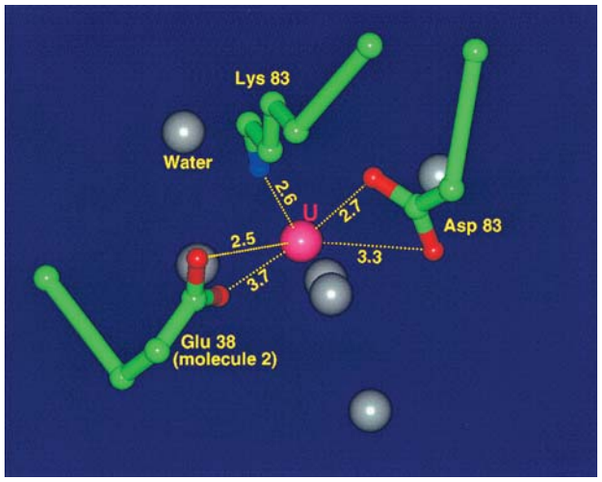

Fig. 12.1.5.1. The binding site for uranyl ions in cytochrome *b*5 (oxidized: 3B5C). The positions of the ligands in the parent crystals are shown; these probably move in the complex.

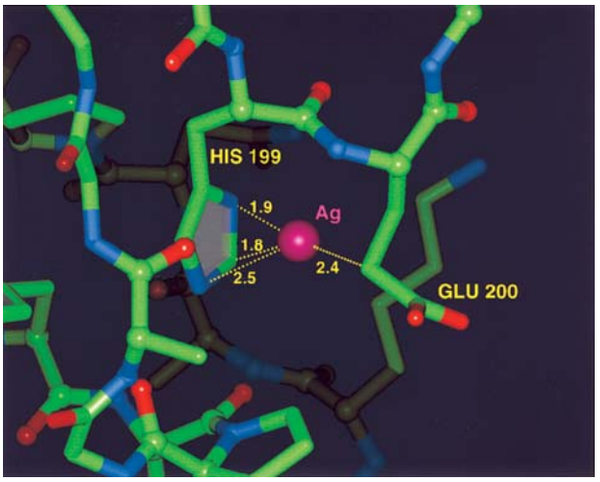

Fig. 12.1.5.3. The binding of a silver ion to immunoglobulin Fab (2FB4). The positions of the ligands in the parent crystals are shown, and these must move in the complex to coordinate the silver ion.

charge on the aromatic groups of organomercurials can give rise to different substitution patterns.

Silver, used as the nitrate, tends to interact with cysteine or histidine (see Fig. 12.1.5.3). In the presence of ammonium sulfate, it probably reacts as the ammonia complex, $Ag(NH_3)_4^+$. Silver ions are less polarizing and less reactive than Hg^{2+} ions; thus they give similar derivatives but often with less disorder, as in glucagon (Sasaki *et al.*, 1975). Where the metal ion displaces a proton, Ag^+ will need to react at a higher pH than Hg^{2+}.

The class B metals *palladium*, *platinum* and *gold* form stable covalent complexes with soft ligands, such as chloride, bromide, iodide, ammonia, imidazole and sulfur groups. The stereochemistry of their complexes depends on the number of *d* electrons present. For instance, the d^{10} ion of Au(I) gives a linear coordination of two [*e.g.* $Au(CN)_2^-$], whereas d^8 ions of Pd(II), Pt(II) and Au(III) are predominantly square planar, giving cationic [*e.g.* $Pt(NH_3)_4^{2+}$], anionic [*e.g.* $Au(CN)_4^-$, $PtCl_4^{2-}$ and $PdCl_4^{2-}$] or neutral [*e.g.* $Pt(NH_3)_2Cl_2$] complexes. These may accept an additional ligand to give square pyramidal coordination or two ligands to give octahedral coordination. The additional ligands are normally more weakly bound. Pt(IV) has a d^6 configuration and forms stable

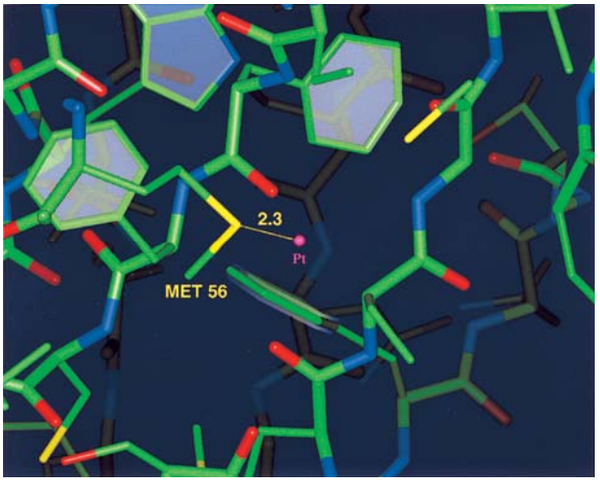

Fig. 12.1.5.4. The binding of $PtCl_4^{2-}$ through a methionine in azurin (1AZU).

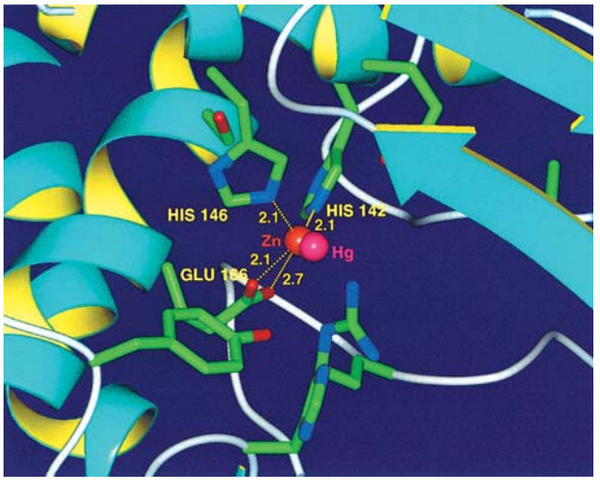

Fig. 12.1.5.2. Mercuric ions replace zinc in thermolysin (3TLN). The mercuric ion is shown superposed on the parent crystal structure; notice that the mercuric ion is slightly displaced from the zinc position due to its larger ionic radius.

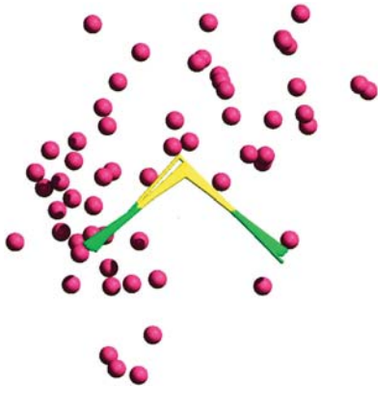

Fig. 12.1.5.5. The relative positions of methionine side chains (carbon: green; sulfur: yellow) in the parent crystals to the binding of platinum (pink) of $PtCl_4^{2-}$. The methionine side chains have been least-squares fitted.

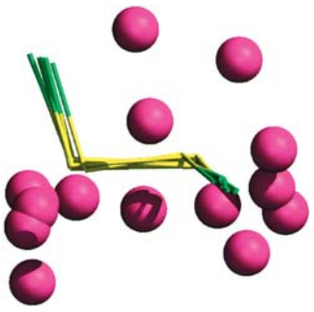

Fig. 12.1.5.6. The relative positions of cystine disulfide bridges (carbon: green; sulfur: yellow) in the parent crystals to the binding of platinum (pink) of $PtCl_4^{2-}$. The cystine side chains have been least-squares fitted, and only those with torsion angles in the range $99.7 \pm 8.3°$ have been used.

octahedral complexes, such as $PtCl_6^{2-}$, with six equivalent covalently bound ligands.

The kinetic and thermodynamic stability of these complexes depends on the protein ligands, buffer, pH and salting in/out agent (Petsko *et al.*, 1978). Anionic groups do not readily react with anionic reagents, such as RS^-, but are attacked more readily by neutral nucleophiles such as *R*SH, *R*-imidazole or RNH_2. The inert cationic group $Pt(NH_3)_4^{2+}$ is most likely to form electrostatic complexes with anionic groups, such as carboxylate. The neutral $Pt(NH_3)_2Cl_2$ molecule, however, can penetrate into hydrophobic areas but requires a stronger nucleophile such as RS^-. In acidic and neutral solutions, $PtCl_4^{2-}$ reacts most commonly with methionine (Figs. 12.1.5.4 and 12.1.5.5), cystine (disulfide) (Fig. 12.1.5.6), N-termini and histidine to form stable complexes. However, methionine reacts faster than histidine. Thus, it is possible to use time as a variable to define specificity. The most popular platinum reagents are listed in Table 12.1.5.4.

In aqueous solution, the square-planar complex $AuCl_4^-$ is hydrolysed to $Au(OH)_4^-$ in about one hour, or in the presence of a protein, reduced to Au(I) by methionine. In ammonium sulfate it probably exists as $AuCl_3(NH_3)$, $AuCl_2(NH_3)_2^+$ and $Au(NH_3)_4^{3+}$. In contrast, $Au(CN)_2^-$ is more stable and normally binds electrostatically. However, on occasions at pH > 6.0, the $Au(CN)_2^-$ complex has bound to cysteine residues by nucleophilic displacement reactions.

Osmium resembles platinum in many ways and typically acts as a class B metal. It occurs in all oxidation states from 0 to VIII, but most usually in III, as in K_3OsCl_6; in IV, as in K_2OsCl_6; in VI, as in $K_2OsO_2(OH)_4$; and in VIII, as in osmium tetraoxide, OsO_4. Higher-oxidation-state compounds tend to be reduced to $OsO_2(OH)_2$ in most crystallization solutions and in the presence of ammonia or halide ion they can become further reduced to cationic or anionic complexes, such as $Os(NH_3)_6^{3+}$ or $OsCl_6^{2-}$. Anionic complexes may be substituted by histidine residues at pH > 7.0 or bound as ion pairs by histidine at pH < 7.0 or protonated amino groups. Cationic complexes tend to bind to negatively charged residues *via* electrostatic interactions.

Iridium is found in all oxidation states from II to VI but commonly exists in III, as in K_3IrCl_6, and IV, as in $(NH_4)_2IrCl_6$. Ir(III) is similar to *rhodium*(III) and is found in a variety of cationic, uncharged and anionic complexes. All Ir(III) complexes are kinetically inert, whereas most anionic complexes of Rh(III) are labile. Ir(IV) is commonly found as the hexahalo complexes IrX_6^{2-} (except iodine), which are also fairly kinetically inert. Cationic [*e.g.* $Ir(NH_3)_6^{3+}$], neutral (*i.e.* $IrCl_3$) and anionic (*i.e.* $IrCl_6^{2-}$) species have proved useful in forming derivatives of protein crystals.

12.1.5.4. *Electrostatic binding of heavy-atom anions*

Positively charged groups of proteins, such as the α-amino terminus, ε-amino of lysine, guanidinium of arginine and imadazolium of histidine, may form ion pairs with heavy-atom anionic complexes. For example, HgI_4^{2-} and HgI_3^- can bind through electrostatic interactions. Anionic metal cyanide complexes tend to be more resistant to substitution and consequently interact electrostatically on most occasions. For example, $Pt(CN)_4^{2-}$ binds at several sites involving lysine or arginine residues in proteins (Fig. 12.1.5.7). $Pt(CN)_4^{2-}$ and $Au(CN)_2^-$ can act as inhibitors by binding at coenzyme phosphate sites.

12.1.5.5. *Hydrophobic heavy-atom reagents*

Since many heavy-atom reagents are hydrophilic, most interactions occur at the protein surface. However, substitution, addition or removal of the non-heavy-atom component(s) of the reagent can alter the hydrophilic–hydrophobic balance and lead to penetration of the core. For example, anionic complexes such as $HgCl_4^{2-}$ and $PbCl_6^{2-}$ are hydrophilic and would not normally enter the protein core, although organometallics, such as *R*HgCl and R_3PbCl (*R* = aliphatic or aromatic), are much more hydrophobic and can do so.

Hydrophobic organomercury compounds of the general formula *R*Hg*X*, where *R* is an aliphatic or aromatic organic group, react with sulfhydryls through displacement of *X*. When *X* is PO_4^{3-}, SO_4^{2-} or NO_3^-, the bond is ionic, making the formation of the cation RHg^+ easier. *R* is often chosen to be a small aliphatic group (*e.g.* CH_3, C_2H_5). However, the presence of a benzene ring enhances the

Table 12.1.5.4. *The five most popular platinum derivatives*

The first column gives the number of times the reagent has been used the analyses included in the heavy-atom data bank.

No.	Compound
287	Potassium tetrachloroplatinum(II)
61	Potassium tetranitritoplatinum(II)
58	Potassium tetracyanoplatinum(II)
57	Dichlorodiammineplatinum(II)
51	Potassium hexachloroplatinum(IV)

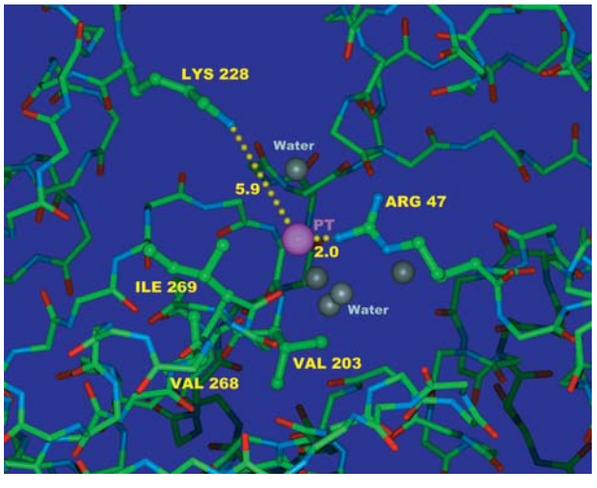

Fig. 12.1.5.7. The binding of $Pt(CN)_4^{2-}$ to aldose dehydrogenase (8ADH).

stability of the heavy-atom reagent. Careful selection of the *X* group can assist penetration into the hydrophobic core. The hydrophobicity of *X* follows the order

$$PO_4^{3-} < NO_3^- < Cl < Br < I < R.$$

*R*Hg*R* (*R* = aliphatic or aromatic) compounds also bind sulfhydryl residues in hydrophobic regions. The mechanism of reaction of methylphenylmercury with buried sulfhydryl groups may involve fast dissolution in the hydrophobic interior of the protein followed by a slow reaction with neighbouring sulfhydryl residues (Abraham *et al.*, 1983). They are difficult to prepare in aqueous solutions; an aprotic solvent, such as acetonitrile, can improve solubility, but this is not normally a problem in high concentrations of organic components, such as PEG, MPD or ethanol.

Inert gases were first used in the analysis of myoglobin. Schoenborn *et al.* (1965) discovered that the hydrophobic site that bound HgI_3^- also bound a xenon atom at 2.5 atmospheres. They proposed that this may be a general way of producing heavy-atom derivatives of proteins. Recently, there has been increasing interest in this idea, which has now been developed to produce well defined derivatives of a wide range of different proteins. Crystals are subjected to high gas pressures. Xenon requires about 10 atmospheres in order to get saturated binding sites. Krypton binds much less strongly and requires around 60 atmospheres. Since the binding of both inert gases is reversible, it is necessary to keep the protein crystals in a gaseous environment in a specialized pressure cell. Such pressure cells have been developed by Schiltz (1997) at LURE. Xenon binds to hydrophobic cavities, with little conformational change and a retention of isomorphism in crystals. Krypton binds at the same sites as xenon, but since it is lighter and needs higher pressure it has been exploited less by protein crystallographers. However, it has a well defined *K* edge at around 1 Å and so has attractions for multiple-wavelength anomalous dispersion.

12.1.5.6. *Iodine*

In addition to their use in isomorphous replacement, iodine derivatives of crystalline proteins have been prepared as tyrosine or histidine markers to assist main-chain tracing and to act as a probe for surface residues. The order of reactivity towards these reactive residues is

$$\text{Tyr} > \text{His} \gg \text{Trp}.$$

I_3^-, I^-, I^+ and I_2 can be generated by several different methods. An equimolar solution of KI/I_2 or NaI/I_2 in 5% (*v*/*v*) ethanol/water solution is often used to generate the anionic species I_3^- and I^-. An oxidizing agent, such as chloramine T, can be added to KI, typically in a concentration ratio of 1:50; alternatively, polystyrene beads derivatized with *N*-chlorobenzene sulfonamide can be used with NaI. Similarly, the addition of excess KI to ICl or OI^- will generate I_3^-, I^- and I^+. To avoid oxidation of iodine solutions, the pH should be less than 5.0. To avoid cracking the crystals, it may be necessary to increase the iodine concentration very slowly and to wash the derivatized crystals in the mother liquor in order to remove free I_2. Mono- or di-iodination of tyrosines can cause disruption of the protein structure either because of the larger size or the breaking of hydrogen bonds due to lowering of the pK_a of the phenolic hydroxyl.

12.1.5.7. *Polynuclear reagents*

The structure determination of large multicomponent systems such as the 50S ribosomal subunit (Yonath *et al.*, 1986) or the nucleosome core particle (O'Halloran *et al.*, 1987) requires the addition of reagents with a greater number of electrons, preferably in a compact polynuclear structure. Such reagents may be either cluster compounds or multimetal centres having metal–metal bonds.

Polynuclear reagents should preferably be covalently bound to one or a few specific sites, either first in solution or later in the crystals. Spacers of differing length can be inserted into the reagent to increase accessibility. Their low solubility in aqueous solutions can often be overcome by dissolving them in an apolar solvent (*e.g.* acetonitrile). Tetrakis(acetoxymercurio)methane (TAMM) and di-*m*-iodobis(ethylenediamine)diplatinum(II) nitrate (PIP) have better solubility in aqueous solutions than other polynuclear heavy-atom compounds.

Polynuclear heavy-atom reagents give an enhanced signal-to-noise ratio in low-resolution MIR studies, but this advantage is offset by the fall-off in scattering amplitude that arises from interference of diffracted waves at higher resolution. In the nucleosome core particle, the scattering reached 50% of its zero-angle value at 7.0 Å, while the relative drop for a single heavy atom was 10% (O'Halloran *et al.*, 1987). Cluster and multimetal reagents that have been successfully employed in protein structure determinations have been reviewed by Thygesen *et al.* (1996).

12.1.6. Metal-ion replacement in metalloproteins

The metal-ion cofactor can sometimes be displaced by dialysis or diffusion by a heavy-atom solution, but usually the cofactor is first removed by a chelating agent (*e.g.* EDTA) or by acidification. These are best carried out on the crystals. Alternatively, the metal

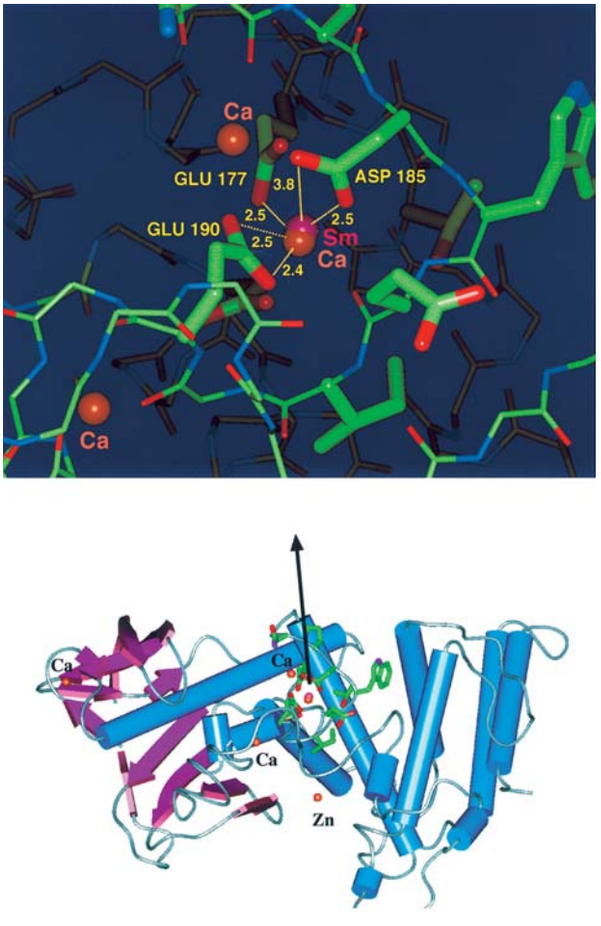

Fig. 12.1.6.1. The displacement of calcium by samarium in thermolysin. The samarium of the heavy-atom derivative is shown superposed on the parent crystal structure.

references