Table 23.2.3.1. *Metal ions associated with proteins*

Metal ion	Concentration in blood plasma (m*M*)	Common cofactors	Hard/soft classification	Common coordination number and geometry	Preferred ligand atom
Na^{+}	138		Hard	6	O
K^{+}	4		Hard	8	O
Ca^{2+}	3		Hard	8	O
Mg^{2+}	1		Hard	6	O
Fe	0.02	Haem	Intermediate	6 (octahedral)	N
Zn^{2+}	0.02		Intermediate	4 (tetrahedral), 6 (octahedral)	N, S
Cu^{2+}	0.015		Soft	4 (tetrahedral)	S
Co^{2+}	0.002		Hard	6 (octahedral)	O
Mn^{2+}	0		Hard	6 (octahedral)	O
Ni^{2+}	0		Intermediate	6 (octahedral)	N
Mo	0	Pterin	Intermediate	6 (octahedral)	S
W	0	Pterin	Intermediate	6 (octahedral)	S
V	0			5 (trigonal bipyramidal)	

hydrogen bonding generally follows a simple pattern in which the carbohydrate hydroxyl accepts a hydrogen bond from a protein amide group while simultaneously donating a hydrogen bond to a protein carbonyl oxygen. Hydrogen bonding to protein hydroxyl groups is observed only infrequently. This pattern is thought to be a result, in part, of the entropic cost of fixing a freely rotating protein hydroxyl group while simultaneously fixing the ligand hydroxyl group. Amides and carbonyls are usually fixed in a planar geometry and thus do not require as much energy to compensate for their loss of entropy in ligand binding.

The vicinal hydroxyl groups of carbohydrates provide an ideal geometry for the formation of 'bidentate' hydrogen bonds, where the pair of hydroxyls interacts with two functional groups of a single amino-acid side chain or the main-chain amide groups of two consecutive residues (Fig. 23.2.2.1). These interactions occur when the adjacent carbohydrate hydroxyls are either both equatorial, or one is equatorial and the other axial. The interatomic distance for the carbohydrate hydroxyl oxygens is ∼2.8 Å in these cases, allowing for a bidentate interaction with the planar side chains of aspartate, asparagine, glutamate, glutamine and arginine. Bidentate hydrogen bonds have not been observed for consecutive axial hydroxyls where the oxygen–oxygen distance increases to ∼3.7 Å.

Carbohydrates often present a disc-like face of non-polar aliphatic hydrogen atoms which proteins recognize through the use of aromatic side chains. The protein aromatic groups are 'stacked' on the flat face of the carbohydrate, thus generating both specificity and binding energy through van der Waals interactions. Tryptophan, the aromatic amino acid with the largest surface area and highest electronegativity, is the most common side chain employed in van der Waals 'stacking' with carbohydrates. The infrequent use of aliphatic groups in the binding of the non-polar carbohydrate faces suggests that the aromatic moieties are employed in a specific manner. The electron-rich electron clouds of the aromatic side chains may provide a strong electrostatic interaction with the aliphatic carbohydrate protons that could not be satisfied by protein aliphatic groups. The anionic character of aromatic side chains is observed in a number of protein–intramolecular (Chapter 22.2) and protein–ligand interactions (see below).

23.2.3. Metals

Metal ions provide a number of important functions in their diverse and ubiquitous interactions with proteins. The most common function for a protein-bound metal ion is the stabilization and orientation of the protein tertiary structure through coordination to specific protein functional groups. In addition to this structural role, metal ions are also often directly involved in enzyme catalysis and protein function. Examples of these functions include redox reactions, the activation of chemical bonds and the binding of specific ligands. Myoglobin, the first protein structure determined by X-ray crystallography, specifically binds molecular oxygen through an iron ion of a haem cofactor. Myoglobin provides a prototypic example of a protein and a metal ion providing a unique and specific functionality through their combination.

23.2.3.1. *Metals important in protein function and structure*

A number of metals are relatively abundant and available in living systems (Table 23.2.3.1) (Glusker, 1991). The most common ions include sodium, potassium, magnesium and calcium. Along with these ions, a large variety of trace metals are also found coordinated to proteins. The structures of protein complexes with some of these trace ions, including iron, zinc and copper, have been studied extensively for some time (Glusker, 1991). More recently, the structures of protein complexes with more unusual ions, such as nickel, vanadium and tungsten, have been determined (Volbeda *et al.*, 1996).

Specificity in the interactions between proteins and metal ions is conferred through each ion's preference for the coordinating atoms and the geometry of the binding site. All four of the more common metals, *i.e.* sodium, potassium, magnesium and calcium, are classified as 'hard' metals, referring to the polarizability of the electron cloud of the ion. The nucleus of a hard metal has a relatively tight hold on the surrounding electrons. These ions lack easily excitable unshared electrons and have a low polarizability. The interactions between these metals and their ligands tend to have the character of ionic interactions rather than the more covalent nature preferred by the 'soft' metals. In general, the hard metals prefer to coordinate with hard acids, such as the oxygen atoms of hydroxyls, carbonyls and carboxyls.

The soft metals have a high polarizability, large ionic radius and several unshared valence electrons. They generally prefer to coordinate with soft acids, such as the thiol and thiol ether groups of cysteine and methionine. The loosely held valence electrons of soft metals tend to favour partially covalent π-bonding with their coordinated ligands. These outer-shell electrons can be donated to the empty outer orbitals of the ligand atom. The partially covalent nature of these bonds yields more stable complexes than the ionic

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