Boron-Binding-Biomolecules: a Key to Understanding the Beneficial Physiologic Effects of Dietary Boron from Prokaryotes to Humans

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1. INTRODUCTION

Boron is an essential element for plants (Lovatt, 1985) and embryological development in fish (Rowe and Eckert, 1999) and frogs (Fort et al., 1999) does not proceed normally in the absence of extracellular boron. There is evidence that higher animals (Hunt and Idso, 1999; Armstrong et al., 2000) and humans (Hunt et al., 1997; Nielsen et al., 1992; Travers et al., 1990) require physiological amounts of boron to support normal biological functions. Despite the progress made in studies of boron essentiality for both plants and animals, the biochemical mechanisms responsible for the beneficial physiologic effects of boron across the phylogenetic spectrum are poorly understood. However, the unique nature of boron biochemistry suggests specific lines of investigation. This review summarises some of the progress made in understanding the essential roles of boron at the molecular level.

2. BORON CHEMISTRY, BONDING, AND COMPLEXATION

Organoboron compounds are defined for this discussion as those organic compounds that contain B-O bonds, i.e., the orthoborates \((B(OR)_3), (RO)B(OR')_2\) and \((RO)B(OR')(OR'')\); \(R = \text{alkyl or aryl group}\) and orthoborates of polyhydric alcohols. Organoboron compounds include B-N compounds, because B-N is isoelectronic with C-C (Greenwood, 1973). Organoboron complexes occur in plants, and are most likely present in animal and human tissues. Experimental evidence to date suggests these organoboron complexes are the result of interaction with either OH or amine groups as described below. For a typical trivalent boron compound, BX₃, including the physiologically important orthoboric acid, the B-X σ-bonding electrons can be regarded as occupying three equivalent \(sp^3\) hybrid orbitals that results in a trigonal-planar configuration of the molecule with the substituents disposed at 120° angles about the central boron atom. However, the boron octet is incomplete because there are fewer electrons available than atomic orbitals. That is, the remaining boron orbital of principal quantum number two, the \(p_z\), is vacant, or at least partially so, and lies perpendicular to the plane of the molecule (Coyle and Stone, 1964). This vacant orbital is available for the formation of a fourth covalent bond to the boron atom. An important consequence of the incomplete boron octet in BX₃ compounds is the availability of their dative electron pair for complex formation (Cotton and Wilkinson, 1972). The electron donor-acceptor interaction forms a coordination complex that involves considerable rehybridisation of the boron orbitals with the final bonding configuration of the boron atom in the complex close to \(sp^3\). The interaction also changes the boron bonding configuration from planar-trigonal to tetrahedral (Coyle and Stone, 1964).

The most probable form of boron after ingestion and subsequent hydrolysis (Greenwood and Earnshaw, 1984) is orthoboric acid (common name: boric acid) \(B(OH)_3\) and it has unique chemical properties that aid in the search for boron-binding biomolecules. For example, boric acid is an exclusively monobasic acid and is not a proton donor, but rather accepts a hydroxyl ion (a Lewis acid) and leaves an excess of protons to form the tetrahedral anion \(B(OH)_4^-\) (Reaction [1]) (Greenwood, 1973):

\[
B(OH)_3 + 2H_2O \leftrightarrow H_3O^+ + B(OH)_4^- \quad \text{pK}_a = 9.25 \ (25^\circ C) \quad (1)
\]

Thus, at typical physiological boron concentrations \((6.0 \cdot 10^{-7} \sim 9.0 \cdot 10^{-3} \text{ mol/L})\) in plants, animals, or humans, inorganic boron is essentially present
only as the monomeric species boric acid $\text{B(OH)}_3$ and as borate $\text{B(OH)}_4^-$ (Weser, 1967). Polyborate species can form near neutral physiological conditions ($\text{pH} \sim 7.4$) when borate concentrations exceed $\sim 0.025 \text{ mol/L}$ (Power and Woods, 1997), an unusually high boron concentration in biological systems, but still lower than that found in the snap bean leaf (0.1 mol/L) (Dugger et al., 1957). Within the normal pH range of the gut and kidney, $\text{B(OH)}_3$ would prevail as the dominant species ($\text{pH} 1: \sim 100\% \text{ B(OH)}_3$; $\text{pH} 9.3: 50\%$; $\text{pH} 11: \sim 0\%$) (Spivack and Edmond, 1987).

3. **BORON BIOCHEMISTRY**

3.1 **Boroesters**

Many biomolecules contain one or more hydroxy groups and those with suitable molecular structures can react with boron oxo compounds to form boroesters, an important class of biologically relevant boron species. Several types of boron esters exist. Boric acid reacts with suitable dihydroxy compounds to form corresponding boric acid monoesters ("partial" esterification) (e.g., Structure 1) that retain the trigonal-planar configuration and no charge.

In turn, a boric acid monoester can form a complex with a ligand containing a suitable hydroxyl to create a borate monoester ("partial" esterification; monocyclic) (Structure 2) but with a tetrahedral configuration and a negative charge. A compound of similar configuration and charge is also formed when borate forms a complex with a suitable dihydroxy compound.

![Structure 1](image)

The two types of boromonoesters can react with another suitable dihydroxy compound to give a corresponding spiro-cyclic borodiester ("complete" esterification) that is a chelate complex with a tetrahedral configuration and negative charge (Structure 3) (Van Duin et al., 1984). A partially esterified tridentate cleisto complex (Structure 4) may be formed.
when a ligand contains three suitably cis-oriented hydroxyl groups (Bell et al., 1986).

Boric acid and boric acid-like structures, instead of borate, are most likely the reactive species with biological ligands, because it is probably easier for a diol to substitute for a relatively loosely bound water molecule associated with boric acid or a boric acid-like structure than it is for the diol to substitute for a hydroxyl ion in borate or a borate-like structure of differences in charge (Van Duin et al., 1984). Thus, the boration of dihydroxy compounds is a three-step process (Scheme 1) that involves, in sequence, the addition of the first substrate hydroxyl group to boric acid to yield an anionic borate monoester ROB(OH)₂⁻, the elimination of one hydroxyl group from ROB(OH)₃⁻ to yield the uncharged boric acid monoester ROB(OH)₂, and the addition of the second substrate hydroxyl group to give the cyclic borate monoester. The third step in this boration process is assumed to be fast and the first two to be rate limiting transition states (Johnson and Smith, 1976).

3.1.1 Environment pH and ligand structure

Of great importance is the fact that borate complexes often have a much lower pKa (e.g. boron-mannitol, 5.2) than free boric acid (9.25)] which means that the pKa of many boron complexes is considerably below physiological pH (Raven, 1980). That is, when boron forms covalent bonds with biological ligands, its pKa is reduced to ~6, and the majority of the boron in ligand complexes are in the negatively charged borate (H₄BO₄⁻) form while occupying the binding site (Weser, 1967). This phenomenon has functional consequences. For example, boric acid is a competitive inhibitor of Streptomyces griseus Protease 3 and there is decreased affinity of the protein for boric acid below pH 7.

\[ \text{(Structure 2)} \]
This is directly attributable to the stability of the enzyme inhibitor complex that is dependent upon an ionising group in the protein with an apparent pKₐ of 6.6. (Bauer and Pettersson, 1974). This indicates that boric acid is an inhibitor of the enzyme at physiological pH by shifting the pKₐ.

Only certain diol ligands can react with boric acid or borate because the resultant borosether must contain O-B-O angles that do not exceed the limits of tolerable strain on the bond. That is, the strongest complexes are formed when a diol contains OH groups oriented in such a way that they accurately match the structural parameters required by a tetrahedrally coordinated boron (Power and Woods, 1997). Typically, ligands that contain adjacent cis hydroxyl groups are most likely to react with B oxo compounds to form a borosether, and the reactivity of boric acid with the ligand generally increases with in proportion to the number of these cisoid groups (Zittle, 1951). For example, boric complexes readily with many cis 1,2-diols to form borodiesters with the boron atom in the tetrahedral configuration because the resultant five-membered ring does not contain any strain. On the other hand, boromonoesters are formed more readily from cis 1,3-diols than from cis 1,2-diols because the former allow much better attachment of the trigonal-planar boric acid to form an unstrained six-membered ring. For this reason boromonoesters of 1,2-diols are not considered likely in aqueous biochemical systems (Weser, 1967).
A boroester is sometimes formed without the presence of a cisoid-diol group because of stereochemistry that limits bond strain. For example, certain ring conformations in trans-1,2-diols favor complex formation with boron (Zittle, 1951). Also, hydroxyl groups arising from lactol formation (in glucose, alpha-D-form only), hydration of COOH groups (in α-hydroxy, e.g. lactic; and aromatic α-hydroxy acids, e.g. salicylic acid), or hydration of ketone groups (in benzil or alloxan) are reactive with B(OH)₃ (Zittle, 1951). In some situations, the stereochemistry is correct for the reaction of borate with the hydroxyl groups in 1,3 positions (e.g., in pyridoxine) (Scudi et al., 1940). Substitution of one or more of the hydroxyl groups coordinated with boron, or substitution of individual hydrogens in those hydroxyl groups gives rise to many classes of cyclic coordination complexes.

\[
\text{M}^+ \left( \begin{array}{c}
\text{O} \\
\text{O} \\
\text{B} \\
\text{O} \\
\text{CH}_2 \\
\text{CH}_2 \\
n
\end{array} \right)
\]

(Structure 3)

Biomolecules possessing vicinal cis-diols or proximal hydroxyls in the correct orientations form boroesters with association constants that vary from weak to highly stable (Van Duin et al., 1984; Thellier et al., 1979; Loomis and Durst, 1992). The esterification reaction that produces boromonoesters (e.g., Structure 1) is easily reversible. That is, the ester is completely hydrolysed when transferred into water. Because boromonoesters are easily hydrolysed when placed in aqueous solutions, it seems reasonable that boromonoesters may be isolated from hydrophobic environments (e.g., the lipid portions of the plasmalemma) where the absence of water shifts the equilibrium to the right (Reaction 2) (Greenwood, 1973).

\[
\text{B(OH)}_3 + 3\text{ROH} \rightleftharpoons \text{B(OR)}_3 + 3\text{H}_2\text{O} \quad \text{(Reaction 2)}
\]

The discovery of the currently recognised boron dependent biomolecules was achieved because the bound boron formed four coordinate covalent bonds with the ligand, creating a thermodynamically stable complex (Structure 3) that is almost undissociable in water (Thellier et al., 1979; Gerrard, 1961). However, many boron interactions with biological ligands are rapidly reversible (Berezin et al., 1967; Hausdorf et al., 1987) and difficult to detect with current techniques.
3.2 N-B Compounds

A variety of important biological complexes are formed when nitrogen acts as an electron-pair donor to fill the vacant boron pz orbital. For example, experimental evidence (Bachovchin et al., 1988) suggests that the mechanism for inhibition of a sub-subclass of enzymes (the serine proteases) by boron involves formation of a covalent bond between boron and a specific nitrogen at the active site of these enzymes. By way of further example, the N\textsuperscript{\textepsilon 2} of histidine-57 of \(\alpha\)-lytic protease and the boron atom of a peptide boronic acid interact to form a covalent bond and give rise to a reversible complex (Structure 5).
4. SPECIFIC BORON-BINDING BIOMOLECULES

4.1 Boron and natural selection of carbohydrate energy sources

There may have been considerable evolutionary pressure exerted to select for carbohydrate energy sources that do not interact with boron. Sugars often form intramolecular hemiacetals and those with five-membered rings are called furanoses and those with six-membered rings are called pyranoses. In cases where either five- or six-membered rings are possible, the six-membered ring usually predominates for unknown reasons (Zubay, 1988). In general, the most stable borate esters are formed when boron reacts with compounds that have cis-diols on a furanoid ring. The furanoid ring structure restricts the mobility of the reactive –OH groups and thereby forces them to stay close to the optimum O-O distance for anionic borodiester formation. Furthermore, the ring oxygen and substituent oxygens in the furanoid ring activate the –OH groups by electron withdrawal (Loomis and Durst, 1992). A ligand with a dihedral angle of 0° to ~40° for the O-C-C-O group is suitable for the formation of stable anionic borate diesters. A corresponding O-O center-to-center distance (2.49 Å to ~2.63 Å) is thought to be present in structures with a furanoid ring (but not a pyranoid ring) (Mazurek and Perlín, 1963, cited in Loomis and Durst, 1992).

Cisoid pyranose rings, as well as cisoid furanose rings, favour the chair conformation and biochemicals with the former ring structure would have to assume the boat conformation to bring the cisoid groups close enough together to react with boric acid (Loomis and Durst, 1992).

Therefore, compounds in a configuration where there are cis-diols on a furanoid ring (e.g., ribose, apiose, and erythritan) form stronger complexes with boron than do compounds configured to have cis-diols predominately on a pyranoid ring (e.g., the pyranoid form of α-D-glucose). Fructose in solution has a large furanose component while glucose is virtually all in the pyranose form (Loomis and Durst, 1991). The relevant cis-diol conformations are not present in glucose and galactose or their immediate derivatives (Raven, 1980). However, the conformations are present in several biologically important sugars and their derivatives (sugar alcohols, -onic and -uronic acids), including some polymers. Common examples include mannitol, mannose, mannan, and polymannuronic acid, ribitol, ribose, erythritol, erythrose and glycerol.
The equilibrium constant for a boric acid complex with D-glucose \( (k_2 = 7.60 \times 10^0) \) is considerably less than that formed with D-ribose \( (k_2 = 1.57 \times 10^3) \) (Power and Woods, 1997). D-glucose reacts with boric acid, probably with the hydroxyl group that arises from lactol formation (Zittle, 1951), but the near absence \((<0.5\%)\) of an \( \alpha \)-furanose form of D-glucose in aqueous solutions (Zubay, 1988) suggests that glucose was selected as the aldose for general energy metabolism because of its lower reactivity with boric acid. Only two natural sugars, ribose and apirose, occur in their physiological derivatives in the strongly borate-complexing erythrofuranose configuration. Thus, the former may have been selected as part of the chemistry of nucleic acid and nucleotide function and the latter for, rather than against, its extraordinary borate-complexing capability.

4.2 Boron and procaryotes and HIV antibiotics

The greater stability of borodiesters, compared to boromonoesters, probably explains why the boron-containing antibiotics were isolated; the structure of the known boron-containing antibiotics, for example, tartrolon B (Structure 6), is characterized by a boron atom bound to four oxy groups as a Boeseeken complex of boric acid (Schummer et al., 1994). Even so, boron can be removed from at least one of these antibiotics (aplasmomycin) by mild acid hydrolysis by using citric acid buffer solution \((pH \, 3)\). This causes slight conformational changes in the molecule and formation of a new compound (desboroaplasmomycin) that does not have the activity of the original (Sato et al., 1978). Boron can be easily reinserted into desboroaplasmomycin by treatment with boric acid at \( pH \, 6 \) and \( 8 \) (Chen et al., 1979). Recently, another related antibiotic, boromycin, was discovered (Kohn et al., 1996) to be a potent anti-human immunodeficiency virus \((HIV)\) antibiotic. It strongly inhibits the replication of the clinically isolated HIV-1 strain and apparently blocks release of infectious HIV particles from cells chronically infected with HIV-1 by unknown mechanisms.

4.3 Plant boron complexes as possible analogs for animal boron biomolecules

Plants require boron and contain organoboron complexes. Apiose, an unusual monosaccharide found in the pectin fraction of a wide range of plant species, can cyclize only as a furanose such that, in aqueous solution, the predominate \((54\%)\) free form is \( \beta \)-D-erythrofuranose, a form that represents the optimal configuration for complexation with borate (Loomis and Durst, 1992).
Rhamnogalacturonan II (RG-II), a small, structurally complex polysaccharide of the pectic fraction of primary plant cell walls, contains apiose. RG-II is present in at least 24 plant species and represents an extreme example of the evolutionary conservation of wall polysaccharide structure. RG-IIIs described above and an atom of boron that cross-links two RG-II dimers at the site of the apiose residues to form a borodiester (Structure 7) (Albersheim et al., 1994). Knowledge that borate ester cross-linking of polysaccharides in vitro is pH-dependent has led to the suggestion that the boron cross-links in RG-II are the “load-bearing,” acid-labile linkages that are hydrolysed by a decrease in wall pH during auxin-induced cell expansion (Loomis and Durst, 1992). Searches for structural roles for boron in animals and humans should include searches for analogs of RG-II. Recently, the technique of matrix assisted laser desorption Fourier transform mass spectrometry (MALDI-FTMS) was used to demonstrate that boron oxo compounds form stable ionic complexes with the polyol ligands mannitol, sorbitol, and fructose in liquid samples of celery phloem sap and vascular exudate and phloem-fed nectaries of peach (Hu et al., 1997). In fact, the MALDI-FTMS data suggested that no free boric acid or borate is present in these phloem saps or vascular exudates.

4.4 Boron binding to biomolecules with adenosine moieties

Although furanoid-containing structures are rare in nature (Loomis and Durst, 1992), there are numerous critically important biomolecules that
contain ribose moieties. Because a ribose is present in all molecules with an adenosine moiety, a capillary electrophoresis (CE) method was recently developed in the author’s laboratory (Ralston and Hunt, *in press*) to examine the binding interaction between boron and molecules with adenosine moieties. The electrophoretic migration of a molecule is primarily determined by its electronic charge (Gunther Sillero and Caeselle, 1992). Therefore, formation of boronesters can be quantitatively detected by monitoring the influence of negatively charged borate on the migration of boron-binding biomolecules in CE. The boron-binding affinity of ligands can be compared by measuring the proportional increases in their relative migration times in the presence of boron at increasing concentrations.

![Structure 7](image)

4.4.1 **Boron and mono-adenosines** (adenosine, SAM, 2’AMP, 3’AMP, 5’ADP, 5’ATP)

S-adenosylmethionine (SAM) (Structure 8) is the predominant methyl donor in biological methylations and is therefore a versatile cofactor in a variety of physiologic processes (Kozarich, 1988). Our laboratory (Ralston and Hunt, *in press*) recently demonstrated that SAM has a far greater affinity for boron than do several other tested monoadenosine species: SAM $>$ 5’ATP $>$ 5’ADP $>$ 5’AMP $>$ adenosine (ADS) $>$ 3’AMP $\cong$ 2’AMP $\cong$ cAMP $\cong$ adenine (ADN) (Ralston and Hunt, *in press*). The rank order of boron binding to these various species indicates that boron binding by the 2’-3’-*cis*-diols of the ribose moiety of SAM was electrostatically stabilised by
the cationic sulphur of the methionine and interaction with the terminal amino and carboxyl groups on its methionine. Boron does not bind to either ADN or ADS because of the lack of ribose or absence of an intact ribose binding site on these respective species.

4.4.2 Boron and nicotinamide adenosine dinucleotide (NAD\(^+\)) and NADH

The pyridine (e.g., NAD\(^+\) or NADP\(^+\)) and flavin (e.g., FAD) nucleotides (Structure 8) are essential co-factors for five sub-subclasses of oxidoreductase enzymes. These co-factors have received special attention as they contain a ribose moiety with a cisoid diol conformation (Smith and Johnson, 1976) that interacts with boron to cause reversible \textit{in vitro} inhibition of the oxidoreductases that require pyridine or flavin nucleotides. In fact, until our laboratory investigated other biomolecules with ribose moieties, NAD\(^+\) (K\(_d\) of 14.4 mM) was recognised as having the highest boron binding affinity of any biomolecule that occurs naturally in animals. Borate interacts with NAD\(^+\) to form an anionic complex where NAD\(^+\) is electrostatically stabilised by the cationic nitrogen of the nicotinamide of NAD\(^+\) (K\(_d\) 14.4 mM), a charge that is not present on NADH (K\(_d\) 26.4 mM) (Ralston and Hunt, \textit{in press}). This need for electrostatic stabilisation also has the probable functional consequence of limiting, at physiological concentrations, the reaction of boron with many ribosyl-containing compounds including most, if not all other, nucleotides.

\begin{center}
\includegraphics[width=\textwidth]{structure8}
\end{center}

(Structure 8)
4.4.3 Boron and di-adenosine polyphosphates

Structurally similar to NAD+ and NADH, the di-adenosine-phosphates (ApnA) all possess a pair of ribose moieties held in close proximity, joined by 2-6 phosphodiesters (Structure 8). Present in all cells with active protein synthesis, ApnA molecules function as signal nucleotides associated with platelet aggregation and neuronal response. The ApnA are putative Aalarmones which reportedly regulate cell proliferation, stress response, and DNA repair (Ogilvie, 1992). At physiologic pH, the adenine moieties of either Ap2A or Ap3A are driven together by hydrophobic forces and stack interfacially (Kolodny and Collins, 1986). This stacking of the base adenines prohibits formation of a diconplex (to resemble Structure 3) and, as our data indicate (Ralston and Hunt, 2001), only allows for simple additive binding between boron and two independent monocomplexes. However, for ApnA with four or more intervening phosphates, boron binding apparently is cooperative, i.e., the adenosine bases do not stack and a unimolecular diconplex binding of boron occurs between the vicinal cis-hydroxyls of the opposing ribose moieties (Structure 8; ApnA borospirane). Thus, boron binding by Ap4A, Ap5A and Ap6A is greatly enhanced compared Ap3A or Ap2A, NAD+, NADH or any of the tested mono-adenosines except for SAM. In summary, we have established that SAM and certain ApnA families bind boron with higher affinities than does NAD+, formerly recognised as the highest affinity boron ligand present in animals.

4.5 Boron and inflammation and serine proteases

The known ability of boron to form covalent bonds with the nitrogen atom of amine groups (as described above) and the observation that boron binds near the coordination iron site of hemerythrin (the nonheme iron-containing, oxygen transport protein of the sipunculid worm, Golfingia gouldii) (Garbett et al., 1971) suggest the possibility of a large array of biochemicals other than polyols that can react with boron to form complexes.

The serine proteases are major proteolytic enzymes (i.e., elastase, chymase, and cathepsin G) released by activated leukocytes that, in addition to degrading structural proteins, have many essential regulatory roles in normal inflammation, including control of the blood fibrinolytic system (e.g., thrombin) and the coagulation system (e.g., coagulation Factor Xa) (Kettner et al., 1971). Boron reversibly inhibits these enzymes as well, but by an entirely different mechanism, the transition-state analog. The boron atom is thought to inhibit the serine proteases by forming a tetrahedral B
adduct that mimics the tetrahedral adduct formed during normal substrate hydrolysis (Berry et al., 1988). The adduct includes a covalent bond between boron and a specific nitrogen at the active site of these enzymes (Structure 5). Nanomolar concentrations of certain synthetic peptide boronic acids, including MeO-Suc-Ala-Ala-Pro-acetamido-2-phenylethaine boronic acid, effectively inhibit chymotrypsin, cathepsin G, and both leukocyte and pancreatic elastase in vitro (Kettner et al., 1984).

Our laboratory has tested the hypothesis that dietary boron helps regulate the normal inflammatory process by dampening the activities of several serine proteases and NADP-requiring oxidoreductases (for respiratory burst-generated reactive oxygen species) in activated leukocytes. We have reported recently (Hunt and Idso, 1999) that physiological amounts of dietary boron have immunomodulatory effects such as reduced paw swelling and circulating neutrophil concentrations, and increased circulating concentrations of natural killer cells and CD8a+/CD4− (T suppressor) cells in rats with antigen-induced arthritis. The results indicate a definite role for physiological amounts of dietary boron in normal immune function.

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