The Journal of Trace Elements in Experimental Medicine 9:153–163 (1996)

Review of Possible Boron Speciation Relating to Its Essentiality

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Boron is one of the very few elements known to be essential in plants and is yet to be unequivocally proven as essential in animals and humans. Animal and human research on essentiality would benefit if the speciation of boron in biological fluids and tissues could be determined. This is complicated by the myriad of functional biomolecules with which inorganic borates can react and by the exceedingly low concentrations of boron present under physiological conditions. This review brings together published literature on the interaction of boron with biochemical systems which bear on the question of its essentiality. Some fundamentals of boron chemistry that are germane to the issue of speciation in living organisms are reviewed. Potential mechanisms of boron action in plants are discussed, with a view toward predicting effects in other organisms. Complexation with polyhydroxyl compounds, a well-known feature of boron chemistry, and interactions with enzymes, cofactors (NAD/NADP), and membranes are proposed as the most likely sites of boron involvement. Non-destructive techniques that might be utilized to directly study boron speciation in biological systems are discussed.

Key words: borate complex; enzyme; membrane; NAD; riboflavin

INTRODUCTION

Boron (B) has been known to be essential in plants since 1923 [1] but remains one of the few such elements not conclusively proven to be essential in animals [2]. It has been theorized that B essentiality arose with the evolution of vascular plants. B also has been shown to be essential in diatoms [3] and in nitrogen-fixing cyanobacteria [4], with some evidence that it is not essential for green algae [5] or for fungi [5,6]. This review will cover aspects of B chemistry relating to the possible interaction of B with key biomolecules. The most likely B species to play a role in plant essentiality will be reviewed, with implications for essentiality in animals and humans.

At the low concentration of B found in normal mammalian tissues and fluids (0.02–0.5 mg/g, 10^{-6}–10^{-5} M) [7,8] and in the absence of interaction with biomolecules, only monomeric boric acid [$B(OH)_3$] and tetrahydroxyborate anion [$B(OH)_4^-$] are expected to be present. Boric acid is a very weak acid (pK_a 9.2). Therefore, at the pH of human blood (7.4), the B would be >98% in the form of free $B(OH)_3$ and only <2% as $B(OH)_4^-$. Boric acid is known to form esters and complexes with a wide variety of mono-, di-, and poly-hydroxy compounds. Such complexes increase the

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Received 5 October 1996; Accepted 5 November 1996

acidity of the boric acid, the classic case being the use of mannitol to increase its acidity to a pK_a of about 5, making boric acid titrable.

Besides higher pH, a number of structural factors enhance the stability of such complexes. In the case of diols, *cis*-1,2 are favored over *trans* or 1,3-diols, and five-membered furanosidal are preferred over six-membered pyranosidal 1,2-diols. Thus, sugars differ markedly in the stability of their borate complexes [9,10]. The presence and nature of a third group in the molecule also can have a stabilizing effect, such as a large substituent providing steric protection against hydrolysis, an adjacent nitrogen, a group having protons providing H-bonding, or a positively charged substituent giving electrostatic stabilization.

Examples of typical biomolecules capable of forming borate complexes are given in Figure 1; these are a) Apiose has been found in the cell wall of many plants and ribose is a critical component of many biomolecules; both form stable borate complexes, being furanosides with *cis*-1,2 hydroxyls [11]. b) As shown by B-affinity chromatography [12], the nucleoside adenosine gives a complex more stable than that of cytosine, due to the adenine moiety being a more bulky substituent. c) A number of neuro-active biomolecules, such as L-dopa and epinephrine, with ortho dihydroxyls also are expected to form borate complexes.

Serine is believed to form a rather stable borate complex; the amino nitrogen can either coordinate or provide stabilization as a positive charge (Fig. 2). The serine-borate complex is considered a transition state inhibitor of γ-glutamyl transpeptidase and is assumed to form under dilute physiological conditions, since serine or borate alone have no effect [13,14]. The borate complex of the coenzyme NAD^{1+} is 15 times stronger than that of NADH due to electrostatic stabilization of the former by the positively charged nicotinamide group (Fig. 3) [15]. The implications of this for B interaction with a number of key biochemical systems is elaborated below.

PLANTS

Some of the functions proposed to account for the essentiality of B in vascular plants are listed in Table I [2]. It has been suggested [11] that the key role of B is cross-linking of the polysaccharide cell wall. Supporting evidence cited was the high concentration of B in the cell wall, the presence of apiose for complexation of B as well as cross-linking, cell enlargement and wall disorganization with B deficiency (-B), and loss of membrane integrity with -B. However, these explanations do not take into account the critically specific effects of B on certain enzymes and on membrane transport. The pentose phosphate pathway for sugar catabolism is stimulated by -B, due to release of inhibition of glucose-6-phosphate and 6-phosphogluconate dehydrogenases. This results in the accumulation of phenols, leading to plant damage [16]. These enzymes are held in check by normal levels of B, probably due to the preferential complexation of cofactor NAD, as shown in Figure 3.

The glycolytic enzyme phosphoglucomutase has been studied in germination and it may also be regulated by B [17]. Involvement of borate deficiency in the NAD/NADH and NADP/NADPH systems also could alter the energy system of the organism. In addition, membrane effects of B are very important. Uptake of K^{1+}, phosphate, and glucose by root cells is inhibited by -B and this is reversed by 10^{-5} *M* added B [2]. The trans-membrane proton gradient is decreased by -B, as is ATPase activity

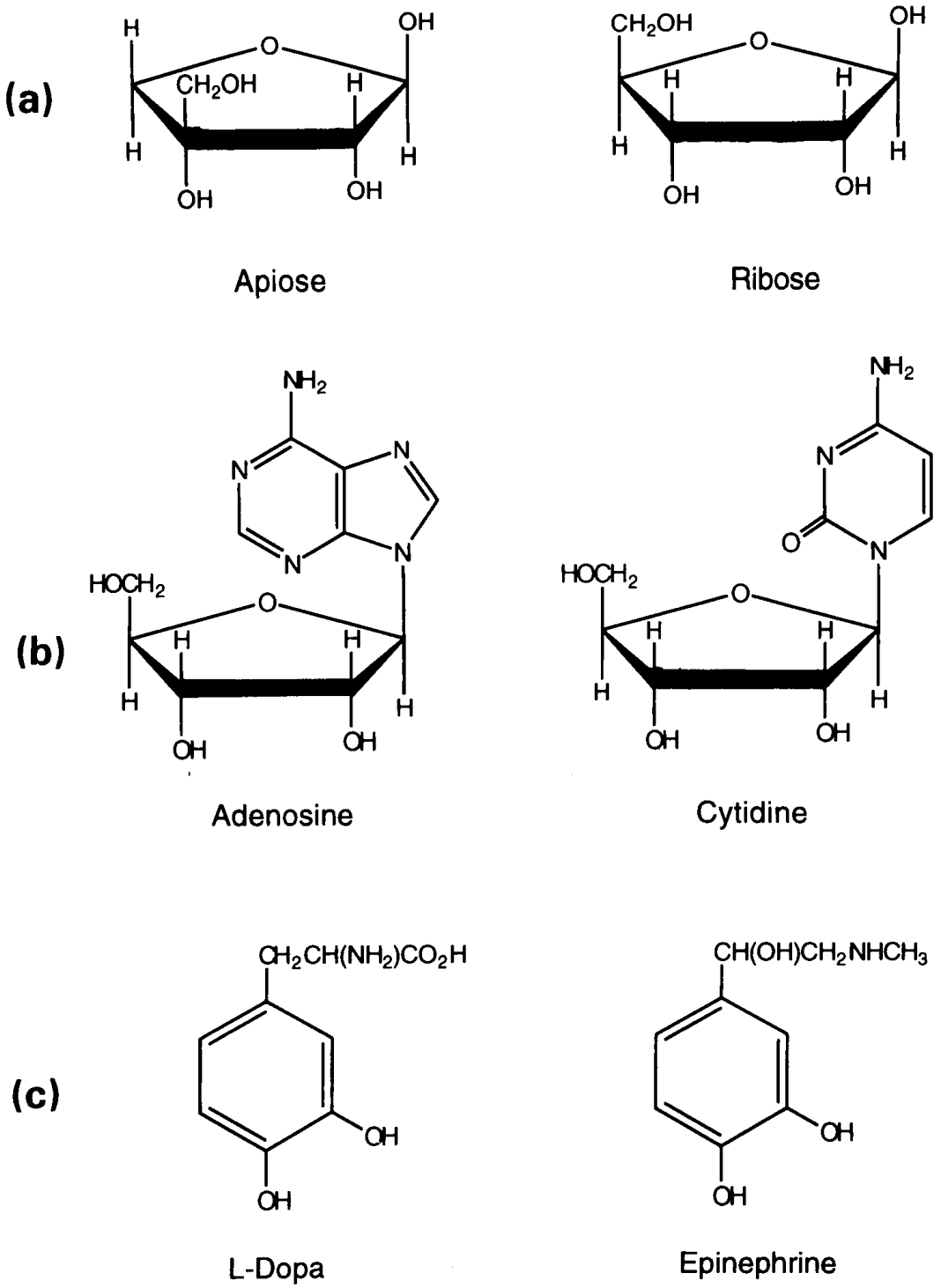

Fig. 1. Biomolecules capable of forming borate complexes.

[18]. B complexation of membrane glycoproteins and/or glycolipids could be involved in the mechanism of these effects. For example, an adjacent glycoprotein is essential for the function of ATPase (see Fig. 4), and the cell coat (glycocalyx) has many sites for such interactions (see Fig. 5).

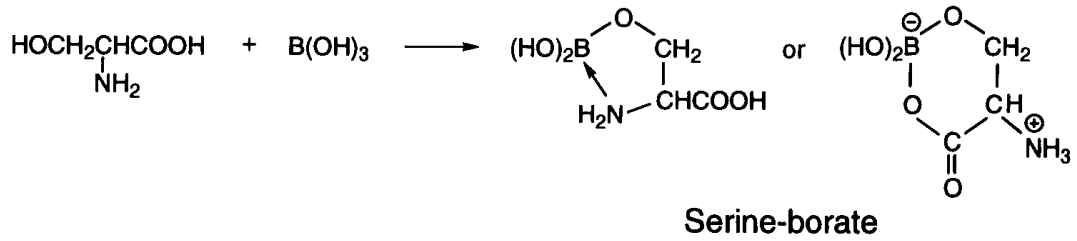

Fig. 2. Two possible forms of the serine-borate complex. Esterification of a simple primary alcohol in dilute, aqueous solution would not be expected unless some special stabilization were available.

OTHER ORGANISMS

In studies of the effects of borate on fungal decay, Lloyd [19] found that the non-substrate polyols sorbitol, ribose, and glucose reversed borate dehydrogenase inhibition. Similar antidote activity has been seen in the reversal of boric acid inhibition of xanthine oxidase activity by added ribose or sorbitol [20] and of alcohol dehydrogenase by sorbitol, ribose, and mannitol [21]. The effectiveness of such polyols is proportional to the strength of their borate complexes. The most efficient diols for reversing the inhibition of 6-phosphogluconate were those with *cis*-adjacent hydroxyls in the vicinity of a positive charge, NAD and nicotinamide mononucleotide (NMN) both being more efficacious than ribose [19].

Macrocyclic tetrols with ideal geometry for forming tetrahedral borate complexes represent the only known B-containing natural products. These are the antibiotics boromycin [22], aplasmomycin [23], and tartrolon B [24]. Tartrolon A (Fig. 6), without the B, extracts B from Pyrex glass to give tartrolon B. The latter inhibits RNA, DNA, and protein synthesis in *Staph. aureus* and in *E. coli*. Tartrolon B is better than A in removing K^+ from *S. aureus* cell cultures, indicating a membrane transport function for the borate complex. Synthetic "cleft" molecules with both a B and a crown ether function can transport alkali metal ions and sugars across a synthetic membrane (Fig. 7).

Boric acid rapidly precipitates the rheumatoid factor from serum of human rheu-

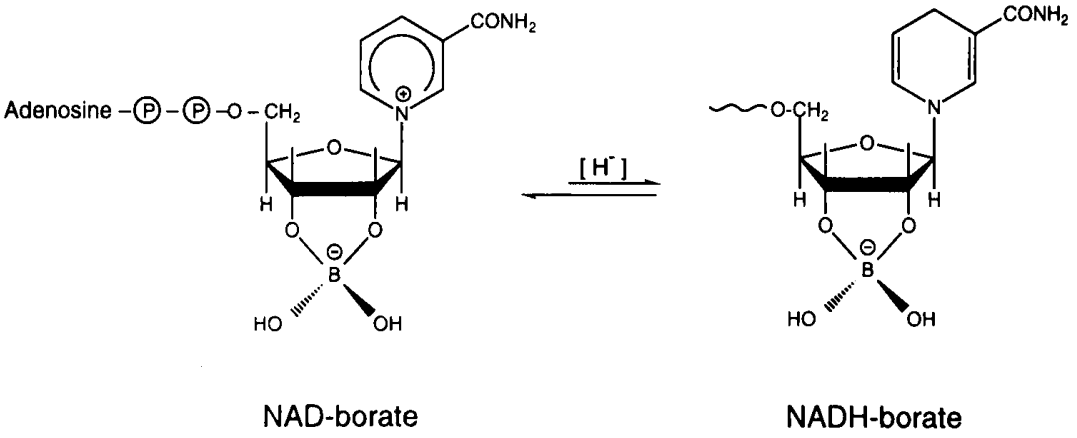

Fig. 3. Borate complexes of NAD and NADH, showing shifting of concentrations due the greater stability of the NAD complex [15].

TABLE I. Biochemical Systems Involving Boron Potentially Related to Essentiality in Plants*

Biochemical system	Method, species used
Sugar transport	Movement of sucrose in intact tomato, snap bean; in excised leaves of French bean.
Cell wall synthesis	Analysis of tobacco wall; glucose incorporation into field bean wall material.
Lignification	Lignin determination in sunflower, tobacco; peroxide level and distribution in field bean, wheat.
Carbohydrate metabolism	Effect of borate on *in vitro* sucrose synthesis, on phosphoglucomutase, and on 6-phosphogluconate dehydrogenase.
RNA metabolism	Incorporation of labeled precursors, mung bean; -B symptoms by base analogs in cotton.
Respiration	ATP levels in sunflower; P incorporation into organic phosphates, field bean.
Phenol metabolism	Effect of B on pentose phosphate enzyme; determination of phenol levels, compare with -B effects.
IAA metabolism	IAA degradation, compare IAA vs. -B, field bean; IAA levels, oil palm; IAA oxidase, sunflower, squash.
Membrane effects	Effect on mung bean bioelectric fields, on ion transport, membrane enzyme, maize; ultrastructure, sunflower.

*See ref [2] for original literature citations.

matics, indicating interaction with the sugars on the macroglobulin [25]. Boronate affinity chromatography can separate glycosylated hemoglobin from the blood of diabetics, pointing to borate complexing of glycoprotein [26].

Borate and boronic acids are known to inhibit a variety of serine proteases, including α-chymotrypsin [8], Subtilisin Carlsberg [27], bovine trypsin [28], and others, as well as the manganese-containing allantoate amidohydrolase [29]. Borate forms a tetrahedral complex at the active site of the enzyme, as shown in Figure 8. Boro-amino acids such as boro-leucine [$(CH_3)_2CHCH_2CH(NH_2)B(OH)_2$] are the most effective inhibitors known for nasal peptidases [30]. The clotting action of thrombin is inhibited by tripeptides terminated by boro-arginine: AA-AA-NH-$CH[B(OH)_2]$-$CH_2CH_2CH_2NHC(NH_2)=NH$ [31–33]. Leukocyte elastase, which destroys elastin causing emphysema, is inhibited by tetrapeptides terminated by a boro-amino acid: $HOOCCH_2CH_2CONH$-AA-CONH-AA-CONH-AA-CONH-AA-$B(OH)_2$ [34].

RIBOFLAVIN

Riboflavin (Vitamin B_2, **1**) is known to form a complex with boric acid which is claimed to be 25 times more water soluble than riboflavin itself [35]. A 1:1 complex was isolated and, as judged by effects on optical rotation, some 2:1 complex also was present in solution. Involvement of the hydroxyl on carbon atom 2 of the ribityl side chain was indicated. The isoalloxazine ring of **1** is hydrolyzed more slowly in solutions buffered with borate vs. unbuffered solution at the same pH [36]. Excessive urinary excretion of **1** has been observed in cases of boric acid ingestion [37,38]. Studies in chickens have shown that dietary supplementation with **1** protects against boric acid toxicity [39]. It has been proposed [37] that formation of a water-soluble borate complex could displace **1** from albumin, which transports it in the plasma.

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