Boron concentration in the Golgi apparatus of [plant type] cells and the possible locations of rhamnogalacturonan-II borate diester cross-linking

1.0 | Introduction

The Golgi apparatus of plant cells is known to be involved in the synthesis of pectins [1], complex carbohydrates that constitute the outermost layer of the cell wall. This layer, the middle lamella, is key to the lives of plants as it forms the interface between their cells and glues adjacent plant cells together. One such pectin is the intriguing **rhamnogalacturonan-II** (**RG-II**), a polysaccharide consisting of 12 distinct glycosyl residues [2]. At the apiosyl residues, RG-II chains are cross-linked by a 1:2 borate-diol diester [3]. As shown in the figure below, four deprotonated hydroxyl groups are coordinated to a central borate atom. When cross-linked, RG-II exists as a dimer; each dimer possessing a single borate cross-link [3]. To find the element **boron** (**B**) actively participating in biochemistry at all is simply fascinating. But of all biomolecules, what a wonder it is to find B cross-linking a complex obscure plant cell wall pectin.

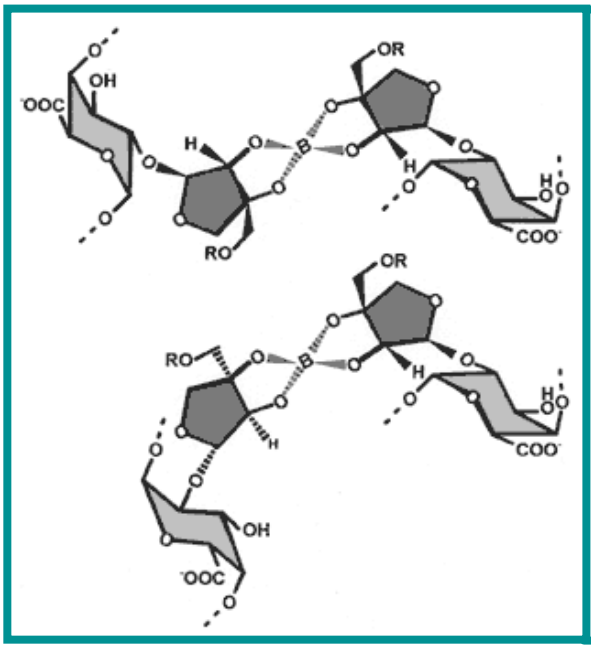

Fig. 1. The two possible isomers of the borate cross-link at the **apiosyl** residues of the RG-II pectin polysaccharide.

Please note that the B atom has a negative charge.

The figure is taken from [3].

2.0 | Proposals and Hypotheses

2.1 | *Golgi apparatus B concentration*

Relatively recently, researchers [4, 5] have concluded that RG-II cross-linking takes place either during synthesis in the Golgi apparatus and/or during secretion, but not after secretion. Since the plant Golgi apparatus is known to synthesize pectins, cross-linking in the Golgi apparatus is certainly a plausible phenomenon. Therefore, we suspect that B concentration would be notably high in the Golgi apparatus. In order to test this hypothesis, we propose measuring the B concentration in the Golgi apparatus (G_B) and comparing it to that in the surrounding cytosolic fluid (C_B). If G_B is higher, this would support the idea that RG-II cross-linking occurs in the Golgi apparatus. If C_B is higher, then RG-II cross-linking either does not occur in the Golgi apparatus or the amount of B needed to cross-link RG-II chains is so unappreciable that a plant cell does not need to stockpile B in the Golgi. Yet a third possibility is that the Golgi apparatus simply imports B when it is needed.*

2.2 | *Location of RG-II borate cross-linking*

In addition to measuring and comparing G_B and C_B, we wish to elucidate the stage of RG-II's "career" in which borate cross-linking is most prevalent, as it could occur during multiple stages (as noted in the preceding section). One possibility (discussed in [5]) is that cross-linking occurs *en route* in Golgi-derived transport vesicles that are carrying RG-II monomers toward the apoplast. Furthermore, the BOR2 transport protein is thought to play a crucial role in inter-vesicular RG-II cross-linking [6] by allowing borate /boric acid to enter the vesicle – see Fig. 8 of [6]. Since RG-II transport via Golgi-derived vesicles falls under the category of "during secretion," we propose testing the criticality of BOR2 to RG-II cross-linking in order to narrow down cross-linking locations.

* We are not aware of any such import mechanism but felt that its existence is worthy of consideration.

If BOR2 proves critical to RG-II cross-linking, then further work must be done in order to elucidate the location where cross-linking occurs. If RG-II cross-linking can occur at reasonable rates without the assistance of BOR2, then cross-linking may not occur in transport vesicles but rather in the Golgi apparatus or at the very moment of exocytosis (see [5] for discussion on all possible RG-II cross-linking locations).

3.0 | Purposes of the Experiment

The first purpose of this experiment is to quantify the B concentration in the Golgi apparatuses in the cells of a given type of plant and compare it to that of cytosol. We will endeavor to find implications in the resulting data for the location(s) of RG-II borate cross-linking.

The second purpose of the experiment is to directly investigate the location(s) of RG-II borate cross-linking. Through investigation of the relative importance of the BOR2 protein to RG-II cross-linking, we will endeavor to narrow down the presently diverse borate cross-linking locations.

4.0 | Tentative Experimental Design

4.1 | *Type(s) of plant(s) to use in experiment*

The type(s) of plant(s) to use as subjects for the experiment is of foremost importance. Our two tentative subject choices are *Arabidopsis thaliana* and/or "Paul's Scarlet" rose (*Rosa* sp.). We propose using *Arabidopsis* because (a) it is a tried-and-tested model organism and (b) the study ([6]) on the roles of BOR2 used this plant. On the other hand, *Rosa* would also be a good choice because the studies ([4, 5]) on the locations of RG-II cross-linking used this plant. A third (but more complex approach), would be to use plants of both types as subjects for the experiment. Nevertheless, I am leaning toward employing *Arabidopsis* in this experiment.

4.2 | *Obtaining and grouping subjects, etc.*

Whatever plants are chosen as subjects, we intend to obtain them in the highest quality condition possible in order to achieve maximum accuracy in the experiment. We will obtain purified Golgi apparatuses from the leaves of the chosen plant(s) through cell fractionation and then purify the resulting sample from other organelles and debris. (This sample will be tested for G_B). We will obtain unperturbed cells by simply peeling off thin, one cell-thick layers from the plant leaves. (This sample will be tested for C_B).

In Part 1 of the experiment, plant cells tested for C_B will be the control group, while those tested for G_B will be the experimental group. Alternatively, the cytosol and Golgi apparatuses could be derived from the same cells. In this scenario, the purified Golgi apparatuses would be the experimental group, while the residual cytosol after cell fractionation would act as the control group.

In Part 2 of the experiment, the control group will be composed of normally grown plants, while the experimental group will be composed of plants with non-functional BOR2. This could be achieved by such methods as gene knockout (mutagenesis) or inhibition of the protein. Unfortunately, I do not know what type of molecule would be best utilized to inhibit BOR2, as there are currently no available PDB structures of the protein. Based on the structure of boron transporter BOR1 (PDB 5L25), it should be a hydrophobic molecule (assuming homology between the two structures), since the channel through which boron diffuses is lined with hydrophobic residues (e.g., Leu). Still, I believe inhibition of BOR2 is a plausible option.

4.3 | *Methods of measuring B concentration*

Two main methods appear plausible to measure B concentration. First, spectroscopy could be used to identify B because the emission spectrum of every element is unique.

Even though cellular boron is not atomic, but found instead as oxides or in molecules such RG-II, the spectroscopy should still be applicable. Nonetheless, I do have some concerns about using spectroscopy in measuring both G_B and C_B. Firstly, the fluid of the Golgi apparatus and the cytosol is filled with obstructive macromolecules such as proteins. Secondly, measuring G_B would necessarily involve destroying the plasma membrane, as it would significantly obstruct spectroscopic efforts. This is an additional difficulty that must be dealt with if spectroscopy is employed.

The second method of measuring boron concentration would involve boron-binding molecules. Fluid samples from Golgi apparatuses and the cytosol would be exposed to a boron-binding molecule and the resulting amounts of B-complex would indicate the concentration. One such molecule that could accomplish this job would be **NAC** (***N*-acetyl-L-cysteine**).

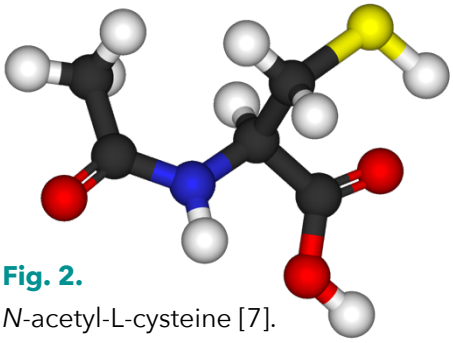

Fig. 2.
N-acetyl-L-cysteine [7].

A toxicological study [8] has concluded that NAC is effective in detoxifying chromate and borate, which is convenient because free-floating cellular boron assumes mainly $B(OH)_3$ (boric acid) / $[B(OH)_4]^-$ (tetrahydroxyborate) – see Fig. 8 of [6].

Modifying NAC by swapping the sulfur for a selenium would produce the compound *N*-acetyl-L-selenocysteine. This may be an advantageous substitution for the present experiment, because the resulting "NAS" molecule might be able to bind borate with greater affinity than NAC and thereby give more accurate measurements of G_B and C_B.

The logic behind this proposal is that selenide (Se^{2-}) is known to be an able detoxifier of mercury (Hg), more so even than S^{2-} or thiol [9]. Therefore, the selenol moiety of "NAS" may confer stronger binding of borate upon the molecule.

Another interesting option would be to use RG-II itself as a boron-binding molecule. Assuming that the reaction with $B(OH)_3$ / $[B(OH)_4]^-$ is spontaneous as depicted below, the amount of dimerized RG-II would indicate B concentration. However, other pH and divalent cations seem to be involved, so this may not be the simplest choice.

Finally, using a borate chelator that fluoresces upon binding its substrate would be a tremendous bonus, since the fluorescence intensity would clearly indicate G_B and C_B.

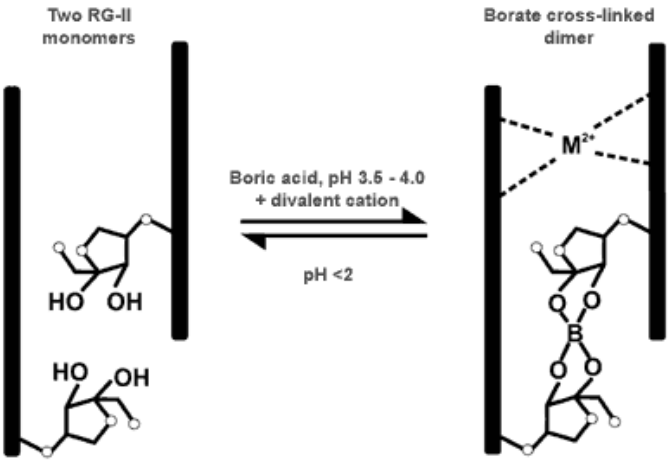

Fig. 3.
Formation of the borate crosslink from boric acid.

The reaction is shown as being spontaneous.

The figure is taken from [3].

To conclude this section, I must pose one intriguing question that has presented itself: Could RG-II dimerization in fact be enzymatic? It has certainly been assumed to be spontaneous, but the possibility of enzyme involvement should still be considered.

5.0 | References

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