Cellulose Biogenesis in Bacteria and Higher Plants is Disrupted by Magnetic Fields

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The value of magnetic orientation of macromolecules in studies of the structure, assembly, and flexibility of polymers and liquid crystals has recently been reviewed [1]. The high degree of orientation produced by magnetic field strengths of under 2 T (Tesla) has yielded much information on the in vitro assembly of fibrin [1], collagen [2, 3], actin [4], and tubulin [5]. Assembly of these molecules in an applied magnetic field provides a more highly ordered product than polymerization in the geomagnetic field alone. The effects of applied homogeneous magnetic fields on polymer assembly in vivo have not been reported.

Externally applied magnetic fields have been shown to interact with many biological systems producing an orienting effect. On a cellular level these responses include oriented growth of pollen tubes [6], magnetotaxis of Volvox [7] and salt marsh bacteria [8], orientation of Chlorella cells and isolated chloroplasts [9], orientation of purple membranes of Halobacterium [10], egg lecithin vesicles [11], and chylomicra [12]. Studies of effects of magnetic fields on cellulose-producing organisms have been largely limited to tropic responses of plants induced by magnetic field exposure.

We have discovered that the ordered aggregation of β-4-glucan into crystalline arrays by two very dissimilar organisms can be disrupted in vivo by magnetic field strengths comparable to those that cause high orientation of macromolecules in in vitro systems. In this preliminary report we present the effects of applied magnetic fields on cellulose biogenesis by the bacterium Acetobacter xylinum (strain ATCC
and oat coleoptiles (Avena sativa L. cv. Garry).

Freeze-fracture of A. sativa coleoptiles incubated in 0.5 – 1.8 T fields indicated that cellulose microfibrils which had been synthesized during magnetic exposure (forming the inner wall layer) were randomly organized and loosely associated with one another (Fig. 1C). Inner wall layers of control plants had highly ordered, closely associated arrays of microfibrils typically found in A. sativa coleoptiles (Fig. 1A) [13]. The random orientation of inner wall layer cellulosic microfibrils produced by magnetic fields resembles the inner wall structure of coleoptile lower hemicylinder cells observed after gravistimulation of oat seedlings [13].

It has been reported that rapidly growing oat, wheat, rye, corn, and cress roots and oat shoots respond to an externally applied magnetic field with a reorientation response and/or a change in growth rate [14 – 17]. In our experiments we observed tropic responses of the same nature as those reported for oat coleoptiles subjected to an inhomogeneous magnetic field [16]. Sperber et al. [6] demonstrated that single-cell pollen tubes from Lilium exhibited orientated growth parallel to a homogeneous magnetic field of 14 T and speculated that magnetic fields act on the plasma membrane in some manner to produce this response. The organization of the inner cell wall microfibrils, believed mediated by plasma membrane “terminal complexes”, is considered to be a primary determinant of cell elongation capability [18] and thus plant growth. We suggest that magnetic disruption of microfibril deposition in Avena functions to reorient inner wall layers causing differential growth directly related to the “magnetotropic” response [15, 16].

Coincident with magnetic field effects on microfibril orientations in A. sativa coleoptiles we observed differences in plasma membrane intramembranous particle (IMP) distributions between treated and control plants. After brief exposure to magnetic fields the plasma membrane contained regions almost devoid of IMPS while adjacent areas contained abundant, tightly packed IMPS (Fig. 1B). Modification of the structure of photoreceptor membranes by magnetic fields has been implicated in aggregation of rhodopsin in outer segments of retinal rods ([19] and references cited therein). Magnetic orientation of lipids within membranes has been described [20, 21], but we are not aware of a previous direct electron microscopic visualization of the effects of magnetic fields on membranes of living cells. If membrane-associated terminal complexes are involved in cellulose microfibril biogenesis by Avena coleoptiles and other plants as has been proposed [22, 13, 23], then we may expect a direct correlation between inner wall layer disorganization and changes in plasma membrane structure observed on exposure to magnetic fields. Alterations in membranes by chemical agents have been shown to disrupt cellulose deposition in the alga Boergesenia forbesii [24]. Furthermore, if close relationships between microtubules and membrane-associated cellulose-synthesizing complexes exist in A. sativa as they have been shown in B. forbesii [25], then the influence of magnetic fields on microtubule assembly and orientation [5] may have a role in the disruption of normal cellulose deposition in Avena.

The gram-negative bacterium Acetobacter xylinum normally synthesizes an organized twisted ribbon of cellulose (Fig. 2A) [26]. In the magnetic field cells produced alternating regions of fine twisted fibrils and disorganized cellulose (Fig. 2B) or other morphologies of naturally occurring “native band material” [28]. The cellulosic nature of the altered material produced by A. xylinum was verified by a binding assay using a highly purified preparation of cellobiohydrolase (CBH)/colloidal gold complex [29]. Under the conditions employed, we have found CBH/gold to bind to β-4-glycans, but not to other β-glycans or to α-linked polymers.

The production of predominantly native band material by A. xylinum cells exposed to magnetic fields was demonstrated at field strengths ranging from 0.05 to 1.8 T (Table 1), in buffers with pH range from 5.0 to 7.0, and in Schramm and Hestrin’s medium [30]. All conditions tested gave results similar to those in Table 1 with higher field strengths of 1.8 T giving a slightly attenuated response.

The response of cellulose-synthesizing A. xylinum cells to magnetic fields could be attributed to a disruption of bacterial membrane particle arrays.
Table 1. Effects of applied magnetic fields on cellulose synthesis by Acetobacter

<table>
<thead>
<tr>
<th>Field strength [T]</th>
<th>Cellulose synthesizing cells* [%]</th>
<th>Normal ribbon</th>
<th>Native band material [28]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>97</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>21</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>20</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>1.8</td>
<td>6</td>
<td>94</td>
<td></td>
</tr>
</tbody>
</table>

* Based on a compilation of several separate exposures and electron microscopic observation of over 100 total cells for each exposure

thought to be associated with bacterial cellulose synthesis [26]. Also, the direct interaction of glucan chains with the magnetic field could explain altered cel-

lulose produced if, during glucan polymerization and extrusion, there exists a mesophase upon which the field acts. A characteristic of liquid crystals is that they can be ordered in magnetic fields [31], thus modifying many properties of the liquid crystalline system. A cellulose-containing cholesteric liquid crystal has been recently reported from Cypria oblonga [32]. Magnetic orientation of collagen has been closely related to the onset of mesophase in solution [2] and F-actin [4] and tubulin [5] polymerized in a magnetic field have been shown to adopt a preferred orientation demonstrating that biological polymers do respond to magnetic fields at the molecular level.

We have demonstrated that exposure to strong magnetic fields induces alteration in cellulose assembly in the gram-negative bacterium Acetobacter xylanum and in Avena sativa coleoptile cells. When incubated in a homogeneous magnetic field of 0.05 to 1.8 T, Acetobacter synthesizes an atypical cellulose material distinct from cellulose ribbons normally produced. Magnetic fields of 0.6 to 1.8 T cause production of highly disordered cellulose microfibril arrangements in the inner wall layers of Avena coleoptile cells and a redistribution of intramembranous particles in the cell plasma membrane. The underlying mechanisms of these alterations in cellulose synthesis are not clear. We suggest that a direct magnetic interaction with β-glucan during crystallization can be advanced to explain the altered cellulose produced by Acetobacter xylanum. If cellulose biogenesis by A. xylanum involves cholesteric crystallization which is reordered by mag-

netic fields this would provide evidence of the involvement of physical self-assembly in bacterial cellulose synthesis. Magnetic field perturbation of the organization of plant cell wall microfibril arrays in Avena sativa inner wall layers is likely the result of more complex mechanisms and could be considered a cause or a result of the rearrangement of plasma membrane and membrane-associated components.

Exposure of cellulose-synthesizing organisms to magnetic fields provides a novel, noninvasive technique for altering cellulose biogenesis (including the nature of the polymer produced and its arrangement into cell walls) and offers a new tool to investigate the complex mechanisms of cellulose synthesis and the control of plant cell growth.

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