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CELL WALL HYDROXYCINNAMATE ESTERS AS UV-A RECEPTORS IN PHOTOTROPIC RESPONSES OF HIGHER PLANTS—A NEW HYPOTHESIS

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Key Word Index—Cell wall ferulate esters; *E/Z* photoisomerism; UV-A receptors; phototropism; coleoptiles; energy transduction; hypothesis.

Abstract—*E/Z* Photoisomerism, in UV-A, of cell wall ferulate and diferulate-carbohydrate esters is suggested as a mechanism for transduction of light energy leading to changes in wall structure and hence water flux, turgor pressure and growth. Unilateral light would cause phototropism.

It has been known for a long time that, in addition to their ubiquitous occurrence in land plants as soluble esters and glycosides, hydroxycinnamic acids, particularly *p*-coumaric and ferulic acids, are found in the insoluble or 'cell wall' fraction also as esters. Smith first identified these acids in hydrolysates of native lignins of plants including wheat and sugarcane [1, 2]. It was subsequently shown that the cell wall fraction of timothy grass and wheat contained appreciable amounts of these compounds [3, 4]; for example 25-day-old seedlings of Kharkov wheat yielded 1.8 mg/g dry wt *p*-coumarate and 4 mg/g dry wt ferulate from cell wall hydrolysis with alkali, levels much higher than those found in the ethanol soluble fraction of these plants [4]. It was suggested, for wheat, that these pools of wall-bound acids act as a reservoir of phenylpropanoid units for lignin biosynthesis or even that they represent the beginnings of lignification itself [4]. Later work confirmed and extended these results [5–13]. A dimer of ferulic acid was identified associated with the water-insoluble pentosans of *Triticum aestivum* [7] and the structural carbohydrates of *Lolium multiflorum* [10]. A ferulate ester of a glucuronoarabinoxylan was isolated from the cell walls of *Zea* shoots; the ferulate content being 3 mg/100 mg carbohydrate [13]. The primary cell walls from exponentially growing cell-suspension cultures of spinach yielded ferulic and *p*-coumaric acids, esterified with galactose and arabinose residues of polysaccharides [12]. Chemical analyses suggested that acidic and netural pectins carry approximately one feruloyl residue per 60 sugar residues. As in earlier studies, it was generally concluded [11, 12] that these acids are involved in lignin biosynthesis, although Fry [12], suggests that a possible role of feruloyl pectin may be in the regulation of cell expansion, possibly through coupling reactions leading to the production of diferulate. Markwalder and Neukom have presented evidence that diferulate forms crosslinks with adjacent polysaccharide molecules and thus reduces their solubility [7].

We would like to propose another role for cell wall bound *p*-coumaric and ferulic acids.* Major unresolved problems regarding phototropism in higher plants concern the chemical identification of the UV-A and the blue light photoreceptors and the mechanism of the transduc-

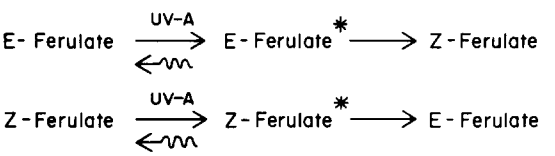

Fig. 1.

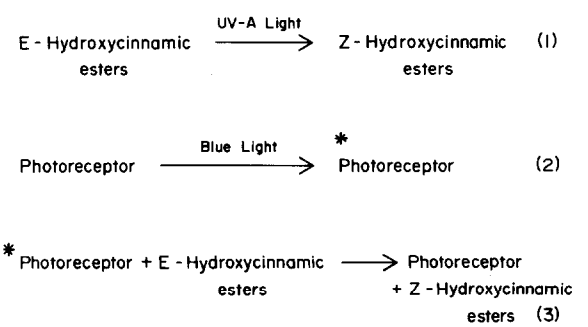

Fig. 2. Direct and indirect photoisomerism of cell wall bound hydroxycinnamic acid leading to growth changes, e.g. phototropism. Photoreceptor = carotenoid/flavin or other suitable photoreceptor. *Photoreceptor = triplet state of the same.

*This hypothesis was presented to the Canadian Society of Plant Physiologists at the Silver Jubilee Meeting held at the University of Waterloo on June 20, 1983 and at the Phytochemical Society of North America meeting held at the University of Arizona, Tucson, Arizona on July 6, 1983.

tion of light energy to physical changes in the properties of cell walls. The vast literature on this topic is replete with contradictions and the models which so far have been proposed to account for this interesting phenomenon are unsatisfactory [14, 15]. For example, in a very recent review [15], Gressel and Horwitz state that the Cholodny–Went hypothesis which implicates changing auxin distribution cannot be general and is still the subject of much debate. The 'blue light response' extends into the UV-A (320–400 nm) and, in fact, some investigators have been able to distinguish between blue light responses and UV-A responses [16, 17]. We will discuss higher plants, particularly the coleoptile of grasses more specifically.

The *E*/*Z* photoisomerism of hydroxycinnamic acids or their esters is a reversible phenomenon, the final proportion of *E* and *Z* isomers depending on the irradiation wavelength. This is shown in Fig. 1. The photoisomerism is rapid, occurring in less than 50 ns with the synthetic ester 2-ethylhexyl-*p*-methoxycinnamate (*p*MCI). It is O_2-independent and probably occurs via a singlet excited state or a very short-lived excited triplet state [18].

The cell walls of grass coleoptiles are fluorescent, the fluorescence extending to the very tip and being somewhat stronger in the epidermal layer. This fluorescence is largely due to ferulate and/or *p*-coumarate present in ester linkages. In etiolated coleoptiles, the acids are present exclusively as the *E*-isomers. Irradiation with UV-A brings about photoisomerism of some of these molecules so that an equilibrium mixture of *E*- and *Z*-isomers obtains after about 15 minutes. In barley coleoptiles the equilibrium of *E* to *Z* isomers is roughly 1 : 0.4. As these acids are bound, either at one end or both, changes in the geometry of a large population of bound molecules would be expected to alter wall structure because of the resulting displacement of macromolecules brought about by the light. These changes in wall chemistry would in turn give rise to changes in turgor pressure and water flux. The effects would be quite rapid. If illumination were unilateral or unequal, unequal growth would follow. Unilateral UV-A light, in other words, would cause a phototropic response because of a change in the relationship of certain critical polysaccharides or lignins and their associated ferulate or *p*-coumarate molecules in the walls. A special role may be seen for diferulate which could be anchored at both ends to different polymers. The apical 350 μm is more sensitive to light than the basal regions where bending occurs [19] and it would become especially important to determine whether photoisomerism occurs in the apex and to what extent.

The blue light response may also be explained in terms of photoisomerism of ferulate and the resulting displacement of macromolecules in the wall except that the cinnamic acids in this case would not be the photoreceptor-molecules. Neither sinapate, ferulate nor *p*-coumarate absorb in the blue region of the spectrum (*ca* 400–450 nm) but their isomerism may be brought about by transfer of the excitation energy of a blue light absorbing triplet sensitizer, such as a carotenoid or a flavin, two well known contenders for the role of photoreceptor for the phototropic responses of plants. This is shown in Fig. 2.

It has been demonstrated for example, that the isomerism of the cinnamates can occur via their triplet states in a photosensitized isomerization. Irradiation of 8-methoxypsoralen at 365 nm (at which wavelength there is no detectable absorption of the cinnamates) in the presence of *E*-*p*MCI, leads to about 25% photoisomerization of the latter [18]. The location of the blue light photoreceptor must therefore also be in the cell wall, according to our *E*/*Z* hypothesis and a search should be made for it with this in mind.

If irradiated barley seedlings are returned to dark conditions there is a gradual increase in the ratio of *E* to *Z*-isomers of cell wall bound ferulate suggesting that there may be either a continuous production of *E*-ferulate (wall-bound) or an isomerase which catalyses *E*/*Z* isomerism. The rate of *Z*- to *E*-dark conversion is much more rapid than the *in vitro* process. Continuous changes in amounts of cell wall ferulate and/or *p*-coumarate and in the extent of *E*/*Z* isomerism could therefore explain other UV-A and blue light responses such as solar tracking and the opening and closing of flowers or of stomates. There would be no need to invoke other transduction mechanisms.

A great advantage to this *E*/*Z* hypothesis is that it is amenable to testing. The isomeric forms of the hydroxycinnamic acids are easily identified by paper chromatography [20] or HPLC [11] and are readily isolated from cell wall fractions. The role of diferulate, in particular, would be most interesting to uncover. It becomes critical now to establish in greater detail the associations of the bound hydroxycinnamates with cell wall lignins, pectins and other carbohydrates.

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