Formation of Intramolecular Ferulate Dehydrodimers: A Molecular Modeling Approach

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Introduction

It is becoming clear that the formation of ferulate dimers plays a crucial role in the structure and function of grass cell walls. With improved techniques for the detection of all the dehydrodimers, researchers are finding significantly higher amounts of wall cross-linking than previously assumed. If all of the ferulate dimers are intermolecular (between two polysaccharide chains) in nature, then there would be a significant impact on wall structure. If, on the other hand, intramolecular dimers (between two ferulates on the same polysaccharide chain) were formed, the impact on wall structure would be diminished. The nature of ferulate dimers attachment cannot be elucidated by normal solvolysis techniques used to analyze ferulates in walls. Release of the ferulate dimers by alkaline hydrolysis eliminates all structural information about how they were once attached within the wall. One approach is to digest cross-linked walls with hydrolytic enzymes and look for xylose oligosaccharides that have both ends of a ferulate dimer attached. This would be a tedious process. Furthermore, one may well be seeking something that does not exist. As an alternative, we have used molecular modeling to predict the likelihood of intramolecular formation of ferulate dimers.

Materials and Methods

The CAChe system from Oxford Molecular Group was used to build carbohydrate models containing two side chains of ferulated arabinose. Molecular structures were optimized to the lowest energy using MM2 molecular mechanic parameters. The initial structure contained 15 xylan residues with the ferulated arabinose residues on xylose 3 and 12 (see Fig. 1); this was considered the base structure. Experimental structures consisted of moving the side chain from xylose 12 to 4, minimizing this structure and coupling the two ferulates by 8-*O*-4, 8-5, 5-5 or 8-8 linkages and repeating the minimization. This cycle was repeated with the second side chain on xylose 5, 6, 7, then 8. The xylan backbone was either locked in its initial energy configuration or allowed to relax during the minimization procedures. If any structure gave a higher energy than the base molecule, the resulting minimized structure was tweeked by altering the structure and repeating the minimization. This prevented the molecule from becoming stuck in a local minimum that would not reflect the true minimized optimum structure. For some structures which appeared to produce low energy optimum structures with an intramolecular diferulate linkage, energy maps were constructed of major dihedral angles to determine if the molecule would naturally reach this configuration.

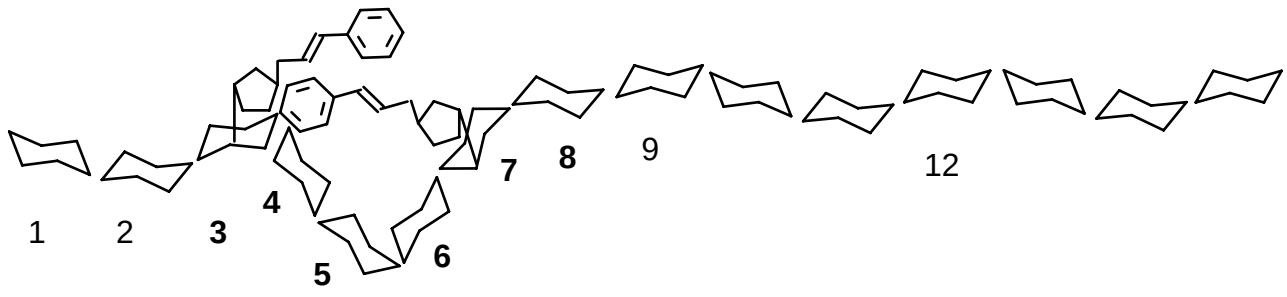

Figure 1. Schematic representation of a xylan fragment used in molecular modeling experiments. The left xylose residue is the non-reducing end of the molecule. Specific structural details have been left off to simplify the diagram. Numbers at the bottom of the diagram represent the numerical order of xylose residues used in the modeling experiments.

Results and Discussion

All structural configurations in which the xylan was allowed to fully relax produced energy minimizations nearly the same as the starting configuration before coupling the ferulates (any energy difference less than 20 Kcal was considered not to be an unfavorable energy configuration). However, with this set of parameters, formation of dehydrodimers at position 3-7 and 3-8 resulted in a buckling of the xylan backbone in order to achieve a low energy structure. Even the 8-8 dimer at position 3-6 resulted in some distortion of the backbone (Fig. 2). As might be expected, when the xylan backbone was held rigid, no ferulate dimers could produce a low energy configuration at positions 3-7 or 3-8, except for the 5-5 dimer at position 3-7. Our interpretation of these results is that, if ferulate monomers are attached to a xylan backbone with more than 3 xylose residues between them, it would not be possible to form intramolecular dimers. This is based on experimental evidence which suggests xylans prefer to stay in rigid rod type configuration with a three fold screw axis (no abnormal flexing to achieve coupling).

The one exception was the formation of the 5-5 dimer at position 3-7. This particular arrangement of ferulate monomers positions them in a configuration that is conducive to 5-5 coupling. This is of interest in that we cannot produce 5-5 coupled dimers using model compounds such as ethyl ferulate and wall peroxidases in free solution. Perhaps the formation of 5-5 dimers within wall matrices requires an exact positioning of the ferulate monomers.

For ferulate monomers in positions 3-4, 3-5 and 3-6, there is the potential for formation of dimers as long as the individual molecules can freely rotate around the axis of the xylan backbone. To check this possibility we have generated energy maps of the major dihedral angle changes that must occur to allow dimer formation. In most cases, it appears that sufficient energy barriers exist to prevent them from achieving the proper orientation necessary for coupling to form any of the dimers. The only configuration that appears feasible would be the 8-*O*-4 or 8-5 dimers which are in position 3-4. Ferulates in this position overlap sufficiently to allow coupling with minimum rotation along the xylan backbone axis.

Conclusions

Based on the modeling predictions combined with experimental evidence, intramolecular formation of ferulate dimers would appear to be restricted to positions 3-4 (dimers 8-*O*-4 and 8-5) or positions 3-7 (5-5 dimer). The latter is especially interesting since it may explain the unexpected preponderance of the 5-5 dimers in grasses.

Impact

Increased understanding of cell wall cross-linking chemistry affected by dimerization of ferulates will aid in the development of strategies for improving forage plants. These strategies may include genetic selection for or against specific traits, molecular modification of enzyme activity, or development of post-harvest treatments (specialized hydrolytic enzymes) to increase wall degradation.

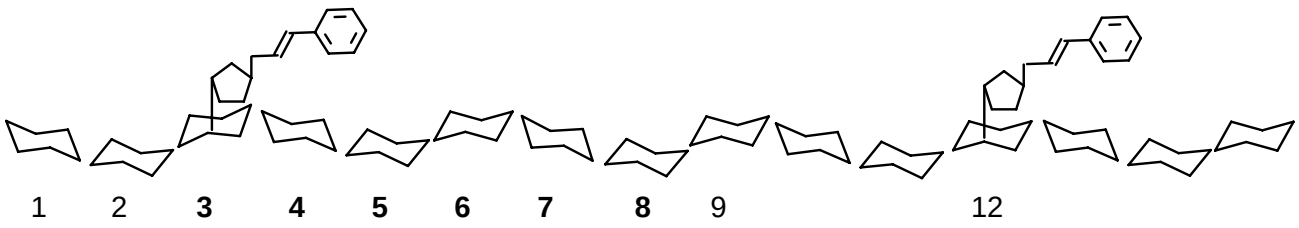

Figure 2. Schematic representation of a distorted xylan backbone when ferulate coupling is attempted with 4 (3-7) or more xylose residues between the ferulates.