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Note

Synthetic methyl hexagalacturonate hapten inhibitors of anti-homogalacturonan monoclonal antibodies LM7, JIM5 and JIM7

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Abstract

A range of synthetic methyl hexagalacturonates were used as potential hapten inhibitors in competitive-inhibition enzyme-linked immunosorbent assays (ELISAs) with anti-homogalacturonan monoclonal antibodies LM7, JIM5 and JIM7. The selective inhibition of these antibodies by different haptens provides insight into the structures of the partially methyl-esterified pectin epitopes of these widely used monoclonal antibodies.

Keywords: Pectin; Homogalacturonan; Monoclonal antibodies; LM7; JIM5; JIM7

1. Introduction

Homogalacturonan (HG) is an abundant polymer of the matrix of primary plant cell walls and is the major component of commercial pectic polymers.[1,2] Current evidence indicates that HG is usually synthesized in a largely methyl-esterified form in the Golgi apparatus and that it can be de-esterified in the cell wall by the action of a class of enzymes known as pectin methylesterases (PMEs).[1,2] PMEs form a large multigene family in the model plant species *Arabidopsis*.[3] The reason for existence of large numbers of PMEs in plants is far from clear but presumably reflects functional requirements for HG with varying degrees and patterns of methyl-esterification. Determining the functional significance of HG methyl-esterification in cell walls is important for understanding plant biology and also for understanding the functionality of commercial pectin preparations.

In recent years, a number of anti-HG monoclonal antibodies have been generated and used in studies of HG functions.[1,4] Antibodies to completely de-esterified epitopes of HG, such as 2F4[5] and PAM1[6] have been relatively easy to characterize due to the availability of defined fully unesterified oligogalacturonides. However, the current understanding of the epitope structures recognized by the widely used JIM5, JIM7 and LM7 are less clear.[7–9] Studies using model pectins with varying degrees and patterns of methyl-esterification indicated that the HG epitopes bound by JIM5, JIM7 and LM7 are all partially methyl-esterified.[8,9] However, partially methyl-esterified hapten inhibitors that allow a more complete characterization of the epitopes recognized by these antibodies have not previously been available.

We now report the use of a panel of synthetic methyl hexagalacturonates with varying patterns of methyl-esterification that can act as inhibitors of binding of LM7, JIM5 and JIM7. These compounds provide direct insight into the structure of methyl-esterified HG epitopes for the first time.

2. Results and discussion

The five methyl hexagalacturonates with varying occurrences of methyl-ester groups (compounds **1**–**5**) used in this study are shown in Fig. 1. Their structures were

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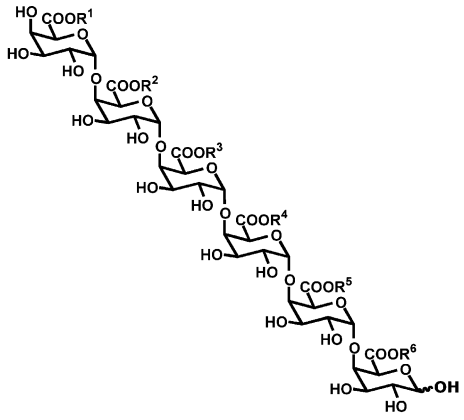

Cmp	R^1	R^2	R^3	R^4	R^5	R^6
1	Me	H	H	H	H	Me
2	Me	Me	H	H	H	Me
3	H	H	Me	Me	Me	H
4	H	Me	Me	Me	Me	H
5	Me	H	Me	H	Me	H

Fig. 1. Structure of methyl hexagalacturonates (compounds **1**–**5**) used as potential haptens. The presence or absence of methyl-ester groups is shown for each residue (R^1 to R^6). In addition, the methyl-esterification patterns of each compound is shown schematically.

verified by MS[10] and their synthesis is published elsewhere.[11]

The effectiveness of the methyl hexagalacturonates in inhibiting the binding of the antibodies was determined using competitive-inhibition ELISAs in which an antigen containing all epitopes (the model pectin F31[8]) was used to coat microtitre plates and was therefore the immobilised antigen. Potential methyl hexagalacturonate hapten inhibitors were titrated in the soluble phase and assessed for their capacity to inhibit binding of antibodies to F31.

The anti-(1→4)-β-D-galactan antibody LM5[12] was used as a control non-HG binding antibody. None of the hexagalacturonides inhibited the binding of LM5 to F31 (Fig. 2). For each anti-HG antibody, different groups of methyl hexagalacturonates were found to be effective inhibitors of binding when present at up to 0.5 mg mL^{-1} as shown in Fig. 2. The concentrations of hapten required to inhibit LM7, JIM5 and JIM7 binding by 50% (IC_{50}s) for all five methyl hexagalacturonates when present at up to 1 mg mL^{-1} are shown in Table 1.

2.1. LM7

Compound **1** was the only effective inhibitor of LM7 binding and is the first hapten inhibitor to be identified for this antibody. The lack of binding inhibition by compound **2** indicates that LM7 requires four unesterified GalA residues between methyl-ester groups-three not being sufficient. This confirms the previously documented sensitivity of the LM7 epitope to polygalacturonase.[8] The LM7 epitope is also sensitive to pectin lyase[8] indicating that optimal binding may require a larger number of flanking methyl-ester groups than occur in **1**.

2.2. JIM5

The binding of JIM5 was inhibited by compounds **1** and **2**, the most effective being **1** which contains four contiguous unesterified GalA residues between two methyl-ester groups. Compound **2** with three unesterifed GalA residues between two methyl-ester groups was also effective. These observations indicate that the JIM5 optimally binds to at least four contiguous unesterified residues between or adjacent to a methyl-ester group. Fully unesterified trigalacturonate is not an inhibitor of JIM5, although fully unesterifed nonagalacturonate is an effective inhibitor.[8] Therefore, the optimal JIM5 epitope may be more than four contiguous unesterified residues adjacent to or flanked by residues with methyl-ester groups. At an equivalent concentration F31 was a much more effective inhibitor of JIM5 binding (Fig. 2) indicating the presence of more optimal epitopes or some conformational feature of the epitopes found in an extended polymer and absent from the hexagalacturonates.

2.3. JIM7

Compounds **3**, **4** and **5** were effective inhibitors of JIM7 binding to F31 with similar IC_{50}s to each other and to F31. These are the first haptens to be identified for this monoclonal antibody and confirm its designation as binding to a relatively highly methyl-esterified epitope.[8] That JIM7 binds to a partially methyl-esterified epitope is indicated by the lack of inhibition of binding by fully methyl-esterified hexagalacturonates.[8] Perhaps the most surprising observation is that, in addition to binding to compounds **3** and **4** which have three and four contiguous methyl-ester groups flanked by unesterified residues, JIM7 binding is also inhibited by **5** with alternating methyl-esterified and unesterified residues. This may indicate that the important features of the JIM7 epitope are methyl-ester groups at every second

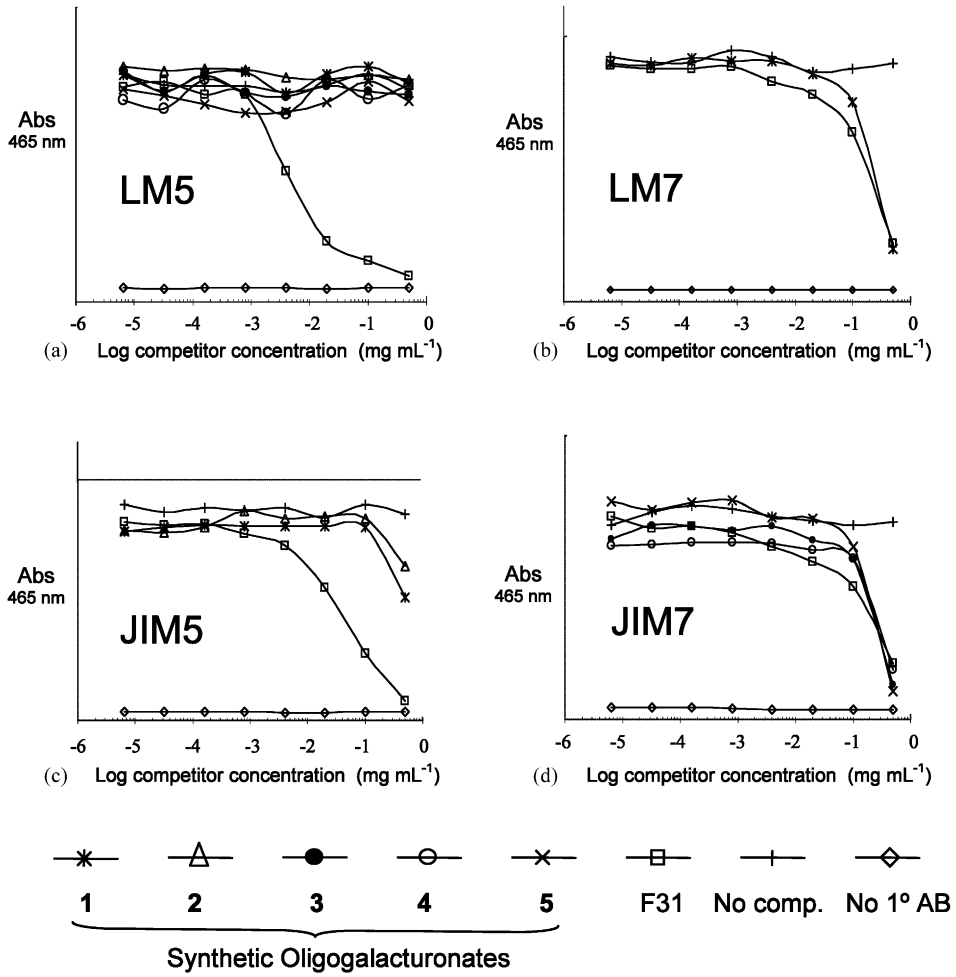

Fig. 2. Competitive inhibition ELISAs indicating the capacity of compounds **1** to **5** to inhibit the binding of anti-HG antibodies LM7, JIM5 and JIM7 to pectin sample F31 used as the immobilised antigen. The anti-(1→4)-β-D-galactan antibody LM5 was used as a control. All five compounds are shown in the LM5 graph but for clarity only compounds that were effective as inhibitors are shown for LM7, JIM5 and JIM7 along with F31 as a soluble antigen, no competitor added and no primary antibody in the assays.

Table 1
Binding of anti-HG antibodies JIM5, JIM7 and LM7 to methyl-hexagalacturonates **1** to **5** in competitive inhibition ELISAs

Compound	LM7	JIM5	JIM7
1	220	600	>1000
2	>1000	900	>1000
3	>1000	>1000	200
4	>1000	>1000	230
5	>1000	>1000	260

Results are expressed as IC_{50}s which is the concentration of hapten (μg mL^{-1}) required to effect a 50% inhibition in binding of an antibody to the immobilised antigen.

residue and no preference for the esterification state of the intervening residue. Such preferences may reflect the nature of the interaction of the JIM7 antibody with the predominant 2_1 helical conformation that is found in HG chains.[1]

In summary, the availability of methyl hexagalacturonates has allowed the identification of partially methyl-esterified haptens for three anti-HG antibodies for the first time. LM7 and JIM5 bind to unesterified GalA residues with adjacent or flanking methyl-esterified residues. In contrast, JIM7 binds to methyl-esterified residues with adjacent or flanking unesterified GalA residues. This has extended our understanding of the binding specificities of these antibodies which is important when considering the application of these antibodies to plant materials.

3. Experimental

Competitive-inhibition ELISAs were performed as described elsewhere.[9]

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References

1. Willats, W. G. T.; McCartney, L.; Mackie, W.; Knox, J. P. *Plant Mol. Biol.* **2001**, *47*, 9–27.
2. Ridley, B. L.; O'Neill, M. A.; Mohnen, D. *Phytochem.* **2001**, *57*, 929–967.
3. The arabidopsis genome initiative, *Nature* **2000**, *408*, 796–815.
4. Knox, J. P. *Int. Rev. Cytol.* **1997**, *171*, 79–120.
5. Liners, F.; Thibault, J.-F.; van Cutsem, P. *Plant Physiol.* **1992**, *99*, 1099–1104.
6. Willats, W. G. T.; Gilmartin, P. M.; Mikkelsen, J. D.; Knox, J. P. *Plant J.* **1999**, *18*, 57–65.
7. Knox, J. P.; Linstead, P. J.; King, J.; Cooper, C.; Roberts, K. *Planta* **1990**, *181*, 512–521.
8. Willats, W. G. T.; Limberg, G.; Buchholt, H. C.; van Alebeek, G.-J.; Benen, J.; Christensen, T. M. I. E.; Visser, J.; Voragen, A.; Mikkelsen, J. D.; Knox, J. P. *Carbohydr. Res.* **2000**, *327*, 309–320.
9. Willats, W. G. T.; Orfila, C.; Limberg, G.; Buchholt, H. C.; van Alebeek, G.-J. W. M.; Voragen, A. G. J.; Marcus, S. E.; Christensen, T. M. I. E.; Mikkelsen, J. D.; Murray, B. S.; Knox, J. P. *J. Biol. Chem.* **2001**, *276*, 19404–19413.
10. Korner, R.; Limberg, G.; Christensen, T. M. I. E.; Mikkelsen, J. D.; Roepstorff, P. *Anal. Chem.* **1999**, *71*, 1421–1427.
11. Clausen, M. H.; Madsen, R. *Chem. Eur. J.* **2003**, in press
12. Jones, L.; Seymour, G. B.; Knox, J. P. *Plant Physiol.* **1997**, *113*, 1405–1412.