Immunology:

# Self-association of an Insect β-1,3-Glucan Recognition Protein Upon Binding Laminarin Stimulates Prophenoloxidase Activation as an Innate Immune Response

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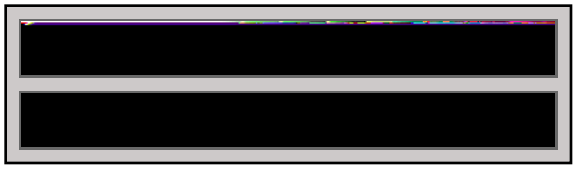

tural studies have included proposed modes for binding of □-1,3-glucan by N-□GRP. The crystal structure of N-□GRP

FIGURE 1.

its elution volume with those of molecular weight standards (Fig. 1*A*). The two-dimensional $^{15}$N-$^{1}$H HSQC spectrum for $^{15}$N-labeled N-□GRP2 showed excellent chemical shift dispersion (Fig. 1*B*), and the backbone chemical shifts of $^{1}$H, $^{15}$N, and $^{13}$C nuclei were assigned using a series of the multidimensional heteronuclear NMR methods; 94% of the backbone amide resonance of 109 non-proline residues could be assigned. The secondary structures of N-□GRP2 were predicted using the TALOS program (19) and the chemical shift assignments of $^{13}$C□, $^{13}$C□, and $^{13}$C□ resonances (Fig. 1*C*), which agreed well with those of *Pi*-N-□GRP determined by solution NMR spectroscopy (12). A three-dimensional structural model of N-□GRP2 predicted using the I-TASSER server (C-score □ 0.83 □ 0.08, expected root mean square deviation □ 2.7 □ 2.0) (20, 21) aligned very well with N-□GRP from other insects, with root mean square deviations of the backbone C□ atoms

between N-□GRP2 and *Pi*-N-□GRP (Protein Data Bank 2KHA (12)) being 0.38 Å (Fig. 1*D*).

*Binding to Laminarin Induces the Association of N-□GRP2 Molecules*—We carried out a series of NMR chemical shift titration experiments to study interactions between N-□GRP2 and laminarin. Laminarin is a water-soluble □-1,3-glucan with □-1,6-glucan branches. The □-1,3 to □-1,6 cross-linkage ratio varies depending on the biological source. Laminarin from *L.*

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We recently demonstrated using solution NMR and analytical ultracentrifugation methods that *Pi*-N-□GRP forms a soluble, high molecular mass complex upon binding to laminarin (12). To directly compare the effects of laminarin on N-

*lanes 3*), this band is of substantially weaker intensity than that for the soluble complex, probably suggesting a nonspecific inter-monomer cross-linking. This cross-linking experiment provides further evidence for the soluble complex of N-□GRP2 and laminarin, and demonstrates that the complex encom-

In the crystal structure of *Plodia* and

iment suggests that the mode of interaction between N-□GRP2 molecules bound to laminarin is consistent with that observed in the crystals of N-□GRP formed with laminarihexaose, which may indicate that binding of N-□GRP2 to laminarin chains promotes their association as a partial triple helical structure (although the □-1,6 branches would interfere with the helical structure formation to some extent). When the molar ratio of protein to carbohydrate is high (at the low [C]/[P] ratio), we predict that multiple N-□GRP2 molecules interacting with a helical form of laminarins assemble through protein-protein

The insoluble complex of N-□GRP2 and laminarin may mimic assembly of □