

## AN 'ALLOSTERIC-TYPE' EFFECT DEMONSTRATED BY A LECTIN FROM THE RED ALGA GRIFFITHSIA FLOSCULOSA

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Lectins are glycoprotein or protein molecules which bind to carbohydrate residues on cell-surfaces and macromolecules. As the binding process is frequently highly specific, lectins have found widespread use in such fields as biochemistry and biomedicine for the identification, location or isolation of receptors on human or other tissues (Thiele & Arndt 1982).

The binding activity of most lectins may be inhibited by prior exposure of the lectin to carbohydrate molecules (often simple hexoses) which specifically block the binding site on the lectin, thereby preventing subsequent combination of the lectin with a similar structure in a receptor. This report describes an opposite effect; that of stimulation of lectin binding to receptors by molecules which might be expected to be inhibitory.

Aqueous extracts of the red alga Griffithsia flosculosa were prepared and investigated by methods described previously (Rogers et al 1986). Proteins in the crude extract were precipitated with 80% ammonium sulphate, dialysed against phosphate buffered saline pH 7.3 (PBS) and concentrated by ultrafiltration. The concentrated extract was then titrated against papainised human erythrocytes using PBS, incorporating  $2 \text{ g l}^{-1}$  Tween 80, as diluent for both the extract and cells. A titration value of 1:8 (score 24) was obtained. Haemagglutination inhibition studies showed that the glycoproteins, bovine sub-maxillary gland mucin, porcine mucin and fetuin were inhibitory and that an extensive range of mono-, di- and trisaccharides were non-inhibitory at 100 mM concentration. A surprising observation was that N-acetyl- $\alpha$ -D-glucosamine (GluNAC) enhanced agglutination of the erythrocytes by the lectin.

This enhancing effect was investigated by preparing ten different diluents each incorporating a known concentration of GluNAC. These concentrations ranged from 1 mM to 100 mM. The other characteristics of the diluents are described above. Each of the ten diluents was used to titrate the Griffithsia lectin against papainised erythrocytes suspended in identical diluent to that used for the lectin.

These studies showed that the enhancing effect of GluNAC was concentration-dependent over a range of 10 mM to 50 mM. For example, 10 mM GluNAC increased the titration value to 1:16 and the titration score to 35, while 50 mM GluNAC increased the titration value to 1:64 and the score to 100. Further studies have shown that the effect is maximal at 50 mM, as concentrations of GluNAC above this figure show decreasing enhancement. The effect is totally absent at 400 mM concentration.

None of the results presented here enable a conclusion to be made as to whether GluNAC affects the lectin or the receptor or both. Although no reports have appeared describing modification of erythrocyte surfaces by "free" GluNAC, one suggestion of an "allosteric-type" effect on lectins by simple molecules has been made (Cammue & Peumans 1985).

Cammue, B.P.A., Peumans, W.J. (1985). Abstract book of 7th International Lectin Meeting, Vrije Universiteit Brussels, p15.

Rogers, D.J. et al (1986) In: Lectins, Biology, Biochemistry, Clinical Biochemistry Vol 5. Walter de Gruyter, Berlin, 155-160.

Thiele, H.G., Arndt, R. (1982) J.Clin.Chem.Biochem. 20: 123-134.