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Sulphydryl groups in the maintenance of the activity of binding protein for N-1-naphthylphthalamic acid from *Acer pseudoplatanus* cells

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Abstract

Binding protein for N-1-naphthylphthalamic acid (NPA), an auxin transport inhibitor, was studied by analysis of the effects of reactions which modify particular amino acid side chains upon their binding activity. Na_2SO_3, N-ethylmaleimide (NEM) and dithiobisnitrobenzoic acid all inhibited the specific binding of NPA to its binding protein from *Acer pseudoplatanus* L. cells. The presence of 10^{-6} M Na_2SO_3 in the binding assay reduced the affinity of the binding protein to NPA from K_d of 1.5×10^{-8} M to K_d of 2.1×10^{-8} M, while concentration of the binding protein was not significantly changed. When the same analysis was applied to NPA binding to the NEM-treated membrane particles, it was found that NEM inactivated binding without changing the affinity for NPA. This study revealed the importance of sulphydryl group(s) in the maintenance of NPA binding protein activity.

Additional key words: amino acid side chain, auxin, transport carrier.

Introduction

Binding proteins for N-1-naphthylphthalamic acid (NPA) were first described by Lembi *et al.* (1971). NPA appears to block auxin transport on auxin efflux carriers (Rubery and Shedrake 1974, Rubery 1979) and so stimulates net auxin uptake by cultured cells, tissue segments and membrane vesicles (Hetel 1983, Xing *et al.* 1988, Xing 1991). A high affinity and low capacity NPA binding protein was detected in *Acer pseudoplatanus* L. cells and these binding proteins were shown to have an important role in the regulation of IAA levels and, consequently, of cell division in

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Abbreviations: DTNB - 5',5'-dithio-bis-2-nitrobenzoic acid; NEM - N-ethylmaleimide; NPA - N-1-naphthylphthalamic acid

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these suspension-cultured cells (Elliott *et al.* 1988, Xing *et al.* 1992). In our present study, the binding protein will be further characterized by analysis of the effects of reactions which modify particular amino acid side chains upon their binding activity.

Materials and methods

The origin of *Acer pseudoplatanus* L. cell suspension culture and general techniques of the culture have been described previously (Elliott *et al.* 1988, Xing *et al.* 1992). The basic culture medium was that developed by Stuart and Street (1969) which contained 2,4-D (4.5×10^{-8} M) as exogenous auxin but no kinetin or urea.

Microsomal membrane fractions were prepared and the basic binding assay was performed as described previously (Elliott *et al.* 1988, Xing *et al.* 1992). For the determination of the effect of Na_2SO_3 on NPA binding, a Na_2SO_3 stock solution at pH 4 were added to the incubation mixture for the binding assay giving required concentrations. In the cases of DTNB and NEM, the isolated membrane pellet was resuspended in DTNB or NEM solution, prepared in extraction buffer at pH 7, for 30 min. The pellet was then sedimented at 75 000 *g* for 45 min and resuspended in pH 4 binding assay buffer. The pellet was then used in the binding assay. Results presented were the average values of at least five independent experiments, each with three replicates. Standard errors were always less than 7 % of the average values and thus omitted from the table and figures.

Proteins were determined according to Bradford (1976).

Results and discussion

Effect of -SH reagents on ^{3}H-NPA binding: The effect of Na_2SO_3, DTNB and NEM on NPA binding was compared. It was found that NPA binding protein was more sensitive to Na_2SO_3 and less sensitive to NEM (Table 1) compared to the observations in *Cucurbita* (Thein and Michalke 1988).

Table 1. Effect of some reagents on NPA binding (data represent means of 4 experiments).

Reagent	Specific NPA binding [% of control]
1 mM Na_2SO_3	2
5 mM DTNB	12
5 mM NEM	49

Inhibition of ^{3}H-NPA binding by Na_2SO_3 at different concentrations: Membrane particles were incubated with different concentrations of Na_2SO_3. The displacement curve of NPA from its binding protein (Fig. 1) showed that the concentration which caused 50 % reduction was 2×10^{-5} M.

Alteration of binding affinity and binding capacity upon Na_2SO_3 or NEM treatment: Scatchard analysis (Scatchard 1949) was applied to the determination of the binding affinity and capacity after treatment with Na_2SO_3 or NEM. This analysis is relevant

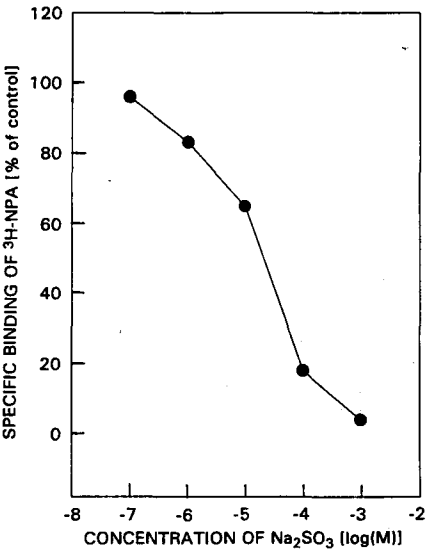

Fig. 1. Specific binding of ^{3}H-NPA upon Na_2SO_3 treatment. Na_2SO_3 stock solution was added to the binding assay mixture giving the indicated final concentrations. NPA binding assays were then performed as described in Materials and methods.

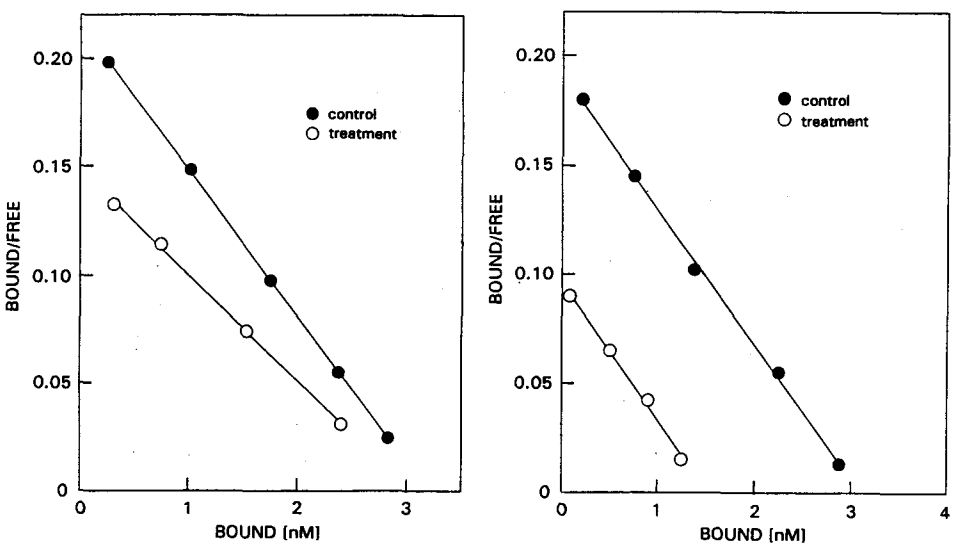

Fig. 2. Scatchard plot showing affinity and capacity of NPA binding proteins after Na_2SO_3 (3×10^{-5} M) treatment.

Fig. 3. Scatchard plot showing affinity and capacity of NPA binding proteins after NEM (5×10^{-3} M) treatment.

to the elucidation of the mechanisms of their inhibitory effects. The presence of 3×10^{-5} M Na_2SO_3 in the binding assay reduced the affinity of the binding proteins to NPA from Kd of 1.47×10^{-8} M to Kd of 2.07×10^{-8} M, while the concentration of the binding proteins in the assay was not significantly changed (Fig. 2). When the same analysis was made on NPA binding to NEM-treated membrane particles, it was found that NEM, which covalently modified -SH groups, inactivated the binding without altering the affinity for NPA (Fig. 3).

Our observations are similar to those demonstrated in *Cucurbita pepo* hypocotyls (Thein and Michalke 1988). Since the covalent modification of -SH groups affected NPA binding proteins while Na_2SO_3 affected them in a different way, Thein and Michalke (1988) suggested that Na_2SO_3 did not act *via* modification of -SH groups. Based on these observations, together with the failure of thiols and arsenite to inhibit NPA binding in *Cucurbita*, they argue against the possibility that bisulfite acted by reducing -SH groups or splitting disulfide linkages. They assumed that its reaction product in H_2O, the HSO_3^- anion, was responsible. This product is nucleophilic, and can bind reversibly to quinones (Malhotra and Hocking 1976), pyridoxal phosphate (Adams 1969) and to the isoalloxane ring of the FAD prosthetic group (Swoboda and Massey 1966, Massey *et al.* 1969). Addition of bisulfite to any of these groups at or close to the NPA binding protein may be able to alter the behaviour of NPA binding protein in the way described here.

Our studies revealed the importance of sulphydryl group(s) in the maintenance of NPA binding protein activity. Previous investigations have concentrated upon structure-activity aspects of ligand-binding protein interactions (*e.g.* Katekar *et al.* 1987). Although less is known of the amino acid residues involved in ligand binding, such information is essential if a sophisticated understanding of plant growth regulator actions is to be obtained.

References

Adams, E.: Reaction of dithionine and bisulfite with pyridoxal enzymes. - Anal. Biochem. **31**: 484-492, 1969.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Elliott, M.C., Hall, J.F., Xing, T., O'Sullivan, A.M., Scott, N.W., Barker, R.D.J.: The role of IAA in the regulation of plant cell division. - In: Bandurski, M., Kutáček, M., Krekule, J. (ed.): Physiology and Biochemistry of Auxins in Plants. Pp. 152-157. SPB Academic Publishing, The Hague 1988.

Hertel, R.: The mechanism of auxin transport as a model for auxin action. - Z. Pflanzenphysiol. **112**: 53-67, 1983.

Katekar, G.F., Geissler, A.E., Kennard, C.H.L., Smith, G.: Recognition of phytotropins by the receptor for 1-N-naphthylphthalamic acid. - Phytochemistry **26**: 1257-1267, 1987.

Lembi, C.A., Morre, D.J., Thomson, K.S., Hertel, R.: 1-N-naphthylphthalamic acid binding activity of a plasma membrane-rich fraction from maize coleoptiles. - Planta **99**: 37-45, 1971.

Malhotra, S.S., Hocking, D.: Biochemical and cytological effects of sulphur dioxide on plant metabolism. - New Phytol. **76**: 227-237, 1976.

Massey, V., Muller, F., Feldbery, R., Schuman, M., Sullivan, P.A., Howell, L.G., Mayhew, S.G., Matthew, R.G., Foust, G.P.: The reactivity of flavoproteins with sulfite: possible relevance to the problem of oxygen reactivity. - J. biol. Chem. **244**: 3999-4006, 1969.

Rubery, P.H.: The effect of 2,4-dinitrophenol and chemical modifying reagents on auxin transport by suspension cultured crown gall cells. - Planta **144**: 173-178, 1979.

Rubery, P.H., Sheldrake, A.R.: Carrier-mediated auxin transport. - Planta **118**: 101-121, 1974.

Scatchard, G.: The attraction of proteins for small molecules and ions. - Ann. N.Y. Acad. Sci. **57**: 660-672, 1949.

Stuart, R., Street, H.E.: Studies on the growth in culture of plant cells. IV. The initiation of division in suspensions of stationary phase cells of *Acer pseudoplatanus* L. - J. exp. Bot. **22**: 96-106, 1971.

Swoboda, B.E.P., Massey, V.: On the reaction of the glucose oxidase from *Aspergillus niger* with bisulfite. - J. biol. Chem. **241**: 3009-3016, 1966.

Thein, M., Michelke, W.: Bisulfite interacts with binding sites of the auxin-transport inhibitor N-1-naphthylphthalamic acid. - Planta **176**: 343-350, 1988.

Xing, T., Zhou, J.H., Elliott, M.C.: Auxin transport carriers. - Plant Physiol. Commun. **24**: 13-17, 1988.

Xing, T.: Biochemistry and molecular biology of auxin receptors: ABP-1, NPA-BP and sABP. - Plant Physiol. Commun. **27**: 7-10, 1991.

Xing, T., Zhang, D.Y., Elliott, M.C.: Characterization and possible function of binding sites for naphthylphthalamic acid in *Acer pseudoplatanus* L. cells. - Chin. J. Bot. **4**: 26-33, 1992.