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Isolation of a protein with cytokinin-receptor properties by means of anti-idiotype antibodies

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A protein from cytosol of 10-day-old barley leaves with cytokinin-receptor properties was isolated and 12 000-fold purified by anti-idiotype antibodies from anti-BA serum. Cytokinin-binding properties of this protein were demonstrated by its competition with Ab_{BA} for immobilized BA in competitive ELISA. In the presence of BA the protein activates in vitro rRNA synthesis in the transcription elongation system containing chromatin-bound RNA-polymerase 1 from barley leaves. The protein with similar properties was isolated from barley leaf cytosol with BA-Sepharose. The control proteins isolated with adenine- and ethanolamine-Sepharose did not possess cytokinin-binding properties and had no effect on RNA synthesis in vitro.

6-Benzyladenine; Anti-idiotype antibody; Cytokinin-binding protein; Activation of transcription in vitro

1. INTRODUCTION

To investigate the phytohormone receptors it is necessary not only to isolate hormone-binding proteins but to demonstrate also the ability of hormone-protein complex to induce a primary hormonal reaction culminated in the final cell response to phytohormone [1–3]. Now CBPs were isolated from wheat germs [4], barley leaves [5] and some other plant materials [3] but the function of all these CBPs is still not clear. Demonstration of the protein fraction in barley leaves capable of inducing cytokinin-dependent activation of RNA synthesis in vitro has given support to the assumed presence of cytokinin-receptor protein(s) in leaf cells mediating hormonal activation of RNA synthesis.

For this reason the aim of our work was to purify and characterize this protein(s). As $Ab_{a\text{-}i}$ were shown to be successful in isolating the receptors of animal hormones [6], we obtained $Ab_{a\text{-}i}$ from the anti-BA serum and used them for CBP purification from barley leaf cytosol.

2. MATERIALS AND METHODS

Barley plants (*Hordeum vulgare* L. cv Viner) were grown in soil boxes in growth chambers (light intensity of 50 W/m^2, 16 h/day, day temperature of 22–23°C, night temperature of 18°C). The first leaves of 8–10-day-old plants were used for CBP isolation.

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Abbreviations: BA, 6-benzyladenine; CBP, cytokinin-binding protein; Ab_{BA}, antibodies against BA; $Ab_{a\text{-}i}$, anti-idiotype antibodies; BSA, bovine serum albumin

The syntheses of conjugates of BA-riboside with BSA and with ovalbumin and the rabbit immunisation procedure were described elsewhere [7].

BA-Sepharose was obtained by immobilization of BA on epoxy-activated Sepharose 6B [8,9] at pH 12. Adenine- and ethanolamine-Sepharose similarly synthesised were used as control sorbents.

Ab_{BA} were isolated from antiserum against BA by chromatography on BA-Sepharose. Non-bound material was eluted with PBS-buffer (10 mM Na-phosphate buffer, pH 7.2, 150 mM NaCl). Ab_{BA} were eluted with 0.1 M glycine-HCl buffer, pH 2.2, saturated with ethyl ester [10]. Isolated Ab_{BA} were immobilized on CNBr-activated Sepharose 4B [9].

$Ab_{a\text{-}i}$ were isolated from anti-BA serum by chromatography on Ab_{BA}-Sepharose. Non-bound proteins were eluted with PBS-buffer and $Ab_{a\text{-}i}$ were eluted with 0.1 M glycine-HCl buffer, pH 2.8. Then $Ab_{a\text{-}i}$ were immobilized on CNBr-activated Sepharose 4B [9].

All procedures of CBP isolation were carried out at 2–4°C. Barley leaves were homogenized in 3–4 vols. of buffer (100 mM Tris-HCl, pH 7.7, 100 mM $MgCl_2$, 250 mM sucrose, 20 mM 2-mercaptoethanol). Supernatant (23 000 × *g*) was passed through Sephadex G-50 column to remove low molecular substances (buffer: 50 mM Tris-HCl, pH 7.7, 10 mM $MgCl_2$, 1 M NaCl, 5 mM 2-mercaptoethanol) and followed by protein fractionation on affinity sorbents. The isolation of CBP on $Ab_{a\text{-}i}$-Sepharose was carried out in the same buffer but NaCl was of 0.25 M. For the elution of protein(s) bound by $Ab_{a\text{-}i}$-Sepharose, 0.1 M glycine-HCl buffer, pH 2.8, was used. The isolation of CBP by BA-Sepharose was held as described earlier [11]. Bound protein(s) was eluted with 0.2 N NaOH. Control proteins from adenine- and ethanolamine-Sepharose were isolated by the same procedure.

Polypeptide composition of proteins was studied by SDS-PAG electrophoresis [12]. The gels were stained according to Oakley et al. [13].

Cytokinin-binding properties of isolated proteins were tested by their ability to displace Ab_{BA} from its complex with immobilized BA in competitive ELISA of cytokinin determination [14–16]. Ab_{BA} associated with immobilized BA were estimated by second anti-rabbit antibodies labelled with horseradish peroxidase. *o*-Phenylenediamine was used as a chromogen. The reaction was recorded at 492 nm.

The interaction of leaf proteins with $Ab_{a\text{-}i}$ was studied by direct

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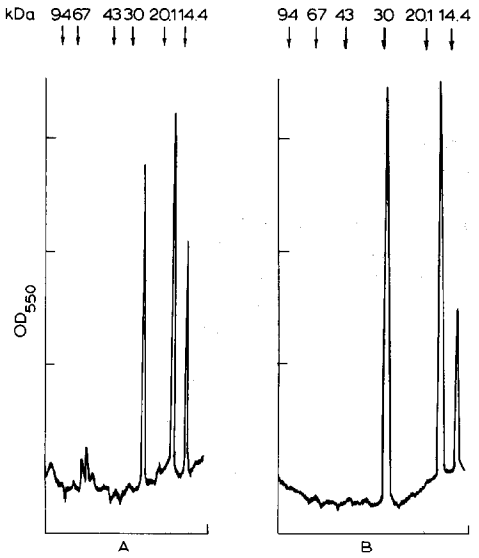

Fig.1. SDS-PAGE electrophoresis densitograms of barley leaf cytosol proteins isolated with Ab_{a-i}-Sepharose (A) and BA-Sepharose (B). Arrows indicate the positions of standard proteins.

ELISA. The proteins to be tested were immobilized on microtitration plate followed by Ab_{a-i} associated with immobilized proteins were estimated by second anti-rabbit peroxidase-labelled antibodies.

The effect of isolated proteins and BA on the transcription in vitro was studied as described elsewhere [8] in systems containing RNA-polymerase I associated with chromatin from barley leaves.

Biochemicals: Sephadex G-50, epoxy-activated Sepharose 6B, CNBr-activated Sepharose 4B, electrophoresis calibration kit were from Pharmacia LKB Biotechnology (Sweden); ovalbumin was from Sigma (USA); BA, BA-riboside, BSA were from Serva (FRG); anti-rabbit peroxidase-labelled immunoglobulins were from N.G. Gamaleya Institute of Epidemiology and Microbiology (USSR).

3. RESULTS AND DISCUSSION

The affinity chromatography on Ab_{a-i}-Sepharose has provided the isolation and 12000-fold purification of barley leaf cytosol protein which could be a potential cytokinin receptor. Fig.1A shows its polypeptide composition. It corresponds to the polypeptide composition of protein isolated from leaf cytosol with BA-Sepharose (fig.1B). In both proteins a polypeptide with molecular mass of 28–30 kDa was observed. The nature of some low molecular polypeptides in both preparations is not yet clear. Perhaps they can be considered as the products of the 28–30 kDa polypeptide partial degradation.

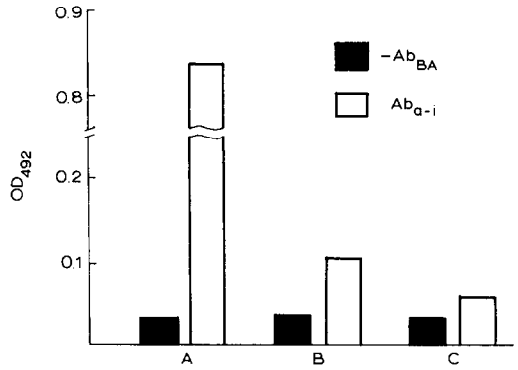

Fig.2. Interaction of Ab_{a-i} with barley leaf proteins isolated with BA-Sepharose (A), adenine-Sepharose (B) and ethanol-amine-Sepharose (C).

Table 1

The effect of proteins isolated from barley leaves, BA, Ab_{a-i} and Ab_{BA} on RNA synthesis in vitro in the system containing chromatin-bound RNA polymerase 1 from barley leaves

The mode of protein isolation	Ab_{a-i}	Ab_{BA}	RNA synthesis (as % to control test)	
			−BA	+BA
Control test (without proteins)	−	−	100	100
	+	−	92	97
	−	+	107	106
Ab_{a-i}-Sepharose	−	−	99	254
	+	−	97	116
	−	+	104	112
BA-Sepharose	−	−	101	245
	−	+	100	88
	+	−	100	74
Adenine-Sepharose	−	−	82	80
Ethanolamine-Sepharose	−	−	98	102

The protein isolated with BA-Sepharose interacted with Ab_{a-i} (fig.2). Hence it has common immunodeterminants with the protein isolated with Ab_{a-i}-Sepharose. Both proteins did not interact with Ab_{BA} used as a control test for non-specific binding of the leaf proteins with immunoglobulins from rabbit antiserum. The control proteins isolated from barley leaf cytosol with adenine- and ethanolamine-Sepharose did not interact with Ab_{a-i}.

The ability of proteins isolated with Ab_{a-i}- and BA-Sepharose to bind BA was shown by their competition with Ab_{BA} for complex formation with immobilized BA in ELISA (fig.3). The decrease of OD_{492} has demonstrated the displacement of Ab_{BA} from their complexes with immobilized BA by proteins isolated from barley

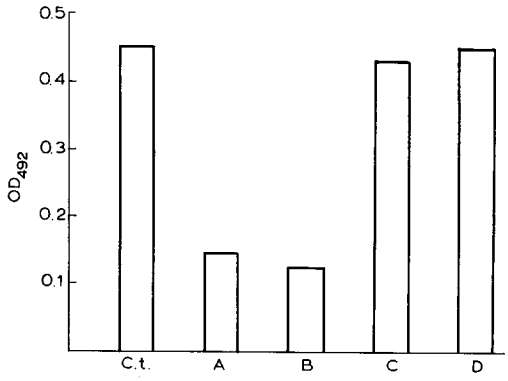

Fig.3. Competitive inhibition of Ab_{BA} interaction with immobilized BA in competitive ELISA by barley leaf proteins obtained with Ab_{a-i}-Sepharose (A), BA-Sepharose (B), adenine-Sepharose (C) and ethanolamine-Sepharose (D). C.t. = control test without protein addition.

leaves. Hence these proteins really possess cytokinin-binding properties. The control proteins isolated with adenine- and ethanolamine-Sepharose did not affect OD_{492} and therefore had no cytokinin-binding properties.

Proteins isolated with $Ab_{a\text{-}i}$- and BA-Sepharose have activated in the presence of BA rRNA synthesis in vitro in the transcription elongation system (table 1). $Ab_{a\text{-}i}$ (as antibodies to CBP) and Ab_{BA} have inhibited CBP-BA induced activation of transcription. Control proteins were unable to activate RNA synthesis in vitro (table 1). Hence a cytokinin-dependent activation of RNA synthesis is really specific for CBP which can be considered as a cytokinin-receptor protein capable of mediating the activation of transcription by cytokinin. Obviously such CBP-BA induced transcription in vitro corresponds to a well-known cytokinin activation of RNA synthesis in vivo in isolated barley leaves [17].

From the data obtained, the conclusion can be drawn that protein(s) with cytokinin receptor properties was isolated from barley leaf cytosol by two independent methods based on its affinity for $Ab_{a\text{-}i}$ and BA.

Future investigation should elucidate the relationship between this CBP-mediated cytokinin-induced activation of transcription and another CBP from barley leaves with an unknown function [5].

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