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# AN INVESTIGATION OF THE MEMBRANOTROPICITY OF THE ETHYLENE- AND CYTOKININ-BINDING PROTEINS FROM COTTONPLANT SHOOTS BY THE FLUORESCENT PROBE METHOD

**Ya. S. Ziyaev, O. N. Veshkurova, A. A. Takanaev, Kh. K. Avlyanov, B. A. Salakhutdinov, and Sh. I. Salikhov**

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A number of hypotheses on the mechanism of the regulatory action of phytohormones assume their interaction with specific receptor proteins, which are subdivided into membrane and intracellular types. The point of view exists that the receptors of the first type, on binding the hormone, change the membrane potential and increase the concentration of secondary messengers [1]. It may also be assumed that they promote the penetration of the phytohormone within the cell and its interaction with the receptors of the second type.

We have previously isolated ethylene- and cytokinin-binding proteins (EBPs and CBPs) from cottonplant shoots and investigated some of their functions [2, 3]. In the present commnication we give the results of an investigation of the membranotropic activities of these proteins.

The membranotropic properties of the isolated proteins were studied by the method of fluorescent probes (the probe used being ANS) in liposomes formed from phosphatidylcholine (PC) as a basic structure-forming component of cells [4]. In each experiment we took 50 $\mu$l of a 0.4% aqueous solution of liposomes, 5 $\mu$l of a 1% solution of 1-anilinonaphthalene-8-sulfonate (ANS) in ethanol, 10 $\mu$l of a solution of ECBs or CBPs with a concentration of $10^{-3}$ M, and 10 $\mu$l of a $4 \times 10^{-6}$ M solution of ethylene in water or 10 $\mu$l of a $1 \times 10^{-6}$ M solution of benzylaminopurine (BAP) in water.

The fluorescence spectra of the aqueous samples of liposomes were recorded on a Mikro spectrofluorimeter using 365 and 475 nm filters, respectively. The kinetic curves were recorded at wavelengths corresponding to the maximum amplitudes of emission. The addition of the EBPs to the probe-labeled membranes led to a rise in the intensity of fluorescence in comparison with its intial value (Fig. 1, *a*), with passage to an intensity plateau after a definite time. On further addition another rise in intensity was observed, but the response of the system was smaller than in the first case. With all subsequent additions of the EBPs a symbatic decrease in the rise of the intensity of fluorecence was seen.

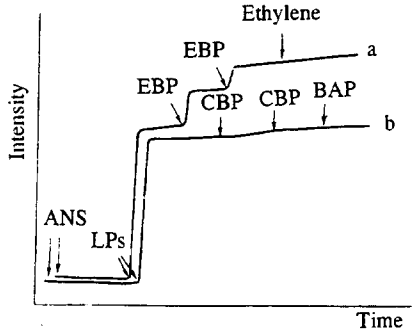


Fig. 1. Influence of EBPs and CBPs on the intesnisty of fluorescence of ANS incorporated into liposomes.

A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax 627071. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 135-136, January-February, 1994. Original article submitted July 13, 1993.

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The pattern observed is connected with the fact that the EBPs, on interacting with liposomes, introduced a certain hydrophobicity into the local microdistribution of the part of the probe molecule that is localized in the most polar region of the membrane surface. In addition, the results obtained indicate that the membrane surface contains a certain number of protein-binding sites the complete occupancy of which involves a process of saturation. When free ethylene was added to the system the intensity of fluorescence did not change. Consequently, the water-soluble EBPs that we had isolated from the cytosol do not belong to the membrane receptor type, even though they exhibit tropicity for a membrane.

When CBPs and (or) benzylaminopurine were added to the liposomes the intensity of fluoresence did not change (Fig. 1, *b*), i.e., CBPs, unlike EBPs, exhibit no pronounced affinity for membranes when investigated under the given conditions.

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