*Chemistry of Natural Compounds, Vol. 30, No. 1, 1994*

# WATER-SOLUBLE ETHYLENE-BINDING PROTEINS OF THE COTTON PLANT: ISOLATION AND FUNCTIONAL ACTIVITY

**Ya. A. Ziyav, O. N. Beshkurova, A. A. Takanaev, and Sh. I. Salikov**

UDC 547.857.581.192.7

*A protein possessing a high affinity for ethylene has been isolated from cottonplant shoots by hydrophobic chromatography on Polikhrom-1 and gel chromatography on TSK gel HW-50F. A number of proofs of the receptor nature of the protein isolated are given.*

Ethylene plays an important role in the development of higher plants. Practiclly all the stage of the development of the plant organism can be regulated by this phytohormone: the germination of the seeds, the initiation of the roots, the ripening of the fruit, and ageing and withering [1] Howver, the biochemical mechanisms of the action of ethylene have been insufficiently widely studied. It is assumed that ethylene binds with specific proteins, forming, like other phytohormones, a hormone-receptor complex activating or inhibiting metabolic processs in the cells.

Protein sites with a high affinity for ethylene have been detected in the leaves, cotyledons, and roots of the kidney bean, pea hypocotyles, and the leaves of tobacco, lemon, and tomatoes, and shoots of the mung bean [2-4]. It has been shown that ethylene-binding proteins (EBPs) are localized mainly in the cytoplasmic membranes and, apparently, cannot be transported into the nucleus for the transmission of the hormonal signal.

In view of this, we assumed that, in addition to membrane-bound EBPs, the existence is also possible of water-soluble EBPs freely moving in the cytosol. To substantiate this assumption, we have studied a fraction of water-soluble proteins isolated from cottonplant shoots. Analysis of the protein fractions obtained by hydrophobic chromatography on Polikhrom-1 in an ethanol gadient (Fig. 1) showed that the greatest affinity for ethylene was exhibited by the fraction eluted with 70% ethanol. The usual expedients for the isolation of highly purified sites of cytokinin- and auxin-binding proteins by affinity chromatography or photoaffinity labeling proved to be ineffective both for us and for other authors [5]. We therefore made use of the capacity of ethylene for forming a fairly stable complex with its receptor.

The fraction eluted by 70% ethanol was saturated with [$^3$H]ethylene and, after incubation for 12 h, the unbound hormone was eliminated by blowing with inert gas, and the product was chromatographed on a column of TSK gel HW-50F. Proteins were detected spectrophotometrically and radiometrically (Fig. 2). As can be seen from Fig. 2, the levels of radioactivity showed that the 4th and 5th peaks possessed affinity for ethylene. It has not yet been possible to analyze the 5th fraction in view of its extremely low yield. When electrophoresis was carried out in PAAG in the presence of sodium dodecyl sulfate, fraction 4 migrated in the form of a single band with a molecular mass of 24 kDa, differing substantially from the membrane EBP isolated previously from *Phaseolus vulgaris* and showing a molecular mass of of the order of 50 kDa [2]. Experiments on binding showed that 1 g of the purified EBP bound 12.4 nmole of [$^3$H]ethlene.

The high affinity for ethylene of the protein that had been isolated makes it a probable candidate for the role of a receptor of this hormone. We therefore required additional proof of the receptor nature of the water-soluble EBP. With this aim, we investigated the influence of ethylene, of the EBP, and of their complex on the synthesis of RNA and protein in isolated cottonplant chromatin (Table 1).

As can be seen from the Table, the highest level of synthesis of RNA and protein was achieved on the addition of the hormone-receptor complex to the incubation medium. The activation of these processes was less pronounced on the addition of ethylene or the EBP and is explained once again by the formation of hormone-receptor complexes through endogenous EBP or endogenous ethylene, respectively, and depends on their concentration. An investigation of the products of translation in

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. 92-96, January-February, 1994. Original article submitted July 13, 1993.

 0009-3130/94/3001-0084$12.50 

TABLE 1. Induction of RNA Polymerase and of Protein-synthesizing Activity by Ethylene, the EBP, and Their Complex

| Variant of the experiment | Synthesis of RNA (inclusion of [$^{33}$P]ATP, %) | Synthesis of protein (inclusion of [$^{35}$S]methionine, %) |
|---|---|---|
| Chromatin | 100 | 100 |
| + ethylene | 150 | 122 |
| + EBP | 125 | 111 |
| + ethylene | 187 | 132 |

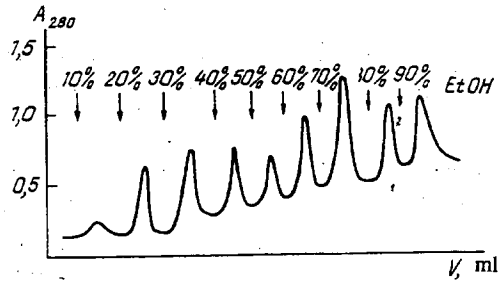


Fig. 1. Hydrophobic chromatography of the water-soluble proteins of the cotton plant.

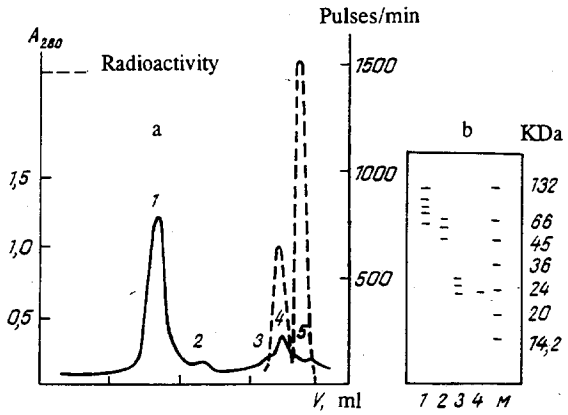


Fig. 2. Gel chromatography of the fraction of water-soluble proteins eluted by 70% ethanol (*a*), and electrophoretogram of the fractions obtained after gel filtration (*b*).

an incubation mixture with isolated chromatin showed that, as compared with a control, two new polypeptides, with molecular masses of 48 and 68 kDa, had appeared in the samples with the hormone-receptor complex. This agrees with the hypothesis that ethylene, on reaching the target cell, induces the synthesis of a specific set of proteins [6]

Thus, the results obtained on the functional activity of the water-soluble EBP of the cotton plant — high affinity for ethylene, activation of the processs of transcription and translation, reproduction of the physiological response in a model system in vitro — confirm the receptor nature of the protein isolated.

## EXPERIMENTAL

**Isolation of the Water-soluble Fraction of Proteins from Cottonplant Shoots.** Three-day cottonplant shoots (100 g) were homogenized at 12,000 rpm in 0.025 M sodium citrate buffer, pH 7.0. Then the homogenate was centrifuged at 20,000 *g* for 45 min. Dry ammonium sulfate to give 70% saturation was added to the supernatant in order to precipitate the water-

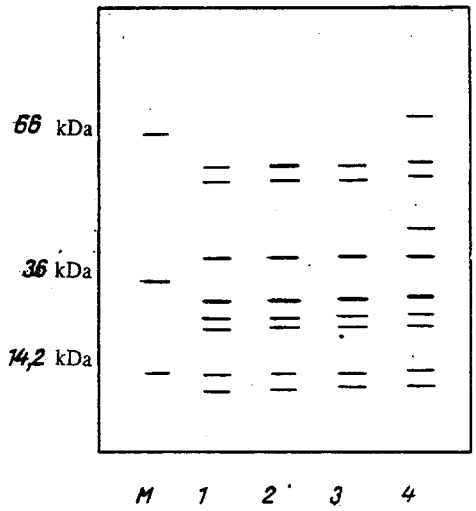


Fig. 3. Electrophoretogram of the translation products: 1) control; 2) in the presence of ethylene; 3) EBP; 4) EBP + ethylene complex; M) marker proteins.

soluble proteins. The precipitate was separated from the solution by centrifugation at 4000 rpm and was dissolved in the initial buffer.

**Hydrophobic Chromatography of the Water-soluble Protein Fraction**. The water-soluble proteins were separated on a 0.8 × 20 cm column with the sorbent Polikhrom-1 at a rate of elution of 20 ml/h, using a stepwise gradient of ethanol: 10–20–30–40–50–60–70–80–90%. Proteins were detected ar 280 nm on a Uvicord-2 instrument. [$^3H_4$]-ethylene with an activity of 32 TBq/mole was obtained as described in [7].

**Analysis of the Binding Capacity of the Protein Fractions**. Aliquots of 100 ml were taken from the protein fractions obtained and were transferred into penicillin bottles with tight closures (rubber stoppers), 0.2 ml of [$^3$H]ethylene was introduced into each and they were incubated at room temperature for 6 h with periodic shaking. The nonbound ethylene was eliminated by purging with gaseous nitrogen for 3 min. To determine nonspecific binding, 1 ml of unlabeled ethylene had previously been added to a control. Then 5 ml of ZhS-8 scintillation liquid was added to each bottle, and radioactivities were measured on a Beta-1 radiometer.

**Gel Chromatography of the Ethylene-binding Proteins** (analytical variant). A fraction eluted by 70% ethanol and containing 2 mg of protein was incubated with [$^3$H]ethylene and, after being purged with nitrogen, the product was separated on a 1.5 × 200 cm column of TSK gel HW-50F. Buffer: 0.025 M Na citrate, pH 7.0; rate of flow 30 ml/min. Detection at 280 nm on a Uvicord-2 and from radioactivity.

**The preparative isolation** of the EBPs was carried out under the same conditions, using the results of the analytical variant, and 3 mg of EBPs was obtained.

**Protein was determined** by Lowry's method [8].

**Electrophoresis** was conducted in a gradient PAAG (from 9 to 25%) with sodium dodecyl sulfate [9].

**The isolation of chromatin and the determination** of RNA polymerase activity were carried out by the method of [10].

As labeled precursors we used [$\alpha$-$^{33}$P]ATP (producd by AP Radiopreparat, Tashkent, with a molar activity of 37 pBq/mole).

**The determination of the protein-synthesizing activity** of the chromatin in the presence of ethylene and its complex with the EBPs and the isolation of the total labeled proteins were carried out as in [11]. We used as labeled marker [$^{35}$S]methionine with a molar activity of 8 pBq/mole (AP Radiopreparat, Tashkent).

## REFERENCES


1. K. Doerffling, Plant Hormones [Russian translation], Mir, Moscow (1985), p. 303.
2. C. J. R. Thomas, A. R. Smith, and M. A. Hall, Planta, **160**, 47 (1988).
3. R. M. Napier and M. A. Venis, J. Plant Growth Regul., **9**, 113 (1990).
4. D. E. Evans, J. H. Dodds, P. S. Lloyd, I. ApGwynn, and M. A. Hall, Plant, **154**, 48 (1982).
5. E. C.Sisler and C. Wood, J. Plant Growth Regul., **7**, 181 (1989).
6. F. Ishige, K. Yamazaki, H. Mori, and H. Imaseki, Plant Cell Physiol., **32**, 681 (1991).
7. Ya. S. Ziyaev, O. N. Veshkurova, A. A. Takanaev, and Sh. I. Salikhov, Dokl. Akad. Nauk RUz, No. 4-5, 53 (1992).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., **193**, No. 3, 265 (1951).
9. U. K. Laemmli, Nature (London), **227**, No. 5259, 680 (1970).
10. O. N. Kulaeva, S. Yu. Selivankina, E. G. Romanko, M. K. Nikolaeva, and A. A. Nechiporovich, Fiziol. Rast., **26**, No. 5, 1016 (1979).
11. B. Wielgat and K. Kleczkowski, Plant Physiol., **81**, 404 (1986).