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SOLID-STATE EXTRACTION SORBENT FOR PHENYLUREA AND TRIAZINE DERIVATIVES BASED ON THE COTTON CYTOKININ RECEPTOR

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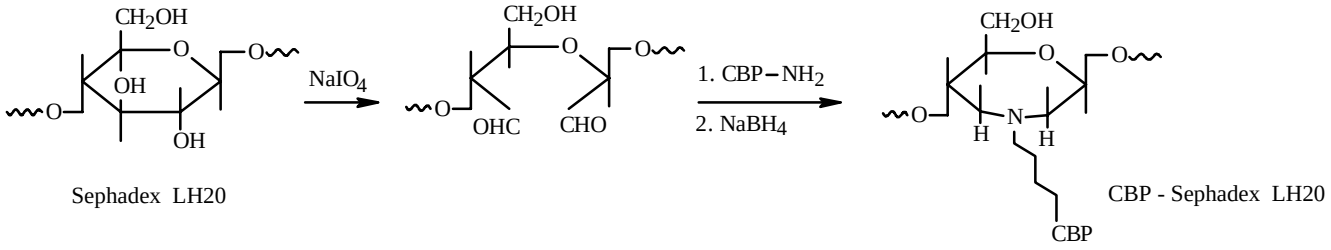
Cytokinin-binding proteins (CBP) from cotton (*Gossypium hirsutum* L.) and beans (*Vigna radiata*) can specifically bind pesticides such as phenylurea and triazine derivatives [1, 2]. We hypothesized that immobilizing CBP on a sorbent could produce a selective solid-state extractant for these classes of pesticides. We selected as the matrix Sephadex LH-20, which is stable in organic solvents [3].

Affinity chromatography on BAP-acetoamidohexylsepharose, which was synthesized by the literature method [4], was used to isolate total CBP from cotton sprouts. According to electrophoresis (Fig. 1), NaCl (1 M) elutes three CBP fractions of mass 70, 57, and 43 kDa. These exhibit different affinities for ^{3}H-BAP with binding constants K_d 3×10^{-5}, 8.5×10^{-6}, and 6×10^{-9} M, respectively. The pure CBP of mass 43 kDa exhibits the greatest affinity for ^{3}H-BAP and was isolated by HPLC over an UltroPack RP18 column using an CH_3CN gradient [5].

The solid-state sorbent was synthesized starting from Sephadex LH-20 and CBP using the scheme:



The synthesis was performed as follows. A 100-mL flask containing $NaIO_4$ solution (0.5 M) was treated with a suspension (20 mL) of Sephadex LH-20 and left for 1 h with gentle stirring. The resulting activated gel was placed in a glass filter-funnel, washed with sodium phosphate buffer (0.01 M) in NaCl (0.15 M, pH 8), and treated with a solution (50 mL) containing CBP (20 mg) in neutral sodium phosphate buffer. The reaction mixture was placed on a rocker overnight in the cold. Then, the gel was stabilized by freshly prepared $NaBH_4$ solution (1%) and decanted with phosphate buffer to remove soluble reaction products. The gel could be stored for several weeks in the cold without noticeable loss of binding activity.

The sorption capacity of the sorbent was estimated using radiometric methods with ^{3}H-dropp (N-[1,2,3-thiadiazolyl-5]-N′-phenylurea), ^{3}H-cotoran [N-(3-trifluoromethylphenyl)-N′,N′-dimethylurea], and ^{14}C-cytodef [N-(1,2,4-triazol-4-yl-3,5)-N′-phenylurea] and chromatographic methods with prometrin (2-methylthio-4,6-bis-(isopropylamino)-*symm*-triazine), diurone (N-[3,4-dichlorophenyl]-N′,N′-dimethylurea), and simazine [2-chloro-4,6-bis(ethylamino)-*symm*-triazine].

Pesticides were concentrated beforehand by placing a suspension (1-1.5 mL) of sorbent in a 2-mL column and passing sample (1 L) containing pesticide or simulant over it. The bound pesticide was eluted by a small volume (2-2.5 mL) of solvent. The concentrated solution was analyzed by HPLC.

The sorption capacity of the column for dropp was 0.45; benzylaminopyrine (BAP), 0.34; cytodef, 0.29; cotoran, 0.16; and simazine, 0.24 mg/mL moist gel.

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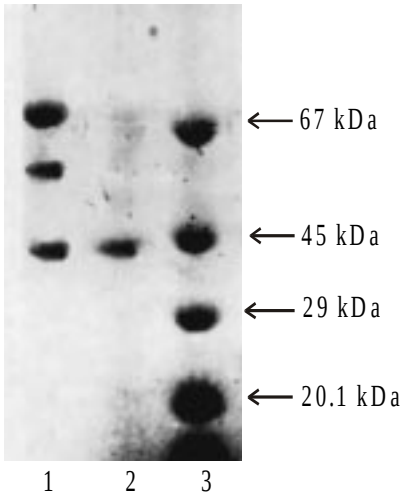

Fig. 1. Electrophoresis in SDS—PAAG: total cytokinin-binding proteins (CBP) after affinity column (1), pure CBP after HPLC (2), marker proteins: BSA (67 kDa), ovalbumin (45 kDa), carboxyanhydrase (29 kDa), trypsin inhibitor (20.1 kDa) (3).

The results indicate that solid-state sorbents based on CBP are promising for preliminary concentration of phenylurea and triazine pesticides from natural samples.

The optimal conditions for solid-state extraction of pesticides were determined by changing the conditions for absorbing and eluting the pesticides, the chemical nature of the pesticide, the volume of the sample used, the ionic strength, and the pH of the eluent.

The results showed that the optimal amount of analyte is a 500-1000-fold excess relative to the sorbent volume. The pH value of the sample has no significance. However, strongly acidic (pH < 2) or basic (pH > 10) solutions can negatively affect the operation of the carrier and immobilized protein.

The amount of pesticide retained by the solid-state sorbent was 80-90%, in certain instances 98%, which agrees well with the literature values [6, 7].

It can be seen that phenylurea and triazine derivatives are preconcentrated during the solid-state extraction. Their content in the sample increases by 500 times. This is adequate to analyze pesticides by HPLC.

The selectivity of the solid-state carrier for various classes of compounds is important in evaluating its sorption properties. As noted earlier, traditional sorbents and membranes based on C_{18}-modified silica gel typically have poor selectivity for various types of organic reagents. Solutions of mixtures of various classes of pesticides were passed through a sorbent column in order to determine the selectivity of the newly synthesized CBP—Sephadex LH-20. C_{18}-Modified silica gel was used as a control. The results indicate that our sorbent is highly selective for pesticides derived from phenylurea and triazine.

Thus, we found that the solid-state sorbent based on immobilized CBP has higher affinity for phenylureas and triazines than, e.g., chloro- and phospho-organic pesticides. This occurs obviously because the interaction involves specific receptor binding that is highly stable and selective for substances with hormonal activity.

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