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BIFUNCTIONAL INHIBITOR FROM CORN CULTIVATED IN UZBEKISTAN

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A bifunctional inhibitor of proteinase and α-amylase from local varieties of corn kernels was isolated and characterized. Its amylolytic and proteolytic inhibiting activities in high-lysine local varieties of corn were determined.

Key words: corn, bifunctional inhibitor, amylolytic and proteolytic activities, high-lysine variety.

Highly specific inhibitors are used as tools for studying the mechanism of action of enzymes and establishing the structures of active centers. They are employed widely in medicine, pharmacology, and toxicology [1, 2].

Certain bifunctional proteinase inhibitors from plants suppress flu virus reproduction [3]. A pathogen-related protein that induces tobacco mosaic virus and a bifunctional α-amylase and trypsin inhibitor from corn were also found to be homologous [4].

We continued our research on physicochemical properties and biological activity by studying proteins from corn cultivated in Uzbekistan. We isolated bifunctional inhibitor proteins from the hybrids Uzbekistan-420, Uzbekistan-306 AMV, Uzbekistan-601 ESV, and their parental varieties [5]. Inhibitors were isolated and purified as before [6]. Extracts from dormant corn kernals had significant activity not only for trypsin (from 68.2 to 96.82%) but also for α-amylase (from 23.03 to 50.76%). Storing the extract at pH 2 and then pH 10 caused much of the protein to precipitate. Such processing enabled a significant quantity of the ballast protein to be separated while retaining the inhibitor activity. The inhibitor was purified by acid—base treatment with subsequent gel chromatography over a column of Sephadex G-75 and ion-exchange chromatography over DEAE-cellulose. Gel filtration confirmed that the resulting inhibitor was homogeneous.

HPLC under denaturing conditions showed that the bifunctional inhibitor from corn contained four subunits of molecular weight 51, 37, 24, and 16.5 kDa.

The activity of the bifunctional inhibitor was estimated from the degree of suppression of trypsin and α-amylase activity (Fig. 1). The method for determining the α-amylase activity was based on a calculation of the amount of starch uncleaved by the enzyme, which was determined photometrically after treating the solution with iodine [7]. The proteinase (trypsin) activity was determined by the literature method [8] using azocasein as the substrate. The unit of enzymatic activity was the amount of enzyme that increased the optical density at 640 nm by one unit in one minute.

Table 1 gives the inhibitor activity of the bifunctional inhibitor from the studied corn varieties.

Table 1 shows that the new local high-lysine corn variety Uzbekistan-420 had low trypsin inhibitor activity. The bifunctional inhibitor activity made it possible not only to select a corn variety but also to judge the food value of the created variety. Furthermore, we made this conclusion based on the amino-acid composition found for variety Uzbekistan-420, for which the lysine content was 4.32% [5].

Immunochemical analysis [9] established the immunological relationship between the bifunctional protease and α-amylase inhibitor from corn kernels and the French commercial product bromelain (Fig. 2). The latter acts as a weight regulator. The central wells in agar plates were filled with antiserum to bromelain; the peripheral ones, antigen. Figure 2 shows the formation of precipitate bands.

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TABLE 1. Activity of Bifunctional α-Amylase and Trypsin Inhibitor from Corn Kernels

Corn variety	Inhibitor activity		Lysine content, %
	amylolytic (α-amylase)	proteolytic (trypsin)	
Mukhabbat	41.66	68.2	2.45
Erkin-2	45.77	85.7	1.18
Uzbekistan 601 ESV (hybrid)	47.36	92.06	2.12
Uzbekistan 203 M	23.03	90.4	2.16
Uzbekistan 205 AMV	23.32	73.0	1.68
Uzbekistan 306 AMV (hybrid)	20.75	96.82	2.46
Line 1812	50.76	80.9	1.29
Line 3928	24.34	82.5	1.64
Uzbekistan 420 (hybrid)	50.13	68.2	4.32

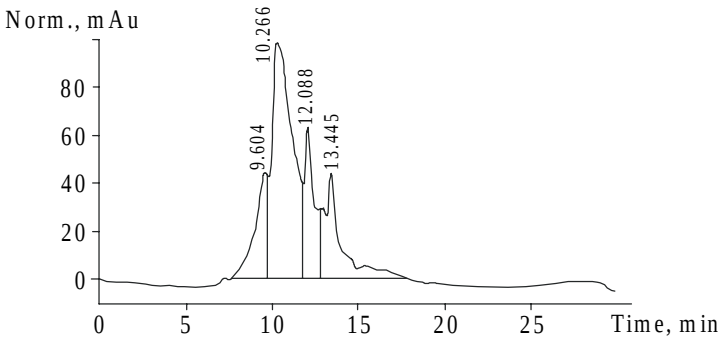

Fig. 1.

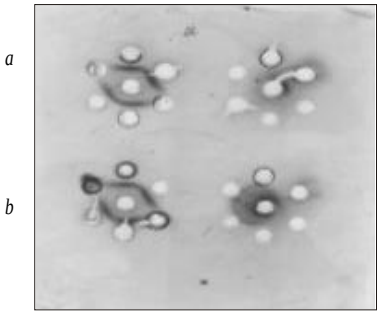

Fig. 2.

Fig. 1. SE-Chromatogram of bifunctional inhibitor from corn in 0.2% SDS solution.
Fig. 2. Immunoprecipitation in agar (1%). Central wells, serum to bromelain; peripheral, bifunctional inhibitor from corn (a) and bifunctional inhibitor from wheat (b).

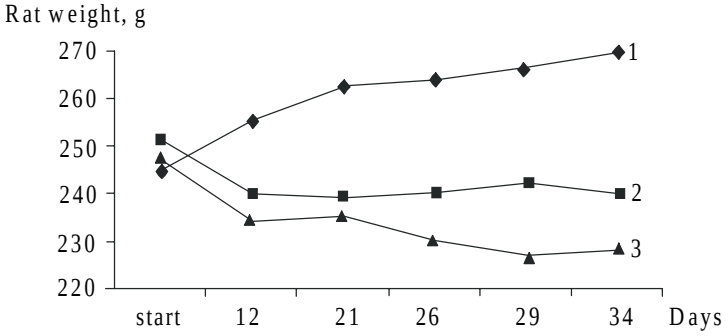

Fig. 3. Dynamics of weight change of mongrel white rats for bromelain and bifunctional inhibitor. Control (1), bromelain (2), inhibitor (3).

Oral administration to animals (rats, 200-280 g) of the bifunctional inhibitor from corn at a dose of 10 mg/kg for three months established that the average weight change (decrease) was practically identical to that for animals given commercial bromelain. The studied bifunctional inhibitor is a weight regulator. Its oral administration did not lead to the death of the animals.

Figure 3 shows that the bifunctional inhibitor from corn causes weight decreases similar to those for bromelain.

Based on the experimental results, we think that a biologically active additive from corn kernels can be used to reduce weight and suppress the appetite. However, in contrast to bromelain, the bifunctional inhibitor from corn kernels, which can suppress the activity of trypsin-like proteinases and α-amylases, is described in the literature as a blood circulation factor [10].

The activities of thromboplastin and the bifunctional inhibitor from corn kernels on blood clotting were compared. Fibrin threads are formed upon addition of thromboplastin and $CaCl_2$ to blood plasma. The thrombus formation time (TFT) averages from 16 to 20 s. With the bifunctional inhibitor, the TFT was 48.3 s; with thromboplastin, 18 s. Therefore, the bifunctional inhibitor from corn kernels affects blood clotting.

Thus, a bifunctional inhibitor of proteinases and α-amylases from local corn varieties was isolated and purified. Its inhibitor activities were determined and can serve as one of the food value indicators of this variety. Immunoenzymatic analysis found that the bifunctional inhibitor from corn is related to the commercial product bromelain.

Animal experiments showed that the bifunctional inhibitor from corn kernels reduces weight. Furthermore, the trypsin and α-amylase inhibitor from corn kernels affects blood clotting.

EXPERIMENTAL

Corn kernels were ground in an electric grinder. The resulting flour was extracted with distilled water in an ice bath (for cooling) for 1 h at a water:flour ratio of 4:1 on a magnetic stirrer. The solid was separated by centrifugation at 3000 rpm for 20 min. The supernatant liquid was acidified with HCl (1 N) until the pH was 2 and stored in ice for 20 min. The precipitated protein was separated by centrifugation under the aforementioned conditions. The supernatant liquid was adjusted to pH 10 using NaOH solution (1 N). The precipitate that formed after 20 min was separated by centrifugation at 3000 rpm for 20 min. The supernatant liquid was dialyzed and concentrated by lyophilization. The concentrated inhibitor solution was placed on a G-75 column equilibrated with tris-HCl buffer (0.05 M). The inhibitor was purified using ion-exchange chromatography over a column (2 × 20 cm) of DEAE-cellulose equilibrated with tris-HCl buffer (0.5 M, pH 8.0) at a flow rate of 30 mL/h. The optical density of the effluent was measured at 280 nm.

The bifunctional inhibitor was studied by HPLC using a Zorbax GF-250 column (4.6 × 250 mm, 4-4.5 μm, Agilent Technologies) and an Agilent Technologist 1100 chromatograph with a four-eluent pump, outgasser, and variable wavelength UV detector. The chromatograph was controlled and the results were processed using Agilent ChemStation programs for the liquid chromatograph. The UV detector was set at 210 nm for the isolated bifunctional inhibitor; flow rate, 0.25 mL/min; mobile phase Na_2HPO_4/NaH_2PO_4 (0.1 M); pH 7.0; SDS solution content (0.2%), injector volume 10 μL, column temperature, 28°C.

The molecular weight of the protein was determined by gel chromatography over a Sephadex G-75 column (1.5 × 90 cm) equilibrated with tris-HCl buffer (0.05 M, pH 8.0) containing NaCl (0.1 M) at 4 mL/h flow rate.

The activity of the bifunctional proteinase inhibitor was determined by the literature method [8]. The activity was established by treating a solution containing trypsin (0.5 mL) with inhibitor solution (30:1). The mixture was stored at room temperature for 1 h, after which the residual enzyme activity for casein was determined. The controls were samples incubated without substrate that were added after adding trichloroacetic acid. The amylolytic activity of proteinase was determined as before [7].

Serum was obtained from immunized animals (rabbits) daily for two weeks. Antigen (bromelain) was administered fractionally, i.e., i.m. and s.c., with each injection. Rabbit blood was collected on the 9-10th day after the last injection. Serum was prepared by storing blood for 2 h at room temperature to separate plasma and then placing it in a refrigerator. On the next day, it was centrifuged at 3000 rpm for 20 min, poured into sterile tubes, preserved with a small amount of boric acid, and stored in a refrigerator.

Immunoprecipitation was carried out on agar (1%) that was prepared by mixing equal volumes of hot solutions of agar (2%) and NaCl (1.7%). After the agar solidified, it was stamped to form wells of a given shape. The wells were filled with antigen and antiserum. The plates were placed in a humid chamber and stored for 5-7 d. They were checked daily for the appearance of precipitation bands.

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