ISOLATION OF A UREASE FROM WATERMELON SEEDS AND THE STUDY OF ITS PROPERTIES

S. I. Dikhtyarev, M. M. Yanina,
R. G. Kuznetsova, B. I. Kurganov,
and V. T. Chernobai

UDC 615.45:615.7.577

The enzyme urease has been isolated from the seeds of the watermelon of the "Ogonek" variety and has been characterized. The molecular weight of a rechromatographed sample of the enzyme has been determined as 480,000. The pH dependence of the activity of the urease and the kinetic parameters of the enzyme have been studied.

In recent years, an acute demand has arisen for a urease enzyme preparation for medicinal purposes. On the one hand, urease has found use in the development of new highly sensitive and highly selective methods for the analytical determination of urea in biological liquids, especially the blood [1, 2], and on the other hand, urease in immobilized form is necessary for creating a compact "artificial kidney" apparatus [3]. We have previously developed a method for obtaining urease from watermelon seeds including the stage of extracting the ground seed, precipitation, and drying with acetone. Urease preparations obtained by this scheme and having a specific activity of 35 μmole of urea in 1 min (EU) per 1 mg of protein can be used for analytical purposes to determine urea in biological liquids. However, to obtain the immobilized preparations used for decomposing urea in an "artificial kidney," urease with a higher specific ureolytic sensitivity must be available. In the present paper we describe a method of obtaining urease with a specific activity of from 140 to 4000 EU/mg of protein, including the stages of extraction, fractionation with ammonium sulfate, precipitation with ethanol, and gel filtration on Sepharose 4B.

Figure 1 shows the course of the salting out of urease from an extract as a function of the degree of saturation of the solution with ammonium sulfate. The urease begins to precipitate at 30% saturation, and up to 40% it is possible to observe some uniform increase in the activity of the urease and the concentration of the protein in the precipitate (curves 1 and 2). After 40% saturation, a marked increase in the urease activity in the precipitate is observed which reaches a limiting value at 50% saturation, with a simultaneous fall in the urease activity and in the amount of protein in the supernatant liquid (curves 3 and 4), which indicates the passage into the precipitate of the bulk of the active enzyme protein. The maximum value of the specific activity of the urease in the precipitate corresponds to an approximately 45% degree of saturation with ammonium sulfate. Under these conditions, the degree of purification is fourfold.

To free the urease from traces of ammonium sulfate, a stage of precipitation with ethanol was introduced (1 ml of the initial solution of urease contained 127 EU of activity and 0,7 mg of protein):

Ratio of solution to ethanol by volume	Amount of protein in the precipitate, %	Specific activity of the urease in the precipitate (EU/mg of protein)	Degree of purification	Ratio of the activity of the protein in the precipitate to the initial activity, %
1:1	45	151	1,07	38
1:1,5	54	272	1,94	82
1:2	55	267	1,90	80
1:2,5	61	217	1,55	77

All-Union Scientific-Research Institute of the Chemistry and Technology of Drugs, Khar'kov. "Vitaminy" Scientific-Production Amalgamation, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 624-628, September-October, 1983. Original article submitted September 23, 1982.

0009-3130/83/1905-0587$07.50

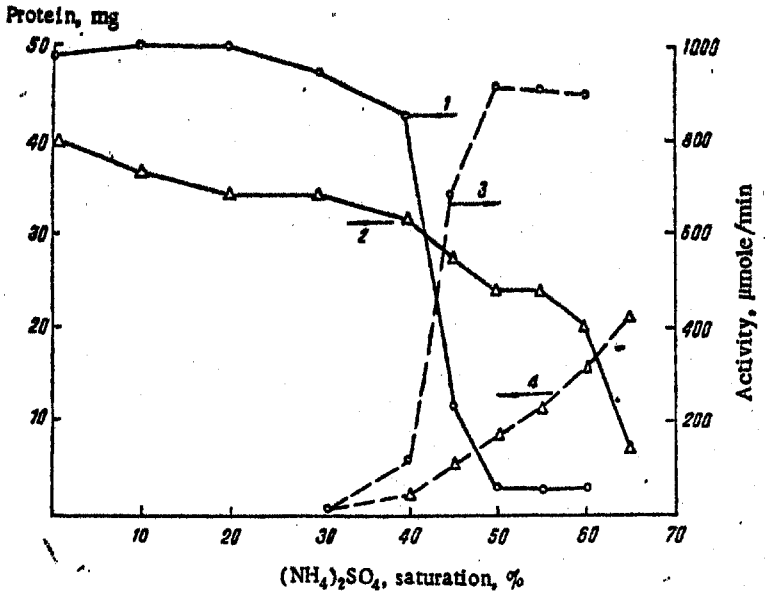

Fig. 1. Salting out of urease from an extracted by saturation with ammonium sulfate solution: 1 and 3) total activity (μmole of urea in 1 min) in the supernatant liquid and in the precipitate, respectively; 2 and 4) protein (mg) in the supernatant liquid and in the precipitate, respectively.

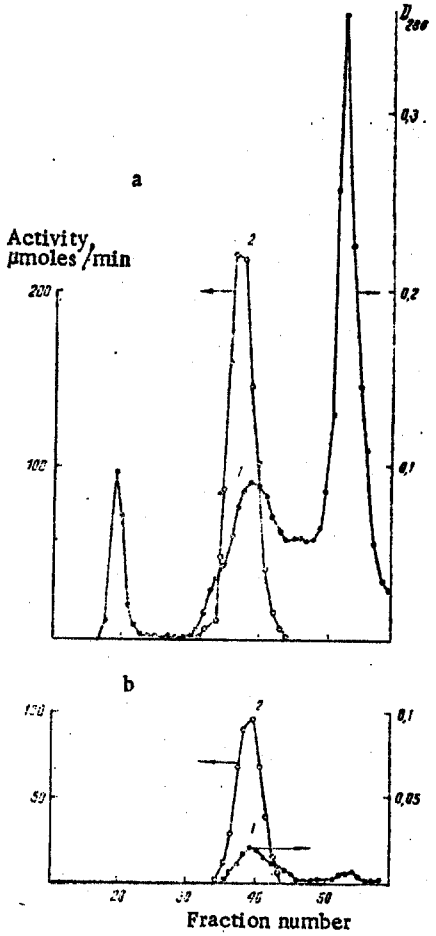

Fig. 2. Gel filtration of urease on a column of Sepharose 4B (1 × 100 cm): a) elution profile of the preparation after salting out with ammonium sulfate and precipitation with ethanol (1.5 ml of a solution with an activity of 2100 μmole of urea per 1 min was deposited on the column); b) rechromatography of fractions 32-43 on Sepharose 4B; 1) optical density at 280 nm in 0.5-cm cell; 2) activity of the fraction (μmole urea in 1 min).

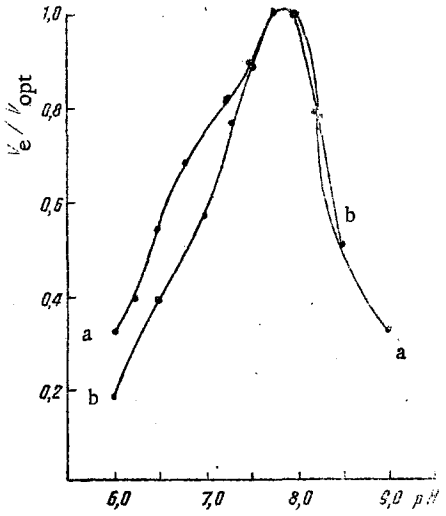

Fig. 3. Dependence of the initial rate of urease-catalyzed hydrolysis of urea on the pH of the solution: a) preparation after salting out the ammonium sulfate and precipitation with ethanol; b) preparation after rechromatography on Sepharose 4B.

As we see, the optimum ratio of the enzyme solution to ethanol is 1:1.5-1:2. In the process of purification with ethanol, the specific activity of the urease rose by an average factor of 1.9, and the activity yield at this stage amounted to 80%. Freeze-dried samples of urease obtained by this scheme of purification had specific activities of 140-160 EU/mg of protein.

Figure 2a shows the elution profile of urease on Sepharose 4B. The urease obtained after precipitation with ethanol was deposited on the column. The elution profile given of urease possessing a higher specific activity basically coincides with the elution profile of urease with a specific activity of 35 EU/mg of protein obtained previously [6], the deviation consisting in a more sharply defined protein peak corresponding to the peak of the urease activity (fractions 32-43). The specific activity at the maximum amounted to 1470 EU/D_{280}. Figure 2b shows the results of the rechromatography of fractions 32-43 on Sepharose 4B. The specific activity at the maximum amounted to 4750 EU/D_{280}, which is at the level of the specific activity of a crystalline preparation of urease obtained from pods of the jack bean [5]. The performance of the calibration of a column of Sepharose 4B with marker proteins of known molecular masses enabled us to considered that the molecular mass of the urease sample after rechromatography was 480,000 ± 3000.

The UV spectrum of the protein peak at the activity maximum (Fig. 2b, fractions 38-40) has the band chracteristic for proteins in the 270-282 nm region due to the absorption of aromatic amino acids, mainly tyrosine and tryptophan.

Figure 3 shows the dependence of the relative activity of the urease (Fig. 2b, fraction 38-40) on the pH. The maximum urease activity is observed at pH 7.8.

Figure 4 shows the dependence of the initial rate of hydrolysis of urea on the concentration of the substrate for samples of urease obtained after fractionation with ammonium sulfate and precipitation with ethanol (Fig. 4a and c) and after rechromatography (Fig. 4b and d). These relationships are described by the Michaelis–Menton equation. To find the parameters of the equation we used the linear anamorphosis

$$V = V_{max} - \frac{K_M \cdot V}{[S]_0}. \quad (1)$$

The values of K_M for the two samples of urease agreed and were $3.5 \pm 0.3 \cdot 10^{-3}$ M. The difference in the values of V_{max} for the purified preparation (3700 ± 50 μmole of urea per 1 min per 1 mg of protein) and also for the unpurified preparation (140 ± 5 μmole of urea in 1 min

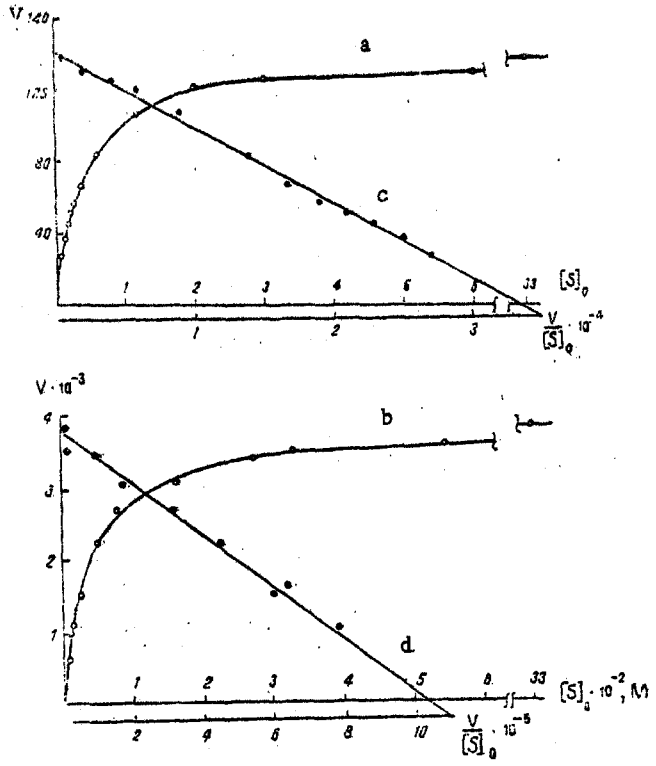

Fig. 4. Dependence of the specific activity of the urease on the molar concentration of urea in the coordinates (V; $[S]_0$) and (V; $V/[S]_0$): a (c) and b (d) are the initial and purified samples of ureases, respectively.

per 1 mg of protein), and for the preparation described previously [6] is due to the exceptionally low amount of active enzyme in the unpurified sample.

EXPERIMENTAL

The source of the isolation of the urease were the seeds of the "Ogonek" domestic variety of watermelon grown in the Experimental Farm of the Ukrainian Scientific-Research Institute of Vegetable Growing and Gourd Cultivation (Khar'kov Province). The ripe seeds were ground in mills by the crushing method. The enzyme was extracted with 0.1 M K phosphate buffer, pH 7.0, containing 10^{-3} M EDTA and 10^{-3} M 2-mercaptoethanol, at room temperature for 2 h. All the subsequent stages were carried out at 4°C. After the separation of the meal and and the freeing of the extract from suspended fine particles by centrifugation, the enzyme was precipitated with a saturated solution of ammonium sulfate. The precipitate was separated by centrifugation at an acceleration of 12,000 × g for 15 min. The precipitation of the urease by ethanol was carried out for 2 h, the resulting precipitate being separated by centrifugation at an acceleration of 2000 × g for 10 min. Gel filtration was performed on a column (1 × 100 cm) filled with Sepharose 4B and equilibrated with 0.01 M K phosphate buffer, pH 7.2, containing 10^{-3} M EDTA and 10^{-3} M 2-mercaptoethanol. The fractions were collected on a LKB Ultrorac automatic collector. The optical density at 280 nm of each fraction of the eluate was determined on a SP-800 spectrophotometer (maximum recorder sensitivity: 1 cm = 0.004 optical density units), and the specific activities of the urease were determined by the pH-stating method [5]. Rechromatography was carried out on Sepharose 4B under the same conditions. The optical absorption spectrum was recorded on a Cary spectrophotometer (Varian). The conditions for determining activity were as follows 0.2 M KCl, 0.13 M urea, 10^{-3} M EDTA, pH 7.8, 36°C. The volume of the reaction mixture was 5 ml and the volume of the buret 0.25 ml, and the titrant was 0.1 N HCl. The calibration of the column of Sepharose 4B (1 × 100 cm) in order to determine the molecular mass of the enzyme was carried out with the aid of the marker proteins catalase, ferritin, and thyroglobulin (Serva). The free volume of the column was determined with the aid of dextran blue (Pharmacia).

SUMMARY

1. A method has been developed for isolating urease from watermelon seeds which includes the stages of extraction, fractionation with ammonium sulfate, precipitation with ethanol, and subsequent lyophilization. A sample of enzyme has been obtained with a specific activity of 140 μmole of urea per 1 minute per 1 mg of protein.

2. It has been shown by gel filtration on Sepharose 4B that the sample contains a component possessing urease activity and characterized by a molecular mass of 480,000.

3. The dependence of the urease activity on the pH and the kinetic parameters of the enzyme has been studied.

LITERATURE CITED

1. I. V. Berezin and A. A. Klesov, Usp. Khim., 14, No. 2, 180 (1976).
2. G. G. Guilbault, in: Comprehensive Analytical Chemistry, Vol. VIII (ed. A. Svehla), Elsevier, Amsterdam (1977).
3. A. Gordon, A. Levin, and L. B. Narantz, Kidney Int., 10, No. 7, 277 (1976).
4. S. I. Dikhtyarev and V. T. Chernoban, Khim.-farm. Zh., No. 10, 97 (1978).
5. R. L. Blakely, E. C. Webb, and B. Zerner, Biochemistry, 8, 1984 (1969).
6. S. I. Dikhtyarev, M. M. Yanina, N. I. Mal'tsev, V. I. Kurganov, and V. T. Chernobai, Khim. Prir. Soedin., 430 (1980).

A STUDY OF THE LIGNIN OF THE SEA ISLAND COTTON PLANT OF VARIETY S-6030

M. K. Mirzakhmedova, L. S. Smirnova, and Kh. A. Abduazimov

UDC 547.992.002.61

The dioxane lignin has been isolated by Pepper's method with a yield of 9.5% on the Komarov lignin from the ripe stems of the sea island cotton plant of variety S-6030. The developed emperical formula has been calculated, the IR and UV spectra have been taken, and the molecular-mass distribution has been studied. The dioxane lignin is polydisperse. $\bar{M}_w$ = 10,100, $\bar{M}_n$ = 5350.

Continuing a study of cotton-plant lignin, we have investigated the dioxane lignin (DLA) obtained from the stems of the sea island cotton plant *Gossipium barbadense* collected after the gathering of the crop, S-6030.

The DLA was obtained by Pepper's method [1] with a yield of 9.5% on the Komarov lignin from the comminuted stems of the cotton plant that had been extracted with ethanol-benzene and washed with hot water. The DLA was purified by two reprecipitations by pouring aqueous dioxane (1:9) solutions of it into absolute ether.

On the basis of elementary analysis and functional-group analysis [2] taking carbohydrates into account [3], the developed empirical formula was calculated:

molecular mass 204.58.

$$C_9H_{8,7}O_{0,59}(OCH_3)_{1,13}(OH_{phe})_{0,29}(OH_{al})_{1,19}(O_{CO})_{0,32}(O_{ar-al})_{0,71}.$$

It can be seen from the formula that the DLA of the sea island cotton plant contains a high level of methoxy groups. On an average, to each phenylpropane structural unit (PPSU) there is one methoxy group and another two to each 5 or 6 PPSUs. The low content of phenolic

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 628-630, September-October, 1983. Original article submitted July 8, 1982.

0009-3130/83/1905-0591$07.50