Chemistry of Natural Compounds, Vol. 29, No. 6, 1993

ISOLATION AND STUDY OF PROTEINASES FROM THE MILKY JUICE OF *Carica papaya* GROWN UNDER HOTHOUSE CONDITIONS IN UZBEKISTAN

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UDC 577.156:634.651

The results are given of the isolation and study of the proteinase complex from the milky juice of papaya grown under hothouse conditions in the Republic of Uzbekistan. It has been shown that the component composition, proteolytic activity, and pH optimum of the action of the complex obtained are similar to those of the complex proteinase preparation from the milky juice of papaya cultivated in the Black Sea littoral of the Caucasus.

Numerous investigations have shown that the milky juice of the papaya *Carica papaya* contains a complex of so-called papainases consisting of a mixture of proteolytic enzymes of the thiol type [1-6].

The use of such modern methods for the separation and analysis of papaya latex as affinity chromatography, HPLC, and immunochemical testing has shown the presence of a least four cysteine proteinases — papain, chymopapain, and two papaya proteinases, A and B, designated in later publications as proteinases III and IV [7-10]. The antiinflammatory, necrolytic, fibrinolytic, and other biological properties exhibited by the individual enzymes or their complex as a whole have proved unique in connection with their wide use.

The greatest interest is presented by the production of medicinal preparations from the papaya proteinases, which is connected with their selective healing action only on the surface tissues of the spine, skin, eyes, etc [11-14]. In view of this, it appeared of interest to study the possibility of isolating and purifying the enzymes from the latex of the fruit of *C. papya* grown under hothouse conditions in the Republic of Uzbekistan with the aim of creating in the future medicinal preparations of practical importance from local raw material.

In the present paper we give results on the isolation of the enzyme complex from papaya latex and of a comparative investigation of its activity and some of its physicochemical properties. In our first experiments we extracted the fresh milky juice with phosphate buffer, with subsequent stepwise fractionation by ammonium sulfate at 45 and 65% saturation [15], which led to the isolation two main components of the latex — papain and chymopapain,with yields of 1.1 and 3.9%, respectively (calculated on the raw material). It must be mentioned that chymopapain is also the predominating component in the latices from papayas of other regions [5, 13]. Subsequently, to isolate the enzyme complex we used a single precipiation with ammonium sulfate at 80% saturation, and in this case the total yield of the complex was 6%.

For comparison with a known complex preparation of proteinases from the milky juice of *C. papaya* grown on the Black Sea littoral of the Caucasus, we performed the chromatographic separation of the complex obtained on CM-Sephadex C-50 under the conditions described by E. A. Kostanova et al. [5]. On comparing elution diagrams, we detected a close similarity of the component compositions both in the profiles of chromatic separation and in the ratios of the peaks (Fig. 1). Three protein peaks were obtained, which we designated as 1-3 and which coincided with the peaks of proteolytic activity. The latter was determined by E. D. Kverzneva's method, using casein as the substrate [16]. The proteolytic activities of fractions 1-3 were 10, 5, and 7 PU/mg, respectively. The activity of the complex itself was 5 PU/mg.

Institute of the Chemistry of Plant Substances, Uzbekistan Academy of Sciences, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 876-879, November-December, 1993. Original article submitted April 10, 1992; revision submitted August 18, 1992.

0009-3130/93/2906-0781$12.50

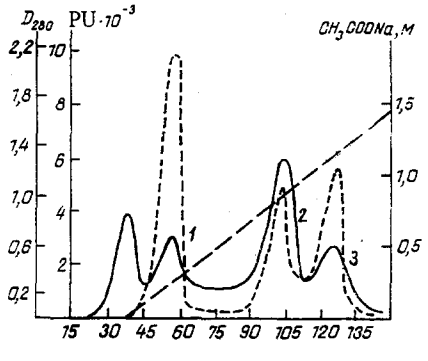

Fig. 1

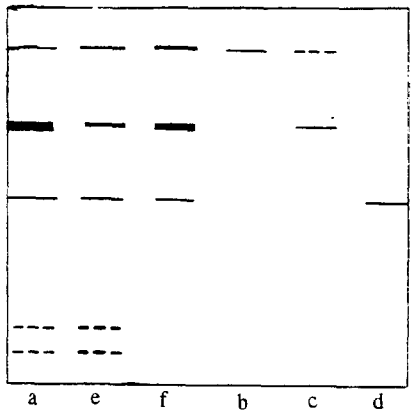

Fig. 2

Fig. 1. Chromatography of the proteinase complex from papaya latex om CM-Sephadex C-50.

Fig. 2. Electrophoretograms of the papaya latex proteinase complex (a), of its individal components (b-d), karipazim (e), and lekozym (f).

A determination of the proteolyic activity of the complex under investigation in the cleavage of casein and azocasein at various pH values [17] showed an optimum activity at pH 6.5-8, which is also close to that for a preparation of the latex from the fruit of the papaya grown in the Caucasus.

A study of the complex that we had obtained and of its individual components was made with the aid of electrophoresis in polyacrylamide gel. The electrophoretic spectrum of our preparation was represented by five protein components, two of which were minor (Fig. 2). For comparison we also investigated with the aid of electrophoresis known medicinal preparations based on papaya proteinases — karipazim (Georgia) and lekozim (LEK, Yugoslavia). It was found that with respect to the numbers of protein bands and their mobilities and intensities all the preparations investigated were similar.

On comparing the results that we had obtained with facts known from the literature, it may be assumed that fraction 1 was papain, and fraction (3) was papaya proteinase III. Fraction 2 possibly consisted of a difficultly separable mixture of chymopapain and papaya proteinase IV, which are close in charge and molecular mass. The separation of these proteinases was achieved by Buttle et al. by the use of affinity chromatography [10, 18].

Thus a study of the proteinase complex from the milky juice of *C. papaya* grown under hothouse conditions in the Republic of Uzbekistan in comparison with that from the latex of the papaya grown in the Black Sea littoral of the Caucasus and also with known medicinal preparations based on papain proteinases (karipazim and lekozim) has shown their close similarity with respect to component compositions and proteolytic activity.

EXPERIMENTAL

To isolate the proteinases we used fresh juice of the fruit of *C. papaya* grown in the hothouse section of the G. Abdullaev kolkhoz [collective farm], Tashkent province, Uzbekistan. Extraction was carried out with 0.1 M phosphate buffer, pH 7.4, in a ratio of 1:5, with centrifugation at 6000 rpm for 25 min. After 45% saturation with ammonium sulfate, the supernatant was centrifuged, and the precipitate of papain was separated off. The new supernatant was acidified with hydrochloric acid to pH 2.0, and, after the resulting precipitate had been removed by centrifugation, the supernatant was brought to 65% saturation with ammonium sulfate. After centrifugation again, the deposit was dissolved in a small volume of 0.02 M acetate buffer, pH 5.0, and was dialyzed against the same buffer, and then against water [15]. The enzyme complex was isolated at 80% saturation with ammonium sulfate. Chromatography on a column (2.5 × 60 cm) of CM-Sephadex C-50 was conducted by the method of [5], with the deposition of 10 mg of the freeze-dried complex in 0.4 M sodium acetate buffer, pH 5.0, on the column equilibrated with the same buffer. After elution of the nonbound material, a linear gradient of sodium

acetate buffer from 0.4 to 1.5 M was used. The rate of elution was 8 ml/h, with the collection of 4-ml fractions in each of which proteolytic activity was determined and the protein content was found spectrophotometrically at 280 nm.

Proteolytic activity was determined with respect to casein and azocasein [16, 19]. Electrophoresis was conducted on plates of polyacrylamide gel by Osterman's method [20]. The processes of isolation and chromatography were conducted at 6-10°C.

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