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PROTEINASES FROM *Carica papaya* LATEX

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Data on the proteinase complex from Carica papaya *latex were reviewed. The properties and applications of the domestic preparation cucumazyme and known medicinal preparations based on papaya proteinases (caripazyme, lecozyme, lecopain) were compared.*

Key words: proteolytic enzymes, latex, preparation, properties, application.

Proteinases from papaya (*Carica papaya*) latex are well known. The discovered proteolytic enzymes are widely used in scientific research, medicine, and the food and light industries [1, 2]. Several experiments showed that papaya latex contains a whole array (complex) of cysteinic proteinases [3-9].

Papaya proteinases from purchased (commercial) dried latex were studied. It has been shown that dry latex contains a complex of thiol proteolytic enzymes, papain; an enzyme mixture known as chymopapains A and B; and two strongly basic proteins, peptidases A and B.

The presence of proteolytic enzymes in both fresh latex from fruit and from plant vegetative organs such as stems, petioles, and leaves has been reported. However, data on the composition of enzymes from latex of various origins are somewhat contradictory [10-12].

In addition to proteinases, papaya latex preparations contain the mucolytic enzyme lysozyme [13, 14]. Papaya lysozyme was the first plant mucolytic enzyme to be purified and characterized [15, 16].

Recently attempts to cultivate papaya along the Black-Sea coast of the Caucases and in greenhouses in the Republic of Uzbekistan were successful [7, 8].

Proteinases occurring in papaya latex have many applications. The development of medicinal preparations based on papaya proteinases is the most interesting. This is due not only to their selective therapeutic activity on damaged tissues but also to the simplicity of enzyme therapy, which has no serious complications and is effective. It seemed interesting to study the possible isolation and purification of enzymes from latex of *Carica papaya* fruit in order to create practically important medicinal preparations.

ISOLATION AND PHYSICOCHEMICAL PROPERTIES OF PAPAYA-LATEX ENZYME COMPLEX

Enzyme preparations isolated from microbial and animal material have been widely acclaimed. However, preparations of proteolytic enzymes isolated from plants are just as promising.

Carica papaya (Cariaceae, papaya) is a perennial that is cultivated in many countries. The aerial parts of papaya contain a complex of proteinases. Shallow incision of latex vessels located under the coverings of green papaya fruit, cuttings, and stalks produces latex. Bound and free phenolic compounds, tannins, organic acids, glucose, flavonoids, lipids, coumarins, steroidal and triterpene saponins, and alkaloids were detected in the leaves [13].

Papaya leaves, stalk bark, and stalk cuttings have been investigated [17]. The content of total alkaloids was determined: 0.025% in leaves; 0.036% in stalk cuttings. According to TLC, both totals are qualitatively the same. The isolated alkaloid was pseudocarpain, which was isolated from an introduced species of papaya for the first time.

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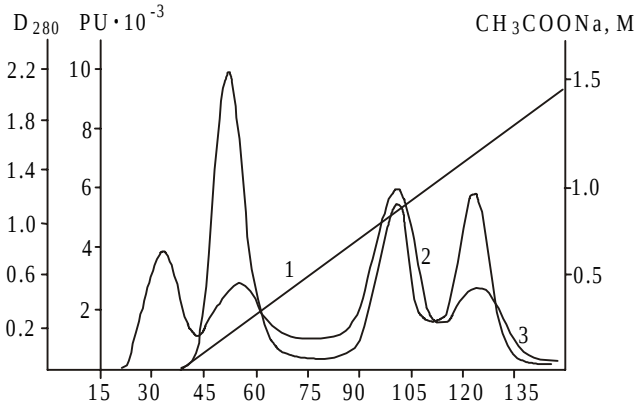

Fig. 1. Chromatography of papaya latex proteinase complex over KM-Sephadex C-50.

The stability to high temperature and the action of urea and other denaturing agents and the high degree of hydrolysis of the proteins lead to the conclusion that the papaya proteinase preparations are widely used in practice. Furthermore, the domestic preparation cucumazyme provides an effective treatment for patients, limiting the importation of the expensive rare enzyme.

Comparisons have shown that the complex proteinase preparation from latex of papaya grown near the Black-Sea coast of the Caucases is similar to latex preparations from papaya fruit grown in other global regions. The specific activity even exceeds that of foreign latex preparations [8]. Furthermore, proteinases of fresh latex isolated from papaya fruit and leaves were also compared [9]. The results are consistent with those previously reported in the literature where it was demonstrated [12] that the main latex components from the vegetative organs are chymopapains. However, papain and peptidases A and B occur significantly less than in fresh fruit latex. The lack of papain in latex of vegetative organs was noted [10]. Furthermore, it was established that only chymopapain B is present in fresh latex from fruit. The papain content in papaya latex from fruit totals 5%, i.e., it is not the main component [18]. Thus, the principal latex proteinases are a heterogeneous mixture of chymopapains A and B and peptidase that occur in latex from both unripe fruit and leaf latex.

Four cysteinic proteinases, papain, chymopapain, and papain proteinases A and B, designated as proteinase III and IV, were identified using modern isolation and analysis methods such as affinity chromatography, HPLC, and immunochemical testing of latex from papaya grown in greenhouses in Uzbekistan [19-22]. The discovery of anti-inflammatory, necrolytic, and fibrinolytic properties for pure enzymes or their complex was critical for their wide practical application. The proteolytic enzymes are valuable because they cleave nonviable denatured proteins while practically not affecting living tissues [13, 23-25].

We obtained results on the isolation of enzyme complexes from papaya latex and compared their compositions, activities, and certain physicochemical properties. Fresh latex of green fruit was extracted with phosphate buffer followed by stepwise fractionation by ammonium sulfate (45-65% saturation) [26]. Two principal latex components were isolated, papain and chymopapain in yields of 1.1 and 3.9%, respectively (per fresh raw material). It should be noted that chymopapain is also the predominant component in papaya latex from other regions [8, 24]. Next, enzyme complexes were isolated by single precipitation using ammonium sulfate (80% saturation). The total yield of complex was 6%.

The resulting complex was compared with the known complex proteinase preparation from latex of *Carica papaya* grown on the Black-Sea coast of the Caucases by chromatographic separation over KM-Saphadex C-50 using the literature conditions [18]. Comparison of the elution profiles showed that the components were similar in both the profile of chromatographic separation and peak ratio (Fig. 1). Three protein peaks, designated by us as 1-3, were observed. These coincided with peaks of proteolytic activity. The proteolytic activity was determined by the literature method using casein as substrate [27]. The proteolytic activity of fractions 1-3 were 10.5 and 7 PU/mg, respectively; the activity of the complex preparation itself, 5 PU/mg.

TABLE 1. Inhibitor Effects on *Carica papaya* Proteinases

Inhibitor	Concentration, $M \cdot 10^{-3}$	Proteolytic activity, %
p-Chloromercuribenzoate	2.5	38
	5.0	0
$HgCl_2$	1.25	27
	2.5	7.7
α-Iodoacetamide	0.1	15
	0.5	0
Ellman reagent	0.2	7.7

Chymopapain is the main component in latex of papaya grown near the Black-Sea coast of the Caucases. Enzymological studies revealed the presence of the mucolytic enzyme lysozyme in the latex. We determined the lysozyme content by gel diffusion [28] based on the ability of lysozyme to cleave the β-1→4 glycoside bond of the mucopolysaccharide layer of the *Micrococus lysodeikticus* cell wall. The method is highly sensitive and simple to use. In our experiments, the content of lysozyme in the latex and lecozyme was less than the amount previously reported [13, 14]. This may be due to the formation of lysozyme complexes with proteins of the analyzed mixture [29]. We used a lysozyme standard with 16% protein [13]. The method enables a comparative analysis of several samples on one plate. The percent lysozyme content in the following preparations was: lecozyme (LEK company, Yugoslavia, 33% total N), 5.3; papaya latex (lyophilized), 4.5; cucumazyme (substance), 9.2; cucumazyme (medicinal form, 25% total protein), 2.4.

The substrate specificity was studied using various inhibitors of the proteinase complex preparations. The enzymatic activity was completely suppressed at a *p*-chloromercuribenzoate concentration of $5 \cdot 10^{-3}$ N. α-Iodoacetamide and Ellman reagent were effective at even lower concentrations. The results for the inhibitor effects on papaya latex proteinases are given in Table 1.

The complete suppression of enzymatic activity by reagents reacting with sulfhydryl groups indicated that the proteolytic activity is due to cysteinic proteinases.

The effect of pH on the activity of proteinases from latex of papaya fruit has shown that the total enzyme preparation is most active toward bovine serum albumin at pH 8-9 and toward casein at pH 8.0 [8]. We determined the proteolytic activity of this complex at various pH values using cleavage of casein and azocasein [30] and demonstrated that the activity is optimal at pH 6.5-8. This also is close to the preparation of latex from papaya fruit grown in the Caucases.

Studies of the proteinase stability at pH 2.5-11 found that the enzymes are stable in the pH range 6-9. The stability remains significant at alkaline pH values. Thus, the activity remains at 70% for pH 11.0. However, a significant loss occurs at pH < 4.0. Nevertheless, the activity is still 40% at pH 2.4 [8]. The complex preparation in this respect differs favorably from pure papain, which is completely inactivated [31].

It is thought that the biocatalyst under natural conditions is located among a large array of other macromolecules. Therefore, the choice of enzyme mixtures is especially interesting. The complex of papaya proteinases, which is certainly promising for medical use, is such a preparation [32]. With respect to the effect of denaturing agents, it is known that papain is a proteolytic enzyme that is highly resistant to them. For example, concentrated urea solutions induce no substantial conformational changes in it [33]. The complex preparation of cysteinic proteinases is also very resistant to the action of urea.

A study of the temperature effect on the stability of proteinases found that an activated preparation is more stable to increased temperature than a preparation that was not activated. Thus, an enzyme preparation activated by incubation at 70°C and pH 7.2 for 30 min retained ~60% of its activity whereas the starting preparation under the same conditions retained 20% [8].

Highly stable covalently modified papain was also prepared by modification with polysaccharides of molecular weight 400 kDa. It retained 80% of the starting activity without changing the optimal pH value and kinetic constants [34].

We continued the study of the enzyme complex and its separate components using electrophoresis in polyacrylamide gel. The electrophoretic spectrum of the preparation contains five protein components, two of which are minor (Fig. 2). Known medicinal preparations based on the papain proteinases caripazyme (Georgia) and lecozyme (LEK, Yugoslavia) were studied by electrophoresis for comparison. As it turned out, the number of protein bands and their mobilities and intensities were similar to the studied preparations.

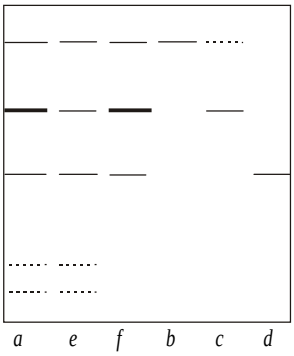

Fig. 2. Electrophoregrams of papaya latex proteinase complex (a), pure components (b-d), caripazyme (e), and lecozyme (f).

A comparison of our data and the literature results indicates that fraction 1 is papain; fraction 3, papain proteinase III. Fraction 2 may be a difficultly separable mixture of chymopapain and papain proteinase IV, which have similar charges and molecular weights. These protinases could be separated using affinity chromatography [35].

The proteinase complexes of latex from papaya grown in the Republic of Uzbekistan, grown near the Black-Sea coast of the Caucases, and known medicinal preparations based on papain proteinases (caripazyme and lecozyme) are very similar with respect to the composition and proteolytic activity.

CURRENT AND POTENTIAL USES OF *Carica papaya* ENZYMES

The enzymes are known to have unique properties as biocatalysts, i.e., a strict specificity and high efficiency. The number of catalytic turnovers effected by an enzyme molecule averages 10^8 per second [36]. The enzymes retain activity over a wide range of pH and temperature values. This makes them attractive for scientific research and practical application.

Various enzymes are used in therapeutic practice. Enzymes of animal origin (trypsin, chymotrypsin, pepsin, lidase, etc.) are most common. A proteolytic enzyme of plant origin, cucumazyme, in contrast with other analogous preparations of animal origin, has distinctive necrolytic, fibrinolytic, and anti-inflammatory activities. Therefore, it has been used in orthopedic, traumatological, surgical, gynecological, and ophthalmologic practice externally and internally, by electrophoresis and phonophoresis methods, as a powder, and in medicinal ointments and pastes.

Medicinal preparations based on papaya proteinases elicit selective therapeutic effects only on damaged tissues of the ocular sclera and spinal cord. Thus, they can be considered unique "chemical" scalpels. Proteolytic enzymes of papaya latex are used extensively in purulent surgery, where the ability of proteases to disintegrate necrotic tissues and purify wounds from scabs without destroying natural biochemical and reparative processes in the wound or damaging healthy tissues are taken into account [37].

Infection is considered to play an important role in the development of purulent-necrotic complications of extremities in sugar diabetes patients. Therefore, the effect of the enzyme preparation of plant origin cucumazyme on the microflora of purulent-necrotic processes was studied and its distinct bactericidal activity was established [38]. Furthermore, the effect of the preparation on the rheovasograph index of vessels in lower extremities of diabetic patients was determined. It was found that it had a distinct fibrinolytic, thrombolytic, and anti-inflammatory action and normalized the condition of the coagulation system and the blood rheology [39].

The application of proteolytic enzymes for therapy of purulent-necrotic diseases of soft tissues occupies a special place in enzymotherapy that helps to disintegrate the purulent masses and purify the wound surface [40].

It has been found that cucumazyme accelerates wound purification from necrotic masses, activates regeneration processes, and shortens the duration of complete healing [41]. It can soften and irrigate connective tissue and blood clots, which catches the interest of practicing doctors, including ophthalmologists studying the effect of the preparation for irrigation of corneal cataracts. The results showed that cucumazyme is an effective enzyme preparation for treating corneal cataracts,

especially in the early stages of the disease, for treating intraocular hemorrhaging, and for avoiding formation of scar tissue in the eye [42-45]. It was noted that *Carica papaya* proteolytic enzymes have a strong effect on eye tissue even at low doses.

The application of proteolytic enzymes for treatment of poisoning by snake venom is a new aspect of their clinical use. The search for effective methods of treating spinal diseases led to the intradisc administration of proteolytic enzymes. Treatment by intradisc administration of papain and lecoozyme to lumbar osteochondrosis patients gave ~80% positive results [13]. Adiminstration of papain (lecopain) for treatment of serious and manifest forms of scoliosis is a new development in vertebrate surgery. Various proteolytic enzymes and, in turn, lidase, have been used for a long time in the treatment of scar-adhesive processes in the brain cortex or spinal cord in neurosurgery in addition to surgical methods. However, the therapeutic effect of them is not always high. Treatment with preparation from the LEK company, lecozyme, was started at the N. N. Burdenko Neurosurgery Institute in 1974. Tests of lecozyme for surgical cosmetology of various facial, neck, and other body scars established the utility of its use for counteracting the formation of rough scars [13].

Thus, recent data indicate that complexes of proteolytic enzymes of *Carica papaya* fruit enzymes are promising and useful. The combination of high proteolytic activity and broad specificity indicates that papaya latex is a valuable source for preparing highly active cysteinic proteinases. The complex of proteolytic enzymes isolated by us from *Carica papaya* fruit latex provided starting material for creating the domestic cucumazyme preparation.

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