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## Evidence of $\beta$ -sheet structure induced kinetic stability of papain upon thermal and sodium dodecyl sulfate denaturation

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**Abstract:** Papain is a protease that consists of  $\alpha$ -helical and  $\beta$ -sheet domains that unfold almost independently. Both, considerable thermal stability and sodium dodecyl sulfate (SDS) resistance of papain have been shown. However, the ability of each domain to unfold upon thermal and SDS denaturation has never been studied. This work shows that fruit papain has slightly higher resistance to thermal inactivation when compared to that of stem papain with a rather high activation energy ( $E_a$ ) of  $223 \pm 16$  kJ mol<sup>-1</sup> and a  $T_m$ 50 value of  $79 \pm 2$  °C. The SDS resistance of fruit papain was estimated by SDS–PAGE analysis and activity staining. It was noted that, in the presence of SDS the protein remained active, unless heat energy was applied in order to unfold papain. Furthermore, it was proven *via* Fourier transform infrared spectroscopy (FT-IR) that an  $\alpha$ -helical domain of fruit papain is more prone to unfolding at elevated temperatures and in the presence of SDS than a  $\beta$ -sheet rich domain. Thermal denaturation of papain without detergent present led to accelerated formation of aggregation specific intermolecular  $\beta$ -sheets as compared to native protein. The presented results are of both fundamental and practical importance.

**Keywords:** thermal inactivation; SDS resistance; secondary structure; FT-IR spectroscopy.

### INTRODUCTION

Papain is a cysteine protease (EC 3.4.22.2) with broad substrate specificity and numerous applications. The preprotein consists of 345 amino acids and it is secreted as zymogen.<sup>1</sup> After cleavage of the activation peptide, the mature enzyme contains 212 amino acid residues that are organized in two domains. The *N*-terminal domain has mainly  $\alpha$ -helical structure, while the *C*-terminal domain

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has an antiparallel  $\beta$ -sheet fold.<sup>2</sup> Commercial papain can be isolated from latex (stem) or papaya fruit. Both commercial enzymatic preparations have broad substrate specificity.<sup>3</sup>

Papain is extensively used as meat tenderizer,<sup>4</sup> in dental caries removal procedures,<sup>5</sup> for preparation of clinically relevant antibody fragments,<sup>6</sup> as a cell dissociation/debris removal agent<sup>7</sup> and as a component in cosmetic preparations.<sup>8</sup> Papain has been used in the detergent industry since the enzyme shows high activity and broad specificity for hydrolysis of peptide bonds.<sup>9</sup> Furthermore, the enzymes from the papain family are known to be sodium dodecyl sulfate (SDS) resistant<sup>10</sup> and very stable enzymes at elevated temperatures.<sup>11</sup>

Elucidation of the basis for the extreme temperature and SDS stability of proteins is both of fundamental and practical importance. Recent studies suggested that a rigid  $\beta$ -sheet fold, which leads to low structural flexibility of a protein, could be responsible for the SDS and protease resistance and might also be responsible for thermal stability of a protein.<sup>10</sup> Since papain has both  $\alpha$ -helical and  $\beta$ -sheet domains, the aim of this study was to check if any of the domains was more prone to SDS denaturation by monitoring the changes in the secondary structures of the protein at elevated temperatures and in the presence of SDS. The kinetic parameters of the thermal inactivation of fruit papain were also estimated.

## EXPERIMENTAL

### *Papain purification*

Fruit papain was purified from a commercial papain preparation (BDH, London, England). Papain was extracted from the dry powder with 100 mM Tris buffer pH 8 containing 100 mM NaCl and 1 mM ethylenediaminetetraacetic acid disodium salt (EDTA) for 30 min at 10 °C. The extract was centrifuged for 15 min at 4000×g at 10 °C and the obtained supernatant was further used. Papain was precipitated from supernatant by addition of 2 volumes of ice-cold acetone followed by incubation at –20 °C for 30 min. The precipitate was separated by centrifugation at 10,000×g for 5 min. at 4 °C, dried and resuspended in 100 mM Tris buffer pH 8 containing 100 mM NaCl and 1 mM EDTA for further purification. Covalent chromatography was performed on Thiol-sepharose 4B (GE Healthcare, Uppsala, Sweden). The matrix was equilibrated in 100 mM Tris buffer pH 8.0 containing 100 mM NaCl and 1 mM EDTA (20 column volumes (CV)) first, and then the sample was applied. Unbound proteins were eluted with 20 CV of 100 mM Tris buffer pH 8 containing 100 mM NaCl and 1 mM EDTA, while bound protein was eluted by addition of 10 mM L-cysteine to the starting buffer. The homogeneity of purified protein was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). The concentration of the purified protein was determined using the Bradford method. Bovine serum albumin (BSA) was used as the standard.<sup>12</sup>

### *Papain activity measurement*

The proteolytic activity of the purified papain was tested using BAPNA (*N*<sup>α</sup>-benzoyl-DL-arginine 4-nitroanilide hydrochloride, Sigma–Aldrich, Steinheim, Germany) as a substrate in a 96-well microplate (Sarstedt, Numbrecht, Germany) as described in Raskovic *et al.*<sup>13</sup> Briefly, substrate solution, 100  $\mu$ L of 1mM BAPNA in citrate buffer (50 mM pH 6 with 2 mM EDTA and 10 mM L-cysteine) was mixed with an aqueous papain solution (25  $\mu$ L) and

incubated at 25 °C. After 60 min, the reaction was stopped by the addition of glacial acetic acid and absorbance at 405 nm was measured.

#### *Thermal inactivation curve*

Papain solution (0.5 mg mL<sup>-1</sup> in 100 mM Tris buffer pH 8 containing 100 mM NaCl, 1 mM EDTA and 10 mM L-cysteine) was incubated at different temperatures ranging from 15 to 100 °C for 30 min. Samples were allowed to cool to room temperature, and percentage of residual activity was determined in each sample using BAPNA. Measurements were performed in triplicate. The percentage residual activity was calculated relative to the sample that was incubated at 40 °C (maximal activity).

#### *Determination of inactivation rate*

Papain solutions were incubated at 60 or 80 °C and aliquots removed at specific intervals (1 min up to 2 h). After cooling to room temperature, the residual activity was measured using the BAPNA assay. The measurements were performed in triplicate.

#### *Determination of the inactivation constants*

Inactivation rate constants for papain were determined at four different temperatures selected from the declining part of the inactivation curve (75, 80, 85 and 90 °C). Papain solutions were incubated at an appropriate temperature, and aliquots were taken at specific intervals, ranging between 1 and 30 min. After cooling to room temperature, the samples were assayed with BAPNA. The residual activity was determined in comparison to the activity of the sample before incubation at the respective temperature. A plot of logarithmic residual activity vs. incubation time (expressed in minutes) was produced in order to calculate the rate constants for the inactivation of papain. Rate constant values were calculated from the slopes of the following linear regression:

$$\log (100A/A_{\max}) = -(k/2.303)t \quad (1)$$

where  $A$  represents the measured absorbance at 405 nm after certain period of incubation;  $A_{\max}$  represents the maximal absorbance before incubation;  $k$  represents inactivation rate constant; and  $t$  represents the incubation time.<sup>14</sup>

*Arrhenius plot.* Activation energy of papain inactivation was determined by plotting the inactivation constants on the Arrhenius plot. Activation energy was calculated from the slope of Arrhenius plot ( $\ln (k / \text{min}^{-1})$  vs.  $T^{-1} / \text{K}^{-1}$ ) in accordance with the equation:

$$\ln k = -E_a/RT + C \quad (2)$$

where  $R$  represents the universal gas constant (8.314 J mol<sup>-1</sup> K<sup>-1</sup>), and  $T$  represents absolute temperature in K. Slopes and their standard errors were obtained from the linear regression curves.<sup>15</sup>

#### *SDS-PAGE and activity staining*

Papain samples for SDS-PAGE and activity staining analysis were prepared by mixing 0.5 mg mL<sup>-1</sup> protein solution in 100 mM Tris buffer pH 8 containing 100 mM NaCl, 1 mM EDTA and 10 mM L-cysteine with adequate volume of reducing sample buffer (60 mM Tris buffer pH 6.8 containing 25 % glycerol, 1 % SDS, 14.4 mM 2-mercaptoethanol, and 0.1 % bromophenol blue) followed by 30 min incubation at various temperatures (60, 65, 70, 75, 80, 85 and 90 °C). The amount of protein loaded on the gel was 15  $\mu\text{g}$  and 0.1  $\mu\text{g}$  in the case of SDS-PAGE and activity staining, respectively. In the case of SDS-PAGE analysis, the samples were resolved in a discontinuous buffer system with a 4 % stacking gel and 10 % resolving gel in a Hoefer Dual Gel Mighty Small SE 245 vertical electrophoresis system

(Hoefer, Holliston, MA, USA) according to Laemmli.<sup>16</sup> In order to detect in-gel proteolytic activity of papain after SDS-PAGE, zymograms were run according to Felicioli *et al.* with some modifications.<sup>17</sup> Briefly, protein samples were applied onto a 10 % resolving gel copolymerized with 0.1 % gelatin. After electrophoresis, the gel was incubated in a 100 mM Tris, pH 8 with 100 mM NaCl and 1 mM EDTA for 16 h, followed by staining with Coomassie Brilliant Blue R-250 (Serva, Heidelberg, Germany).

#### *Fourier transform infrared spectroscopy (FT-IR)*

Infrared spectra were recorded for various papain samples (incubated for a period of 30 minutes at 25, 60 or 90 °C, with or without the addition of 1 % SDS, the same SDS concentration as in the reducing sample buffer for SDS-PAGE), utilizing Fourier transform infrared spectroscopy with attenuated total reflectance (ATR) at 1 cm<sup>-1</sup> resolution (Nicolet 6700 FT-IR, software OMNIC, version 7.0, Thermo Scientific, USA). Papain samples (15 µg) were applied onto a Smart accessory with a diamond crystal (Smart Orbit, Thermo Scientific, USA). The solvent was evaporated under a nitrogen stream in order to obtain a thin ATR film. For each spectrum, 64 scans were collected. The spectrum of the buffer was subtracted from the spectrum of the protein since even water vapor can interfere with the protein absorbance. Criterion for the correctness of subtraction was a flat baseline between 1800 and 2000 cm<sup>-1</sup>.<sup>18</sup>

*Spectral analysis.* Resolution enhanced spectra were generated by Fourier self-deconvolution function using 13 cm<sup>-1</sup> for the full bandwidth at half height (FWHH) and 2.4 for the resolution enhancement factor as described in the study of Byler and Susi.<sup>19</sup> Second-derivative spectra were generated by a seven-point Savitsky-Golay derivative. The positions of the secondary structure peaks were identified from the second-derivative spectra and assigned to specific secondary structures, as described previously.<sup>19,20</sup> Aggregation specific bands were identified from low frequency bands.<sup>21-23</sup>

To compare the secondary structure transition in the papain samples incubated for 30 min at 25, 60 or 90 °C, with or without the addition of 1 % SDS, the ratio between the band intensities from the corresponding frequencies of a specific secondary structure and the amide II band maximum, identified at 1520 cm<sup>-1</sup>, was calculated as described before.<sup>24,25</sup>

## RESULTS AND DISCUSSION

### *Cooperative thermal inactivation of papain*

Fruit papain was purified 8-fold from a commercial preparation in order to remove any inactive protein and colored low molecular weight compounds, which could interfere with the activity estimation assay (Fig. 1). In order to examine the dependence of papain activity and temperature, the residual activity of papain was observed at different temperatures using BAPNA as a substrate. Temperature range was 15–100 °C. After 30 min of incubation at a given temperature, the absorbance of the product was read and an inactivation curve was constructed (Fig. 2). The percentage residual activity was expressed in relation to the activity at 40 °C (maximal activity). The  $T_{m,50}$  value was found to be 79±2 °C, which is comparable to the  $T_{m,50}$  value of around 80 °C determined by differential scanning calorimetry.<sup>11,26</sup> In the incubation temperature region from 60 to 90 °C, the residual activity was reduced from 95 to only 1 %. In this part of the inactivation curve, cooperative loss of activity could be clearly observed due to

denaturation of the protein. Since papain domains unfold almost independently,<sup>26</sup> it seems that loss of activity could be attributed to the transition of one domain. Active site residues (Cys-25, His-159 and Asn-178) are located in the cleft formed between these two domains, thus the destabilization of one domain could lead to inactivation.<sup>11</sup>

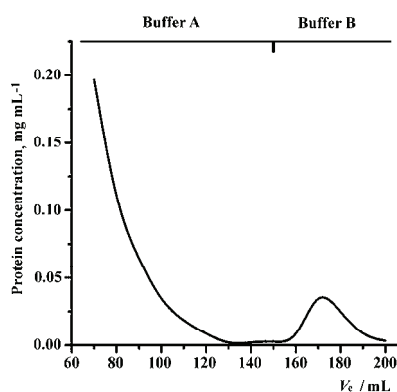


Fig. 1. Chromatographic separation of papain on a thiol-sepharose column. The column was equilibrated with 100 mM Tris buffer pH 8.0 containing 100 mM NaCl and 1 mM EDTA (Buffer A) followed by elution of unbound proteins with the same buffer and elution of papain with the same buffer containing 10 mM cysteine (Buffer B). The chromatography was run at a flow rate of 0.8 mL min<sup>-1</sup>.

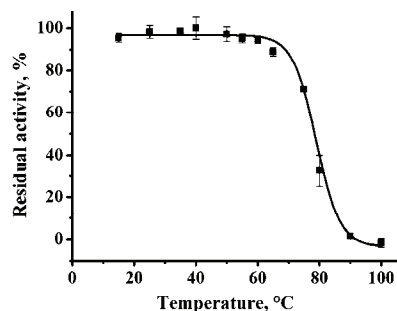


Fig. 2. Thermal inactivation curve of papain determined using BAPNA as a substrate. For creating the curve,  $A_{405}$  was monitored as a function of temperature over the appropriate temperature range (15–100 °C). The residual activity was calculated as the percentage of the maximal activity measured at 40 °C.

#### *Papain inactivation rate at 60 and 80 °C*

The term kinetic stability is used to describe proteins that are trapped in a specific conformation because of an unusually high unfolding barrier that results in very slow unfolding rates.<sup>11</sup> Since inactivation of papain coincided with transition of its tertiary structure, the overall kinetic stability of papain was estimated from its inactivation rates at different temperatures. Papain was incubated at 60 and 80 °C and at specific intervals starting from 1 min up to 2 h, aliquots were removed and the proteolytic activity against BAPNA was assayed. At 80 °C, the activity decline followed first order exponential kinetics (Fig. 3B). When inactivation curve of papain at 60 °C was determined, no decline of activity was registered, indicating stability of the enzyme at 60 °C (Fig. 3A) in the observed time range.

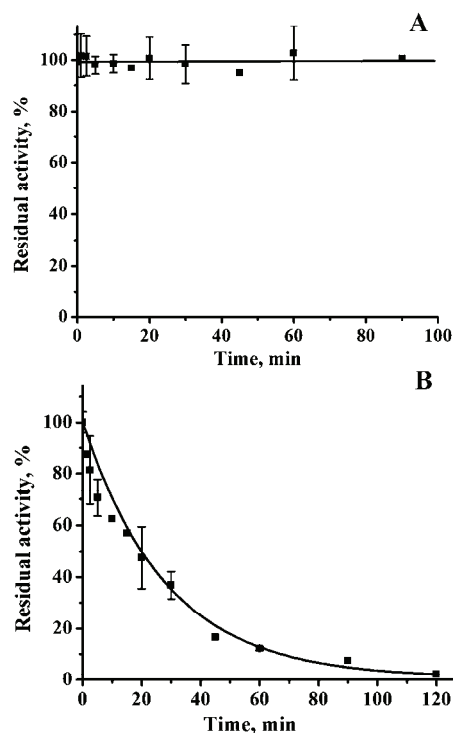


Fig. 3. Kinetics of papain inactivation at: A) 60 and B) 80 °C.

#### *Thermal inactivation of papain*

Thermal inactivation of papain was kinetically investigated in the temperature range 75–90 °C. The results are shown in Fig. 4.

First-order exponential curves for dependence of residual activity of papain on incubation time at temperatures in the range 75–90 °C were linearized by plotting the logarithm of the residual activity as a function of incubation time (Fig. 4A). The inactivation rate constants ( $k / \text{min}^{-1}$ ) were calculated from the linear regression analysis and are given in Table I.

The inactivation rate constants increased approximately 20-fold in the temperature range 75–90 °C. Effect of temperature on inactivation rate is illustrated by Arrhenius plot shown in Fig. 4B.

Temperature dependence of the thermal inactivation of papain, expressed by the activation energy ( $E_a / \text{kJ mol}^{-1}$ ) value, is given in Table I. Fruit papain exhibited slightly higher thermal inactivation resistance when compared to stem papain (the obtained  $E_a$  value for fruit papain was  $223 \pm 16 \text{ kJ mol}^{-1}$ , while for stem papain, it was  $214 \pm 42 \text{ kJ mol}^{-1}$ ).<sup>27</sup> Recently, it was shown that latex and fruit papain differ in both stability and their catalytic properties under acidic conditions and in the presence of ethanol.<sup>3</sup> There is a possibility that fruit and stem papains are not the same enzyme but are isoenzymes. In the case of some other

laticiferous plants (e.g., *Ananas comosus*), different enzymatic profiles could be detected in the stem latex and the fruit regarding molecular weight, amino acid composition, substrate specificity, etc. Thus, stem and fruit bromelains, which belong to the papain family of proteases, could be considered as isoenzymes.<sup>28</sup>

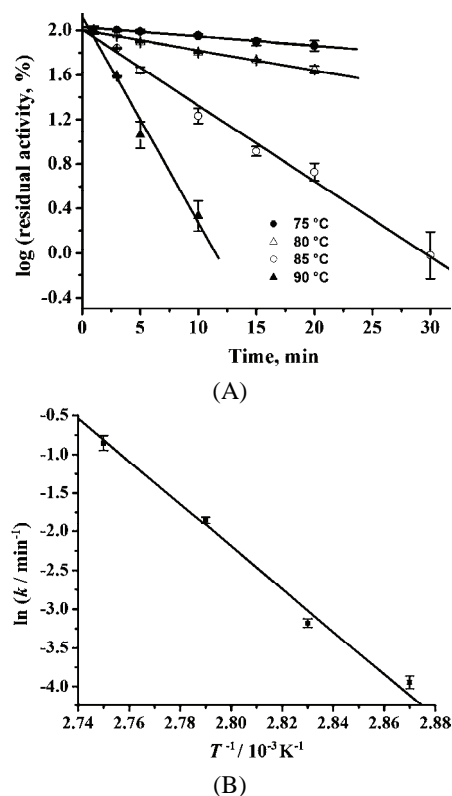


Fig. 4. A) Thermal inactivation of papain in the range between 75 to 90 °C. B) Arrhenius plot showing the temperature dependence of the rate constant of papain inactivation.

TABLE I. Inactivation rate constants ( $k / \text{min}^{-1}$ ) of papain incubated at different temperatures

Temperature, °C	75	80	85	90	$E_a / \text{kJ mol}^{-1}$
$k / \text{min}^{-1}$	$0.019 \pm 0.002$	$0.042 \pm 0.002$	$0.157 \pm 0.007$	$0.43 \pm 0.04$	$223 \pm 16$

The  $E_a$  value of papain ( $223 \pm 16 \text{ kJ mol}^{-1}$ ) was considerably higher when compared to enzymes that are less stable in regards to temperature and presence of SDS. The  $E_a$  values for several enzymes having overall temperature stability below 50 °C and notorious instability in the presence of SDS were found to be 121,<sup>29</sup> 88<sup>30</sup> and 167  $\text{kJ mol}^{-1}$ ,<sup>31</sup> for carbonic anhydrase, urease and amylase, respectively. In the case of proteolytic enzymes, the inactivation  $E_a$  value is very similar to the  $E_a$  value of fruit papain. For example, ficin has an inactivation  $E_a$  of  $210 \pm 22 \text{ kJ mol}^{-1}$ .<sup>27</sup>



### Papain resistance to SDS

Activity staining (Fig. 5) showed that papain was stable at elevated temperatures (up to 60 °C) in the presence of SDS. At the temperatures higher than 60 °C, a gradual loss of activity was observed. Shift in SDS-PAGE mobility of the native (and active) papain in comparison to the denatured one could be explained by the resistance of papain to SDS denaturation. Since native papain showed resistance to SDS, its net negative charge during electrophoresis was decreased in comparison to the net negative charge of denatured papain (mainly originating from the sulfate group of the detergent).<sup>10</sup> It is noteworthy that papain expressed similar thermal stability regardless of the presence of SDS (Figs. 2 and 5). Since such extraordinary SDS stability of proteins was discussed in terms of the kinetic stability of the  $\beta$ -sheet towards SDS unfolding,<sup>10</sup> the propensities of changes of the certain secondary structure elements in the papain molecule upon incubation at elevated temperatures with and without added detergent were explored in the present study.

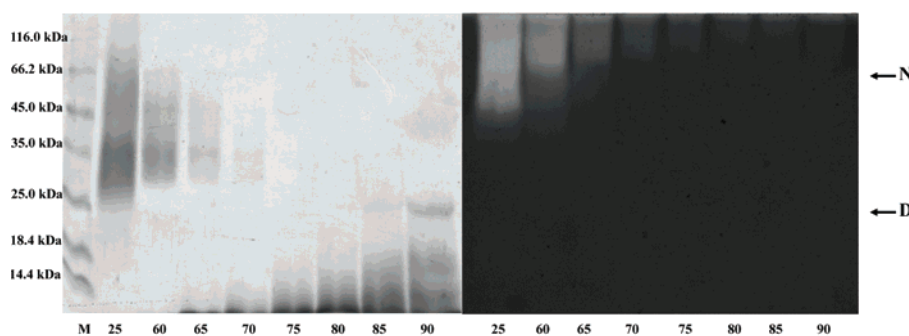


Fig. 5. SDS-PAGE analysis and activity staining towards gelatin of papain incubated at 25 °C and at temperatures from 60 to 90 °C in the presence of 1 % SDS. N – native papain; D – denatured papain.

### Changes in secondary structures of papain

FT-IR spectroscopy was employed for monitoring the changes in the secondary structures. The IR spectra of papain incubated at elevated temperatures with or without SDS are shown in Figs. 6 and 7.

FT-IR spectroscopy is well-established method for the analysis of the secondary structures of proteins. The mostly used spectral region for secondary structure analysis is the amide I band (frequency limits: 1600–1700  $\text{cm}^{-1}$ ), which is almost entirely due to the C=O stretching vibrations of the peptide bonds.<sup>32</sup> Specific maxima within amide I region were identified from second-derivative spectra and assigned to secondary structures:  $\alpha$ -helix 1654  $\text{cm}^{-1}$  and  $\beta$ -sheet 1632  $\text{cm}^{-1}$  according to Byler and Susi.<sup>19</sup> Goormaghtigh *et al.*<sup>20</sup> reported that 1641



$\text{cm}^{-1}$  is a random coil specific frequency for papain, while Byler and Susi,<sup>19</sup> found it at  $1646 \text{ cm}^{-1}$ . In the present study, the random coil band was identified at  $1643 \text{ cm}^{-1}$ . An aggregation specific intermolecular  $\beta$ -sheet conformation was identified at a low frequency at around  $1612 \text{ cm}^{-1}$ .<sup>21-23</sup>

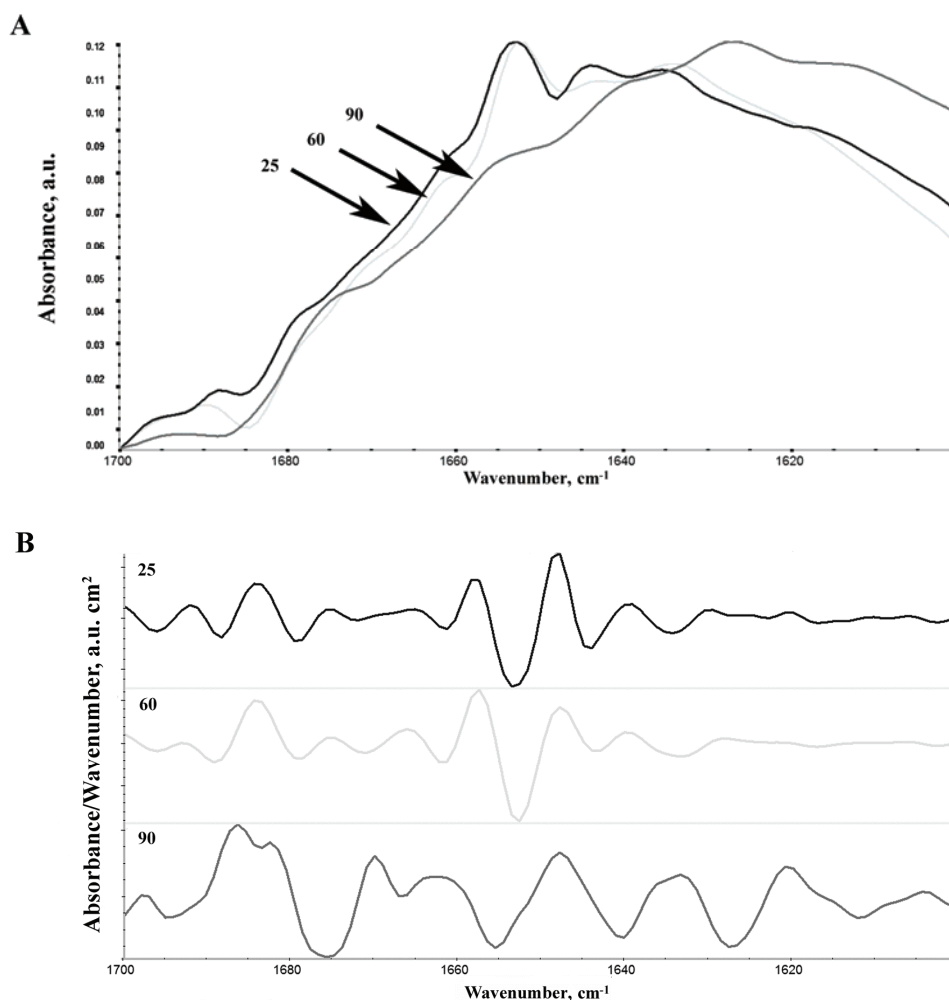


Fig. 6. A) Fourier self-deconvolution IR spectra and B) second derivative spectra of papain incubated at 25, 60 and 90 °C.

Changes in band intensity ratios of certain secondary structures identified within amide I region and amide II band in the IR spectra of papain incubated at elevated temperatures with or without added SDS are shown in Fig. 8.

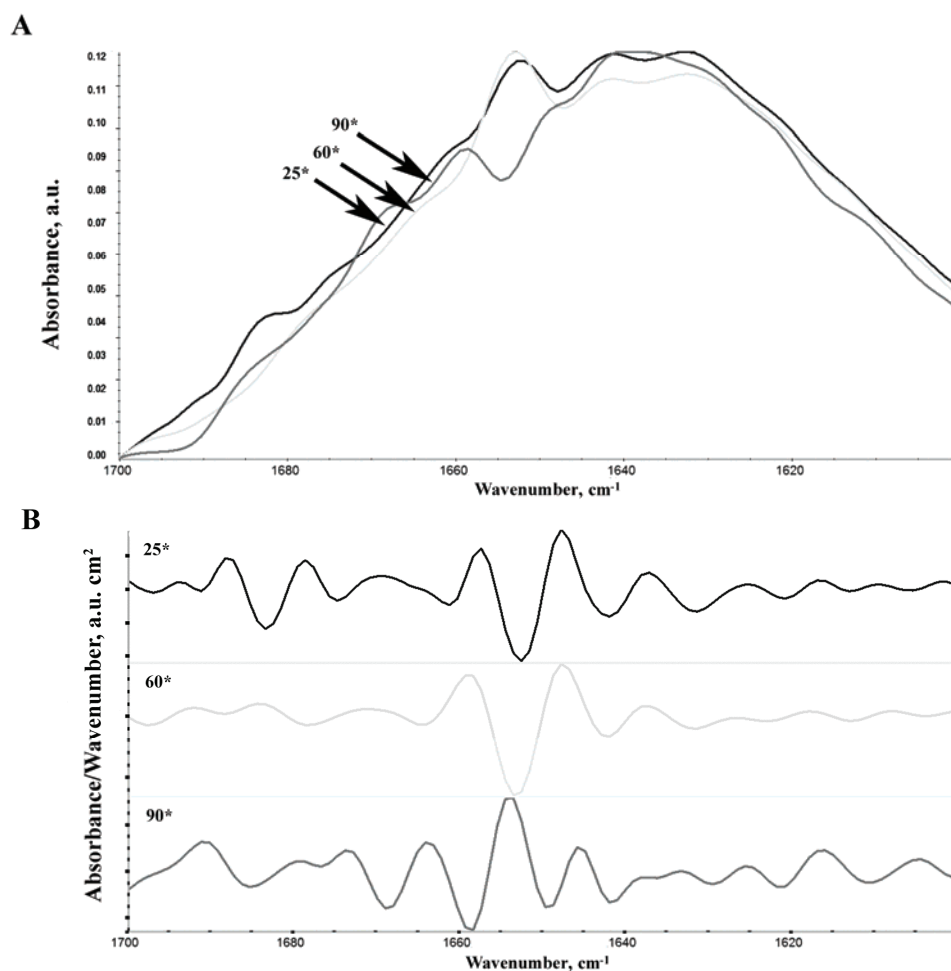


Fig. 7. A) Fourier self-deconvolution IR spectra and B) second derivative spectra of papain incubated at 25, 60 and 90 °C with addition of SDS (25\*, 60\* and 90\*).

Denaturation of papain induced by heat (90 °C) led to lowered contents of native-like structures (ordered:  $\alpha$ -helix; and unordered – random coil), while it accelerated the formation of an aggregation specific intermolecular  $\beta$ -sheet (Fig. 8). Several authors reported that frequencies 1614–1622  $\text{cm}^{-1}$  and sometimes above 1680  $\text{cm}^{-1}$  are aggregation specific frequencies in all  $\alpha$ -helix rich,<sup>22,33</sup>  $\beta$ -sheet rich proteins,<sup>21</sup> and mixed fold proteins,<sup>23</sup> and that they appear due to the formation of intermolecular antiparallel  $\beta$ -sheets prior to aggregation. It was shown in the case of several proteins, including myoglobin,<sup>22</sup> transthyretin,<sup>34</sup>  $\beta$ -lactoglobulin,<sup>21</sup> human growth hormone, human interferon- $\alpha$ -2b<sup>22</sup> and chymotrypsinogen A<sup>23</sup> that heat denaturation induced changes in the amide I region,

corresponding to shift of the intensities of frequency bands of native-like structures toward aggregation specific  $\beta$ -sheet frequency bands. Furthermore, the formation of the aggregates was lowered in the presence of SDS at elevated temperatures (60 and 90 °C, Fig. 8). However, in the case of added detergent it is apparent that elevated temperatures (especially at 90 °C) accelerated the formation of unordered structures, while lowering the content of  $\alpha$ -helix structures. Changes in the  $\beta$ -sheets induced by the presence of SDS were not so pronounced, indicating that denaturation of papain by a combination of heat and detergent started within the  $\alpha$ -helical domain. Lowered stability of the  $\alpha$ -helical domain of papain in comparison to the  $\beta$ -sheet domain was recently reported in case of acid denaturation of papain.<sup>35</sup>

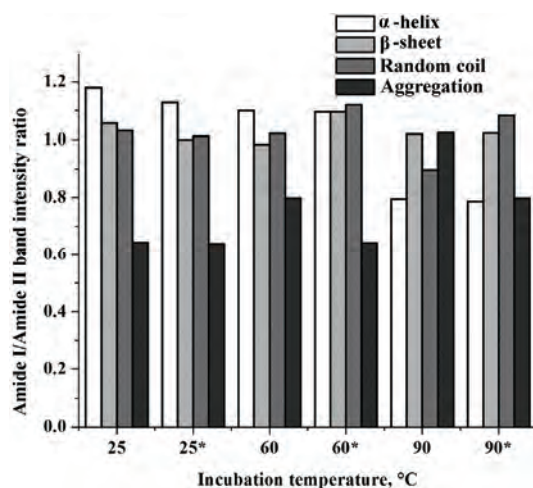


Fig. 8. Changes in intensity ratios of secondary structure specific bands in the IR spectra of papain incubated at 25, 60 and 90 °C (25, 60 and 90) and with addition of SDS (25\*, 60\* and 90\*). The amide I/ amide II band intensity ratios were calculated using the intensity of secondary structure specific band in the amide I region (1654  $\text{cm}^{-1}$   $\alpha$ -helix; 1632  $\text{cm}^{-1}$   $\beta$ -sheet; 1643  $\text{cm}^{-1}$  random coil; 1612  $\text{cm}^{-1}$  aggregation specific intermolecular  $\beta$ -sheet) and the amide II maximum intensity at 1520  $\text{cm}^{-1}$ .

The explanation of SDS resistance of kinetically stable proteins is based on two assumptions. SDS binding appears to rely on transitions between protein conformations, moments of weakness in which the protein is susceptible to SDS binding, thus leading to entrapment.<sup>10</sup> On the other hand, kinetically stable proteins are characterized by unusually low structural flexibility, which lead to suppression of partial unfolding.<sup>36</sup> Thus, kinetically stable proteins, papain among them, infrequently assume open conformations and therefore are resistant to SDS unless provided with energy in the form of heat. With the exception of the papain family of proteases, most of the kinetically stable and SDS resistant proteins exhibit a tight  $\beta$ -sheet fold and tend to oligomerize.<sup>10</sup> However, despite having mixed  $\alpha/\beta$  fold, papain is not the exception to the rule. The obtained results suggest that the  $\alpha$ -helical domain of papain is more prone to unfolding at elevated temperatures and in the presence of SDS than the  $\beta$ -sheet rich domain.

## CONCLUSIONS

In this study, for the first time, the differential SDS resistance of the  $\alpha$ -helical N-terminal domain of papain and the  $\beta$ -sheet rich C-terminal domain is reported. Denaturation of papain by a combination of heat and SDS led to a reduction of the IR band assigned to  $\alpha$ -helix, while preserving the  $\beta$ -sheet content. However, regardless of fruit papain denaturation at 90 °C, the protein exhibited considerable thermal stability and SDS resistance.

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## ИЗВОД

ДОМЕН БОГАТ  $\beta$ -ПЛОЧИЦАМА ЈЕ ОДГОВОРАН ЗА КИНЕТИЧКУ СТАБИЛНОСТ ПАПАИНА ПРИ ДЕНАТУРАЦИЈИ ИЗАЗВАНОЈ ПОВИШЕНОМ ТЕМПЕРАТУРОМ И НАТРИЈУМ-ДОДЕЦИЛ-СУЛФАТОМ

БРАНКИЦА РАШКОВИЋ, НИКОЛИНА БАБИЋ, ЈЕЛЕНА КОРАЋ И НАТАЛИЈА ПОЛОВИЋ

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Папаин је протеаза која у својој структури поседује два домена од којих је један богат  $\alpha$ -хеликсом, а други  $\beta$ -плочицом. Ова два домена се при денатурацији готово независно развијају. За папаин изолован из стабла папаје показана је знатна температурна стабилност, као и отпорност на натријум-додецил-сулфат (SDS). Међутим, подложност сваког од домена да се развијају услед денатурације изазване повишеном температуром или SDS, још увек није проучена. У овом раду је показано да папаин из плода папаје испољава благо повишену отпорност на термалну инактивацију у поређењу са папаином из стабла папаје са прилично високом вредношћу енергије активације ( $E_a$ ) која износи  $223 \pm 16 \text{ kJ mol}^{-1}$  и  $T_m$  50 вредношћу од  $79 \pm 2 \text{ °C}$ . SDS-PAGE анализом и зимограмом одређена је отпорност папаина плода папаје на SDS. Изгледа да протеин задржава активност у присуству SDS-а све док се не обезбеди довољна количина енергије у виду топлоте која ће довести до развијања протеина. Употребом Фурије трансформисане инфрацрвене спектроскопије (FT-IR) показано је да је домен папаина плода папаје богат  $\alpha$ -хеликсом подложнији развијању на повишеним температурама и у присуству SDS од домена богатог  $\beta$ -плочицама. Термална денатурација протеина без присуства детергента доводи до бржег формирања интермолекуларне  $\beta$ -плочице специфичне за агрегацију протеина када се упореди са нативним протеином. Приказани резултати поседују фундаментални значај, али су веома важни и у контексту комерцијалне примене папаина.

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## REFERENCES

1. R. E. Mitchel, I. M. Chaiken, E. L. Smith, *J. Biol. Chem.* **245** (1970) 3485
2. I. G. Kamphuis, K. H. Kalk, M. B. A. Swarte, J. Drenth, *J. Mol. Biol.* **179** (1984) 233
3. M. Esti, I. Benucci, C. Lombardelli, K. Liburdi, A. M. V. Garzillo, *Food Bioprod. Process.* **91** (2013) 595
4. J. Weiss, M. Gibis, V. Schuh, H. Salminen, *Meat Sci.* **86** (2010) 196
5. M. C. Lopes, R. C. Mascarini, B. M. C. G. da Silva, F. M. Flório, R. T. Basting, *J. Dent. Child.* **74** (2007) 93

6. R. Flanagan, A. Jones, *Drug Saf.* **27** (2004) 1115
7. O. Kaiser, P. Aliuos, K. Wissel, T. Lenarz, D. Werner, G. Reuter, A. Kral, A. Warnecke, *PLoS One* **8** (2013) e80490
8. Y. C. Sim, S. G. Lee, D. C. Lee, B. Y. Kang, K. M. Park, J. Y. Lee, M. S. Kim, I. S. Chang, J. S. Rhee, *Biotechnol. Lett.* **22** (2000) 137
9. S. S. Khaparde, R. S. Singhal, *Bioresour. Technol.* **78** (2001) 1
10. M. Manning, W. Colon, *Biochemistry* **43** (2004) 11248
11. H. A. Sathish, P. R. Kumar, V. Prakash, *Int. J. Biol. Macromol.* **41** (2007) 383
12. M. M. Bradford, *Anal. Biochem.* **72** (1976) 248
13. B. Raskovic, O. Bozovic, R. Prodanovic, V. Niketic, N. Polovic, *J. Biosci. Bioeng.* **118** (2014) 622
14. G. E. Anthon, D. M. Barrett, *J. Agric. Food Chem.* **50** (2002) 4119
15. G. E. Anthon, Y. Sekine, N. Watanabe, D. M. Barrett, *J. Agric. Food Chem.* **50** (2002) 6153
16. U. K. Laemmli, *Nature* **227** (1970) 680
17. R. Felicioli, B. Garzelli, L. Vaccari, D. Melfi, E. Balestreri, *Anal. Biochem.* **244** (1997) 176
18. S. E. Glassford, B. Byrne, S. G. Kazarian, *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **1834** (2013) 2849
19. M. D. Byler, H. Susi, *Biopolymers* **25** (1986) 469
20. E. Goormaghtigh, V. Cabiaux, J. M. Ruysschaert, *Eur. J. Biochem.* **193** (1990) 409
21. X. L. Qi, C. Holt, D. McNulty, D. T. Clarke, S. Brownlow, G. R. Jones, *Biochem. J.* **324** (1997) 341
22. D. Ami, A. Natalello, G. Taylor, G. Tonon, S. M. Doglia, *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **1764** (2006) 793
23. A. A. Ismail, H. H. Mantsch, P. T. T. Wong, *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **1121** (1992) 183
24. J. Buijs, W. Norde, J. W. T. Lichtenbelt, *Langmuir* **12** (1996) 1605
25. T. H. Lee, S. Y. Lin, *Process Biochem. (Oxford, UK)* **46** (2011) 2163
26. S. Solís-Mendiola, A. Rojo-Domínguez, A. Hernández-Arana, *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **1203** (1993) 121
27. G. I. Katsaros, P. Katapodis, P. S. Taoukis, *J. Food Eng.* **91** (2009) 42
28. S. Ota, E. Muta, Y. Katahira, Y. Okamoto, *J. Biochem.* **98** (1985) 219
29. B. Kanbar, E. Ozdemir, *Biotechnol. Progress* **26** (2010) 1474
30. W. D. Savage, L. S. Wei, J. W. Sutherland, S. J. Schmidt, *J. Food Sci.* **60** (1995) 164
31. J. K. P. V. Yadav, *J. Biosci.* **34** (2009) 377
32. A. Barth, C. Zscherp, *Q. Rev. Biophys.* **35** (2002) 369
33. F. Meersman, L. Smeller, K. Heremans, *Biophys. J.* **82** (2002) 2635
34. Y. Cordeiro, J. Kraineva, M. C. Suarez, A. G. Tempesta, J. W. Kelly, J. L. Silva, R. Winter, D. Foguel, *Biophys. J.* **91** (2006) 957
35. R. Fosado-Quiroz, A. Rojo-Domínguez, *Protein J.* **30** (2011) 184
36. S. S. Jaswal, J. L. Sohl, J. H. Davis, D. A. Agard, *Nature* **415** (2002) 343.