CCXXXV. HYDROLYTIC PROPERTIES OF *CARICA PAPAYA* LATEX AND LATEX PREPARATIONS

BY MAX FRANKEL, REGINA MAIMIN AND BENJAMIN SHAPIRO

From the Hebrew University, Jerusalem

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WILLSTÄTTER and his co-workers [1924; 1926], on the basis of experiments with commercial preparations, defined papain as an enzyme which splits proteins but does not attack peptones except on activation, e.g. by the addition of HCN or H_2S. Subsequently, more activators of this kind became known, and numerous papers dealing with activation, inhibition and their mechanism have been published. As full reviews are available (cf. e.g. *Ann. Rev. Biochem.* vols. I—VI), the extensive literature on this subject need not be discussed here.

Little attention seems to have been paid in these researches to natural conditions, most of the experiments having been carried out with commercial preparations and only a few with sap of stored fruits. We undertook therefore to study the hydrolytic activity of latex tapped from trees growing under natural conditions and of latex preparations. As far as we are aware no such investigation utilizing an adequate technique has been carried out before. Attention may, however, be directed to the work of Ambros & Harteneck [1929], who studied the enzymic properties of the sap of *Carica papaya* grown in a hothouse in Germany. They found that the hydrolytic activity of the latex depended upon the state of development of the fruit. Almost ripe fruits yielded latex which split only proteins and was without effect on peptones, whereas latex from unripe fruits showed activity towards both proteins and peptones, and latex from very young fruits showed "full activity".[1] It was further found by the same authors that press juice obtained from fruit flesh after complete removal of the milky sap contains an activator which enhances the hydrolytic activity of the latex or of commercial papain to "full activity". This activator is thermolabile, being destroyed on boiling. As will be seen, their findings differ in essential points from our own.

I. *Hydrolytic activity of natural latex (milky sap) of* Carica papaya

Latex of *Carica papaya* was obtained by tapping hanging fruit of trees grown in natural surroundings at the Palestine Government Horticultural Station in Jericho. The enzymic properties of this latex were studied in experiments on the hydrolysis of gelatin and Witte's peptone. It was found, in contradiction to the accepted definition of papain activity, that the latex hydrolyses Witte's peptone as well as gelatin even without previous treatment with HCN or any other reagent. Generally, moreover, the degree of hydrolysis with gelatin was found to fall short of that with peptone. Treatment with HCN stimulated both actions (cf. Table I and partly also Table II).

In the first days of keeping, increase in the activity towards gelatin and often diminution of the activity towards peptone, accompanied by distinct autolysis

[1] The term "full activity" indicates that addition of HCN does not enhance the activity towards proteins and peptones.

of the latex, could be observed, but activity towards peptone never fell to zero.

Table I. *Hydrolytic activities of latex of* Carica papaya *towards gelatin and Witte's peptone and the change on keeping*

Increase in ml. *N*/10 NaOH per 2 ml. reaction mixture after 48 hr.

	Substrates			
	Gelatin		Witte's peptone	
	Without HCN	With HCN	Without HCN	With HCN
(1) Latex (*a*) immediately after tapping	0·18	0·45	0·22	0·54
Latex (*b*) immediately after tapping	0·24	0·48	0·36	0·48
Latex about 2 days after tapping (large fruit)	0·17	0·53	0·29	0·58
Latex about 2 days after tapping (small fruit)	0·09	0·34	0·24	0·46
(2) Latex (*c*) 2 days after tapping	0·21	0·34	0·19	0·42
Latex (*c*) 4 months after tapping	0·68	0·65	0·30	0·63
(3) Latex (*d*) 4 days after tapping	0·11	0·37	0·21	0·49
Latex (*d*) 15 days after tapping	0·20	0·24	0·09	0·44

Reaction mixtures according to scheme given under "Experimental".

No dependence of latex activity or activability by HCN on the state of development of the fruit, as found by Ambros & Harteneck [1929], could be observed. As a rule latex from fruits of different stages of development showed qualitatively identical hydrolysing properties. The quantitative variations observed seemed to be of an individual nature and not clearly related to the state of development.

II. *Hydrolytic activity of preparations obtained from latex*

Besides natural latex, various latex preparations obtained by different treatments were studied.

(1) *Treatment with ether.* The ether-soluble part of the latex was removed by extraction and the aqueous suspension remaining was dried *in vacuo* at room

Table II. *Hydrolytic activities of different samples of ether-treated latex obtained in solid form in comparison with Papayotin Merck 1: 350. Influence of storage on activity*

Increase in ml. *N*/10 NaOH per 2 ml. reaction mixture after 48 hr.

	Substrates			
	Gelatin		Witte's peptone	
	Without HCN	With HCN	Without HCN	With HCN
Natural latex (*a*)	0·17	0·53	0·29	0·58
(*a*) after treatment with ether	0·44*	0·49*	0·48*	0·55*
Natural latex *ca.* 14 days after tapping (*c*)	0·20	0·39	0·50	0·78
(*c*) treated with ether (*d*)	0·33	0·49	0·59	0·74
(*d*) after 6 months' storage	0·26	0·52	0·40	0·60
(*d*) after 12 months' storage	0·32	0·47	0·42	0·71
Ether-treated latex (*e*)	0·97*	1·13*	1·64†	1·64†
Ether-treated latex (*f*)	0·36*	0·43*	0·42*	0·49*
Ether-treated latex (*g*)	0·14	0·39	0·21	0·53
Ether-treated latex (*h*)	0·18	0·35	0·33*	0·41*
Papayotin Merck (1 : 350)	0·11	0·40	0·14	0·51

* Almost "fully active". † "Fully active".
For reaction mixtures see under "Experimental".

temperature. By this method, a solid residue (I) which dispersed well in water and showed a slightly higher enzymic activity than the natural latex was obtained. The activity towards peptone remained higher than towards gelatin. Several such preparations showed "full activity" towards peptone and almost "full activity" towards gelatin. Storage of dried preparations during some months did not cause any considerable loss in enzymic activity.

(2) *Fractionation of the aqueous suspension by centrifuging.* The aqueous suspension obtained by ether extraction of the latex was separated by centrifuging into two fractions:

(*a*) The supernatant fluid showing all the enzymic properties of natural latex (*vide* Table III) and giving a strong colour reaction for —SH compounds with sodium nitroprusside.

(*b*) The centrifugate which initially exhibits activity to both gelatin and peptone. On repeated washing with distilled water, however, the ability to split peptone disappears, and with it the sodium nitroprusside reaction becomes negative, whereas the activity towards gelatin is retained. Both substrates are split by the wash-waters. On HCN treatment gelatin hydrolysis by the centrifugate is enhanced and activity towards Witte's peptone acquired.

Table III. *Hydrolytic activities of* (*a*) *supernatant fluid and of* (*b*) *washed centrifugate obtained from ether-treated latex on centrifuging*

Increase in ml. *N*/10 NaOH per ml. reaction mixture after the indicated hours

	Substrate: Gelatin					
	Without HCN			With HCN		
Time (hr.)	2	20	48	2	20	48
Supernatant fluid from latex 1	0·15	0·34	0·37	0·21	0·47	0·55
Supernatant fluid from latex 2	0·13	—	0·24	0·17	—	0·57
Centrifugate from latex 1	0·07	0·19	0·26	0·20	0·38	0·44
Centrifugate from another latex	—	0·10	0·14	—	0·46	0·50
	Substrate: Witte's peptone					
	Without HCN			With HCN		
Time (hr.)	2	20	48	2	20	48
Supernatant fluid from latex 1	0·27	0·59	0·66	0·32	0·65	0·75
Supernatant fluid from latex 2	0·17	—	0·35	0·24	—	0·74
Centrifugate from latex 1	0·02	0·04	0·06	0·12	0·30	0·35
Centrifugate from another latex	—	0·01	0·04	—	0·22	0·28

Reaction mixtures according to scheme given under "Experimental".

It therefore appears that by the procedure described a natural latex fraction showing all the features ascribed to "papain" on the basis of experiments with commercial preparations has been obtained. This preparation resembled the usual description of "papain" also in so far as its behaviour gave some indication of a time requirement for maximum activation by HCN, as found for commercial papain by Willstätter & Grassmann [1924]. According to them, maximum activation is only obtained when HCN and enzyme are allowed to interact for some time before mixing with the substrate solution. With natural latex no indication for such a time requirement of activation could be demonstrated.

(3) *Treatment of the supernatant fluid with ethyl alcohol.* By treating the supernatant fluid (2*a*) with 96 % alcohol, a white precipitate (III) was obtained. After several washings with alcohol, the enzymic properties of this precipitate

(III) were examined. It proved to be more active than its mother-liquor towards gelatin but less active towards peptone. HCN treatment did not enhance gelatin cleavage any longer, i.e. the precipitate showed "full activity" towards gelatin (*vide* Table IV). When the residue obtained on evaporating the alcohol-soluble fraction of the aqueous solution (2*a*) was added to (III), the hydrolytic power of the system was weakened and the property of "full activity" towards gelatin was lost. HCN treatment now enhanced gelatin cleavage up to the degree found with (III). It appears therefore that the supernatant fluid contains an inhibitor of gelatin cleavage which is removed by alcohol, and which is annulled by HCN. After alcohol extraction accordingly, HCN no longer acts as activator for gelatin cleavage.

Table IV. *Hydrolytic activity of the precipitate obtained from supernatant fluid by alcohol*

Increase in ml. *N*/10 NaOH per 2 ml. reaction mixture after 48 hr.

	Substrates			
	Gelatin		Witte's peptone	
	Without HCN	With HCN	Without HCN	With HCN
Supernatant fluid sample 1	0·24	0·56	0·35	0·65
Ppt. obtained from supernatant fluid 1	0·42	0·46	0·14	0·59
Ppt. (*b*) from another supernatant fluid	0·39	0·36	0·18	0·52
Ppt. (*b*) + alcohol-soluble fraction of its supernatant fluid	0·29	0·44	0·19	0·60

Reaction mixtures as in previous experiments.

The experiments reported above suggest that activation or inhibition of gelatin cleavage and of peptone cleavage, respectively, are not identical processes. Thus alcoholic extraction which raised the hydrolytic power in respect of gelatin to "full activity" even caused a slight loss in peptone hydrolysis.

III. *Hydrolytic action on other substrates*

The hydrolytic power towards substrates, other than gelatin and Witte's peptone, of the ether-treated latex as well as of an alcoholic precipitate prepared from stored latex was compared with that of a commercial preparation of papain (Merck 1 : 350) (cf. Tables V and VI). The ether-treated latex could split native ovalbumin, but, contrary to previous findings by other authors, so also could commercial papain, though to a smaller degree. Addition of HCN considerably enhanced the activity of both preparations. Serum globulin and serum albumin were also split, but activation by HCN in respect of these substrates was less marked.

The casein and gelatin peptones used as substrates were obtained by peptic digestion, whilst silk peptone and Witte's peptone were the commercial preparations generally used in experiments of this kind by previous authors. Little is known about their manufacture and it is possible that they are prepared by hydrolysis with dilute acids. It is therefore of some importance that these peptones of different origin and preparation do not differ qualitatively among themselves in respect of hydrolysis either by our preparations or by commercial papain.

It may be briefly mentioned here in anticipation of a later paper that the degree of activity of commercial papain towards different peptones depends

Table V. *Hydrolytic activities of ether-treated latex towards different substrates in comparison with that of Papayotin Merck (1: 350)*

Increase in ml. *N*/10 NaOH per 2 ml. reaction mixture after 96 hr.

	Substrates							
	Gelatin	White of egg	Native oval-bumin	Native serum globulin	Witte's peptone	Gelatin peptone	Peptone of silk	Casein peptone
Latex treated with ether	0·29	1·96	0·31	0·14	0·35	0·29	0·31	0·37
Latex treated with ether + HCN	0·56	1·90	0·80	0·20	0·73	0·56	0·63	0·60
Papayotin Merck (1 : 350)	0·12	1·07	0·20	0·16	0·21	0·24	0·21	0·40
Papayotin Merck (1 : 350) + HCN	0·38	2·12	0·95	0·40	0·58	0·44	0·52	0·51

Reaction mixtures as usual. Papayotin Merck: 1 ml. of a 10% suspension; *vide* note, Table VI.

Table VI. *Hydrolytic activities of an alcoholic precipitate towards different substrates*

Increase in ml. *N*/10 NaOH per 2 ml. reaction mixture after 120 hr.

	Substrates						
	Gelatin	Native serum albumin	Native serum globulin	White of egg	Gelatin peptone	Peptone of silk	Casein peptone
Alcoholic precipitate	0·70	0·26	0·13	2·02	0·42	0·33	0·49
Alcoholic precipitate + HCN	0·88	0·30	0·23	3·21	0·62	0·73	0·71

Reaction mixtures as usual. The exact concentrations of native serum albumin, native serum globulin and white of egg were not determined. The alcoholic precipitate was prepared from an old latex (sodium nitroprusside reaction positive only in presence of KCN).

upon the ratio substrate-enzyme concentration. This fact may explain why papain was described by previous authors as being without effect on peptones.

IV. *The question of the natural activator*

As mentioned above, according to Ambros & Harteneck the fruit pulp press juice should contain a natural thermolabile activator of gelatin and peptone cleavage and should sometimes be itself proteolytic. We repeated their experiments and examined the fruit pulp press juice in respect of proteolytic activity and activating effect on natural or ether-treated latex and on commercial papain. No stimulation of peptone hydrolysis on addition of juice was found. As regards gelatin hydrolysis, there was, except in a few cases, no activation due to the juice. A clear explanation of these differences between the experimental findings of Ambros & Harteneck and our own is lacking. Significance may attach to the fact that Ambros & Harteneck worked with fruits grown in a hothouse, while we used fruits grown under natural conditions. However, the following observations may be reported. It has been explained above that the supernatant fluid obtained on centrifuging the ether-extracted latex retains the hydrolytic properties of the latex itself. On boiling, the supernatant fluid loses its hydrolytic activity but shows activating properties as regards peptone cleavage. On adding boiled supernatant fluid to the centrifugate, which itself does not split peptone, the system becomes effective also towards peptone. This

activator does not, however, enhance gelatin cleavage by the centrifugate. On the contrary, in some cases addition of boiled supernatant fluid to the centrifugate even diminished the activity towards gelatin.

Table VII. *Activating effect of the boiled supernatant fluid on the splitting of peptone by the centrifugate*

Increase in ml. $N/10$ NaOH per 2 ml. reaction mixture after the indicated number of hours

	Substrate: Gelatin								
	Without HCN					With HCN			
Time (hr.)	2	18	24	42	48	2	18	42	48
Centrifugate 1	0·12	—	0·28	—	—	—	—	—	—
Centrifugate 1 and boiled supernatant fluid 1	0·06	—	0·10	—	—	—	—	—	—
Boiled supernatant fluid 1	0·03	—	0·02	—	—	—	—	—	—
Centrifugate 2	—	0·09	—	0·17	—	—	0·40	0·41	—
Centrifugate 2 and boiled supernatant fluid 2	—	0·05	—	0·09	—	—	0·30	0·41	—
Boiled supernatant fluid 2	—	0·02	—	0·03	—	—	0·04	0·02	—
Centrifugate 3	—	0·10	—	—	0·14	—	0·46	—	0·50
Centrifugate 3 and boiled supernatant fluid 3	—	0·20	—	—	0·23	—	0·17	—	0·40
Centrifugate 4	—	—	0·34	—	0·32	—	—	—	—
Centrifugate 4 and boiled supernatant fluid 4	—	—	0·35	—	0·35	—	—	—	—

	Substrate: Witte's peptone								
	Without HCN					With HCN			
Time (hr.)	2	18	24	42	48	2	18	42	48
Centrifugate 1	0·05	—	0·07	—	—	—	—	—	—
Centrifugate 1 and boiled supernatant fluid 1	0·15	—	0·20	—	—	—	—	—	—
Boiled supernatant fluid 1	0·04	—	0·04	—	—	—	—	—	—
Centrifugate 2	—	0·04	—	0·07	—	—	0·22	0·27	—
Centrifugate 2 and boiled supernatant fluid 2	—	0·12	—	0·18	—	—	0·22	0·29	—
Boiled supernatant fluid 2	—	0·06	—	0·05	—	—	0·03	0·07	—
Centrifugate 3	—	0·01	—	—	0·04	—	0·22	—	0·28
Centrifugate 3 and boiled supernatant fluid 3	—	0·30	—	—	0·26	—	0·24	—	0·26
Centrifugate 4	—	—	0·06	—	0·06	—	—	—	—
Centrifugate 4 and boiled supernatant fluid 4	—	—	0·38	—	0·42	—	—	—	—

Reaction mixtures as usual.

This indicates that the aqueous fraction of the latex contains a thermostable activator of cleavage of peptone but not of gelatin. In respect of the chemical nature of the peptone cleavage activator it may be significant that the supernatant fluid shows a strong colour reaction with sodium nitroprusside and that this reaction also persists after boiling.

Unlike our activator, that found by Ambros & Harteneck in the fruit pulp press juice was completely destroyed by boiling. It is noteworthy that the natural activator described here is confined in its effect to peptone cleavage. This fact, as well as the behaviour of the precipitate obtained by alcohol treatment of the supernatant fluid (cf. Table IV), suggests that activations of the latex in respect of gelatin and peptone cleavages respectively are not identical processes.

These questions as well as that of the chemical nature of the activator will be dealt with in a future paper.

Experimental

The latex was obtained from the source mentioned under I. The fruits were scratched with a horn spatula and the milky viscous liquid was collected in flasks. Generally within a short time it turned into a gel. Latices of fruits of different sizes were collected separately. The latex was found to contain about

11 % of solids; 1·7 % of latex weight could be extracted by ether; 8–9 % could be precipitated by alcohol. The fresh latex showed a strong colour reaction with sodium nitroprusside. It decolorized methylene blue in Thunberg flasks.

The substrates used were (*a*) gelatin "Golddruck"; (*b*) peptone "Witte" (Schering-Kahlbaum); (*c*) ovalbumin, prepared by us according to Sørensen's method; (*d*) serum albumin obtained from serum by saturating with $(NH_4)_2SO_4$, washing and dialysis; (*e*) serum globulin, by half saturation with $(NH_4)_2SO_4$, washing and dialysis; (*f*) native white of egg; (*g*) peptone *ex* casein (prepared by peptic digestion of casein); (*h*) gelatin peptone (obtained in the same way as (*g*)); (*i*) silk peptone (J. D. Riedel A.G.).

The buffer solution was *M*/5 disodium citrate, *p*H 5, prepared according to Sørensen, this *p*H having been found by Willstätter & Grassmann [1924] to be optimum for gelatin cleavage.

HCN activation. In the first stages of the present investigation HCN activation was carried out according to Willstätter & Grassmann [1924], i.e. by allowing the HCN to act on the enzyme for 2 hr. before adding the substrate solution; later it was found that this procedure did not increase the activation, and accordingly the substrate was added immediately after the HCN.

Reaction mixtures. The reaction mixtures contained generally: 5 ml. 3 % substrate solution, 1 ml. enzyme suspension equivalent to 1 g. latex, 5 ml. buffer solution, 2 ml. 3 % HCN (or water) and water to 15 ml. 3 ml. toluene were added.

Determination of hydrolysis. The solutions were kept at 37° and the rate of hydrolysis determined by Sørensen's formaldehyde titration with *N*/10 NaOH in 2 ml. portions using microburettes. In all cases appropriate controls were carried out; the figures in the tables are the observed changes for 2 ml. reaction mixture after deduction of the control values. It was ascertained that the formaldehyde titration gives practically the same results as the titration of Willstätter & Waldschmidt-Leitz with alcohol.

The hydrolysis with all the substrates was mainly effected within the first 24 hr., and was practically ended after 48 hr.

Summary

1. Latex of *Carica papaya* splits both gelatin and peptone.

2. No dependence of activity or activability by HCN on the stage of development of the fruit was observed.

3. Different preparations obtained from latex were studied:

(*a*) Ether-treated latex showed a higher activity towards peptone and gelatin than the untreated latex. Several dried preparations showed "full activity" towards peptone and almost "full activity" towards gelatin.

(*b*) Ether-treated latex was separated by centrifuging into

(i) supernatant fluid, exhibiting the enzymic properties of natural latex, and

(ii) centrifugate showing the features generally attributed to "papain", i.e. activity towards gelatin, and activity towards peptones only on activation.

(*c*) On treatment with alcohol, the supernatant fluid (i) yields a precipitate which is "fully active" towards gelatin, but less active than the supernatant fluid towards peptone.

4. A number of different proteins and peptones were studied in respect of their hydrolyses by our preparations and by commercial papain.

5. No activator of cleavage of gelatin or peptone was found in the fruit pulp press juice.

6. The supernatant fluid contains a thermostable activator of peptone but not of gelatin cleavage.

7. Activations of gelatin and peptone cleavage respectively appear to be distinct processes.

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