OXIDATION OF THIOLS AND ASCORBIC ACID IN THE LATEX OF PAPAYA

By C. V. Ganapathy and B. N. Sastri

(From the Department of Biochemistry, Indian Institute of Science, Bangalore)

Received July 10, 1939

It was pointed out in an earlier paper[1] that the fresh latex of papaya (*Carica papaya*, Linn.) contains a remarkably large concentration of sulphydryl compounds (amounting to nearly 2 per cent. calculated as glutathione) and that practically the whole of it is in the reduced form. The actual glutathione content of the latex is about 0·2 per cent. It has now been found that vitamin C co-exists with glutathione and that the whole of it is also in the reduced condition.

Considerable attention has been devoted to the elucidation of the mechanism, present in the tissues, responsible for maintaining glutathione in the SH-form. Hopkins and Elliot[2] showed that liver tissue contains thermolabile catalysts responsible for the reduction of glutathione. Glucose dehydrogenase,[3] a system present in the intact mammalian erythrocytes[4] and the Warburg-Christian enzyme[5] are all known to be capable of reducing GSSG to GSH. It is of interest to enquire whether the papaya fruit also contains thermolabile catalysts, which function in an analogous manner and incidentally ascertain the nature of protection afforded to vitamin C against oxidation. A preliminary communication on the subject appeared in *Current Science*.[6]

Experimental

I. The System GSSG $\rightleftarrows$ GSH

(1) *The influence of aeration on the SH concentration of the papaya latex.*—5 gm. samples of the fresh latex, drawn from plants grown in the Institute nursery, were weighed into a series of wash bottles (300 c.c. capacity) dispersed in 40 c.c. phosphate buffer (pH 7·4) and aerated by sucking in air with the help of a filter-pump. At intervals of 2 hours the contents of the bottles were transferred to a glass mortar, rubbed with trichloracetic acid (20 per cent.) and sand, and filtered on a Buchner. The residue was repeatedly extracted with trichloracetic acid until the filtrate gave a negative test with nitro-prusside. The combined filtrate was made up to 100 c.c., sufficient water being added to bring the final concentration of the trichloracetic acid to 10 per cent. 5 c.c. aliquots were used for the estimation of SH. Two methods were employed for the purpose:—(1) Iodometric titration according to the method of Kuhnau[7] and (2) the colorimetric

method employing the photoelectric colorimeter. This method developed in our laboratory has been described elsewhere.[8]

The results obtained with different samples of latex are given in Table I.* The figures represent c.c. of N/100 iodine required for titrating the SH groups present in one gram of the latex. Temperature 25° C.

TABLE I

Aeration Time (Hrs.)	0	2	4	6	8	10	12
Sample 1	8·2	8·4	8·0	4·4	..	..	2·4
,, 2	10·0	10·1	8·8	7·6	6·8	..	..
,, 3	7·5	7·5	6·7	5·1	..	..	..
,, 4	7·8	7·7	6·6	5·4	4·5	3·24	2·4
,, 5	6·8	6·7	5·1	3·2	..	..	..
,, 6	7·3	7·4	5·7	4·2	..	2·1	..
,, 7	6·7	6·6	4·9	3·3	..	..	..
,, 8	8·4	8·4	6·6	4·8	..	..	1·9
,, 9	7·1	7·1	6·3	5·1	..	2·5	..
,, 10	8·8	8·8	7·2	6·0	5·2	..	2·4
,, 10†	7·5	7·4	5·9	4·8	3·8	..	..

(2) *The influence of preliminary heating of the latex on the oxidation of SH groups during aeration.*—In the second series of experiments the latex was dispersed in phosphate buffer (pH 7·4) as before, raised to a temperature of 50° C., at which temperature, it was maintained for 60 minutes, cooled

* The titre values tabulated in this section have to be corrected for the presence of ascorbic acid. The main conclusions obtained from these experiments, however, will not be affected, as the titre value for ascorbic acid remains constant until almost all the thiols are oxidised, as will be shown in the subsequent part of the paper. The values for the SH concentration determined by the photoelectric colorimeter are not subject to this error.

† The figures refer to the values calculated in terms of $\frac{N}{100}$ iodine obtained with the photoelectric colorimeter; only results for sample 10 are given here. The difference between the values by the two methods is due to the ascorbic acid.

and aerated. Subsequent treatment was the same as before. The results obtained are shown in Table II.

TABLE II

Aeration Time (Hrs.)	0	2	4	6	8
Sample 1	7·5	5·3	4·2	2·3	1·2
,, 2	6·7	4·3	3·0	1·9	0·8
,, 3	7·0	5·1	4·0	2·4	..
,, 4	5·8	3·7	2·5	1·3	0·2

The results in the first series of experiments show that there is no fall in the SH concentration during the first two hours of aeration, after which there is almost a linear fall. In a few instances, there is a tendency towards a slight increase in the SH concentration. When the latex is heated to 50°, prior to aeration, however, there is a steady fall in the SH content from the very commencement of aeration. The SH content remains at a constant level during aeration only if a reducing mechanism capable of reconverting the SS to the SH form is present in the latex. If such a mechanism is present, addition of fresh SS compounds should lead to an increase in the SH concentration of the reaction mixture on incubation.

(3) *Influence of the added SS compounds on the SH concentration of the latex.*—The effect of addition of SS compounds prepared from the latex itself, was examined in the third series of experiments. The thiols were extracted by boiling the fresh latex with water and oxidised with iodine or hydrogen peroxide. In the case of iodine the quantity required for oxidation was determined by direct titration; with H_2O_2, the aqueous extract of the latex was raised to pH 9 by adding NaOH, then H_2O_2 was added and after oxidation was completed (as shown by negative test with nitroprusside) the excess of H_2O_2 was removed by boiling. The resulting solution was finally brought down to pH 7 by hydrochloric acid and used. A solution of the SS compounds (1 per cent. concentration) was mixed with fresh latex and after 4 hours incubation, the SH content was again determined by iodometric titration. The results given in Table III clearly show that there is an increase in the SH content over the initial value, thereby showing that the SS compounds have been reduced. In a parallel series of experiments, where the SS compounds were added to samples of latex dispersed in buffer and previously heated to 50° C. for one hour, the increase in the SH content was markedly less.

TABLE III

Sample	Fresh latex		Latex heated prior to incubation	
	Initial titre	Increase in titre	Initial titre	Increase in titre
1	8·8	0·8	8·9	0·3
2	7·2	1·0	7·3	0·4
3	7·1	1·1	7·2	0·4

(4) *Experiments with the press-juice from papaya fruit pulp.*—100 c.c. of the fresh juice from the pulp of papaya fruit were incubated with a solution containing SS compounds (2·5 per cent. concentration) for 4 hours and the SH content estimated. In the control series, the juice was boiled prior to incubation. The results tabulated in Table IV show that there is a marked rise in the Iodine titre indicating the presence of reducing systems in the juice. The thermolabile nature of the reducing systems is shown by the fact that this increase in titre becomes markedly less when the juice is heated prior to incubation.

TABLE IV

Sample	Time of incubation Hrs.	Increase in titre c.c. N/100 iodine	
		Fresh juice	Heated juice
1	2	2·0	0·8
2	4	5·1	1·3
3	4	5·2	1·4
4	2	2·4	0·9
5	2	3·7	1·1
6	4	7·2	1·8
7	2	4·1	1·2
8	4	6·0	1·8

II. Oxidation of Ascorbic Acid Present in the Latex.

Vitamin C exists along with the thiols in the latex. That glutathione affords protection against the oxidation of vitamin C by hexoxidase or copper was shown by Hopkins and Morgan.[9] The observations reported in this section of the paper support the main conclusions of Hopkins and co-workers.

(1) *Influence of aeration on the concentration of thiols and vitamin C of the papaya latex.* The lay-out of the experiments was exactly similar to that described in the earlier part of the paper. 5 gm. latex were weighed out into each of 6 wash-bottles (300 c.c. capacity) and air bubbled through. At intervals, the vitamin C (dye titration)[10] and SH concentrations were separately determined in aliquots of the tichloracetic acid extracts. The results are tabulated in Table V. The values for vitamin C and thiols are given in c.c. of N/100 iodine corresponding to 1 gm. of latex.

Table V

Time (Hrs.)		0	½	1	2	4	6
Sample 1	Vitamin C	0·6	..	0·6	0·6	0·5	..
	SH	5·5	..	5·7	5·6	3·6	..
,, 2	Vitamin C	0·3	0·3	0·3	0·3	0·3	0·2
	SH	5·4	5·2	5·3	5·1	3·3	2·6
,, 3	Vitamin C	0·8	0·9	0·8	0·8	0·6	0·6
	SH	9·3	9·5	9·6	9·6	7·0	5·1

(2) *Effect of preliminary heating of the latex on the course of oxidation of vitamin C and thiols.* The previous experiment was repeated with samples of latex dispersed in phosphate buffer and heated at 50° C. for one hour prior to aeration.

Table VI

Time (Hrs.)		0	1	2	4	6
Sample 1	Vitamin C ..	0·6	0·6	0·6	0·5	0·3
	SH ..	5·5	4·0	4·1	2·1	0·5
,, 2	Vitamin C ..	0·8	0·8	0·8	0·7	0·6
	SH ..	6·1	5·0	3·5	2·3	1·0

(3) *Influence of thiol compounds of papaya latex on the oxidation of ascorbic acid by copper.*—To 50 c.c. of a boiled aqueous extract of the latex was added pure crystalline ascorbic acid in solution (70 mg.) and aerated after adding a trace of copper. The vitamin C content as well as the concentration of the thiols were determined in aliquots at intervals of 10 minutes. Table VII gives the results obtained. The values for vitamin C and SH are given in terms of N/100 iodine. There is complete protection of ascorbic acid from oxidation by copper as long as there is even a small amount of the thiols present in the solution.

TABLE VII

Aeration time in mins.	Vitamin C	SH
0	2·9	3·0
10	2·8	1·8
20	2·8	1·0
40	2·2	0·0
60	0·6	0·0

Conclusions and Summary

The presence in the latex, and more especially in the pulp of the papaya fruit, of a system responsible for maintaining the thiol compounds in the reduced condition is clearly established. Its thermolabile character is indicated by the observation that a preliminary heating of the latex dispersed in buffer or the press-juice from the pulp to a temperature of 50° C. for a period of 60 minutes, renders it inactive. The system present in the papaya, in these respects, is analogous to that in the liver described by Hopkins. Further work is called for in order to determine the exact nature of the mechanism.

The significance of this finding lies in the fact that the reducing system provides a regulating mechanism for the proteolytic processes in the plant, the concentration of the SH compounds which function as the natural activators of papain being conditioned by the activity of this system. It also helps, indirectly, to maintain the ascorbic acid present in papaya in the

reduced condition, as the thiol compounds afford complete protection to the vitamin against oxidation by hexoxidase or copper as previously shown by Hopkins, and confirmed by us.

LITERATURE CITED

1. Ganapathy and Sastri .. *Proc. Ind. Acad. Sci.*, 1938, **8**, 399.
2. Hopkins and Elliots .. *Proc. Roy. Soc.*, 1931, **109B**, 58.
3. Mann .. *Biochem. J.*, 1932, **26**, 785.
4. Meldrum .. *Ibid.*, 1932, **26**, 817.
5. ———— .. *Ibid.*, 1935, **29**, 108.
6. Ganapathy .. *Curr. Sci.*, 1938, 6, 451.
7. Kuhnau .. *Biochem. Z.*, 1931, **230**, 353.
8. Ganapathy and Sastri *Proc. Ind. Acad. Sci.*, 1938, **8**, 399.
9. Hopkins and Morgan .. *Biochem. J.* 1936, **30**, 1446.
10. Birch, Harris and Ray *Ibid.*, 1933, **27**, 590.