87. The Effect of Oxidants and Reductants on some Sugar Hydrolysing Enzymes

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Leibowitz [1925] and Leibowitz & Mechlinsky [1926] advanced the theory of a specific gluco-maltase in taka-diastrase distinct from yeast maltase and from taka-sucrase [Hestrin, 1940]. According to Weidenhagen [1932; 1933] there is only one maltase identical with gluco-sucrase. Leibowitz & Hestrin [1938; 1939] found that taka-maltase and sucrase can be differentiated through the greater thermostability and acid stability of the former.

In order to differentiate further between the two hydrolysing capacities of taka-diastrase, we studied the effect of oxidation and reduction on the enzymic preparation.

EXPERIMENTAL

In our experiments we used commercial samples of taka-diastrase, Parke-Davis and Co.

As oxidant and reductant we used H_2O_2 and $\text{Na}_2\text{S}_2\text{O}_4$ respectively.

The enzyme solutions were prepared by dissolving 2.5 g. of the commercial product in 15 ml. distilled water in a cellophane bag. The solution under toluene was dialysed at room temperature for 1 day against running tap water and 1 day against distilled water, which was changed several times until the solution was free of reducing constituents. The solution was filtered and brought to 20 ml.

4.5 ml. samples of the enzyme solution were mixed in test-tubes, with 0.5, 1 and 1.5 ml. H_2O_2 30% (perhydrol Merck 'speciale pour le Tropique') respectively; all the samples, including a control solution without H_2O_2 , were brought to 6 ml. with distilled water. After 24 hr. at room temperature, the H_2O_2 was removed by dialysing all solutions in cellophane bags for 2 days against distilled water, which was changed several times. The samples again were brought to the same volume.

The same procedure was used for preparing an enzyme solution treated with hydro-sulphite ($\text{Na}_2\text{S}_2\text{O}_4$). A 3% $\text{Na}_2\text{S}_2\text{O}_4$ of Schering-Kahlbaum was used.

As *substrates* we used 4% solutions of maltose and sucrose (Schering-Kahlbaum) dissolved in Sørensen's phosphate buffer of pH 4.5 for maltose and pH 5.5 for sucrase.

The enzyme mixture was made by mixing 0.5 ml. of the enzyme solution with 4.5 ml. substrate solution in glass stoppered test tubes. The incubation temperature was 37°.

The hydrolysis of the sugars was determined according to Lehmann-Maquet-Schoorl in 1 ml. portions of enzyme mixture removed after different time intervals and introduced into 1 ml. 2% Na_2CO_3 solution (to stop the enzyme activity).

Effect of H_2O_2 on the rate of maltose and sucrose hydrolysis. As shown in Figs. 1 and 2, taka-maltase is far less affected than sucrase. The inhibition of taka-sucrase is not substantially increased with increasing concentration of H_2O_2 .

Effect of $\text{Na}_2\text{S}_2\text{O}_4$ on the rate of maltose and sucrose hydrolysis. Figs. 3 and 4 show that whereas taka-maltase is slightly stimulated by $\text{Na}_2\text{S}_2\text{O}_4$, taka-sucrase is inactivated, the inactivation increasing with the quantity of $\text{Na}_2\text{S}_2\text{O}_4$.

In order to determine whether the effects of H_2O_2 and $\text{Na}_2\text{S}_2\text{O}_4$ respectively are reversible, each once-treated solution was subjected to treatment with the reactant not previously used. Thus taka-diastrase treated with H_2O_2 was subsequently treated with

Fig. 1. Taka-maltase. ● control; ○ 0.5 ml. H₂O₂ 30%; ■ 1 ml. H₂O₂ 30%; × 1.5 ml. H₂O₂ 30%.

Fig. 2. Taka-sucrase. ● control; ○ 0.5 ml. H₂O₂ 30%; ■ 1 ml. H₂O₂ 30%; × 1.5 ml. H₂O₂ 30%.

Fig. 3. Taka-maltase. ● control; ○ 0.5 ml. Na₂S₂O₄ 3%; ■ 1 ml. Na₂S₂O₄ 3%; × 1.5 ml. Na₂S₂O₄ 3%.

Fig. 4. Taka-sucrase. ● control; ○ 0.5 ml. Na₂S₂O₄ 3%; ■ 1 ml. Na₂S₂O₄ 3%; × 1.5 ml. Na₂S₂O₄ 3%.

$\text{Na}_2\text{S}_2\text{O}_4$, and vice versa. 5 ml. of the original dialysed solution of taka-diastrase was treated for 24 hr. with 1.5 ml. 3% $\text{Na}_2\text{S}_2\text{O}_4$ dialysed until free of hydrosulphite, treated with 1.5 ml. H_2O_2 30% for 24 hr. again dialysed and brought up to the same volume with distilled water. In a second experiment the order of treatment was reversed.

Table 1. *Taka-maltase*

Hours	mg. Cu		
	I	II	III
0	31.6	31.6	31.6
3	32.2	32.8	31.6
6	32.2	33.7	31.6
24	36.2	37.5	33.7

Table 2. *Taka-sucrase*

Hours	mg. Cu		
	I	II	III
0	0.00	0.00	0.00
3	1.57	0.00	0.00
6	4.60	0.00	0.00
24	19.55	0.00	0.00

I, Control. II, $\text{Na}_2\text{S}_2\text{O}_4$ 3% and subsequently H_2O_2 30%. III, H_2O_2 30%, $\text{Na}_2\text{S}_2\text{O}_4$ 3%.

The results (Tables 1 and 2) show a striking difference in the behaviour of the two enzymes: in the case of maltase only the effect of the reagent used first was manifested. It follows that treatment of the maltase, whether by H_2O_2 or $\text{Na}_2\text{S}_2\text{O}_4$ renders the enzyme insensible to the effect of the other reagent. In the case of sucrase, an accumulation of inhibiting effects of both reagents was observed, so that by treatment of taka-diastrase with H_2O_2 and $\text{Na}_2\text{S}_2\text{O}_4$ or by $\text{Na}_2\text{S}_2\text{O}_4$ and H_2O_2 in sequence a complete inactivation of the sucrase was attained, whereas the maltase still retained its activity.

In order to ascertain how sucrase from a different source is affected by the oxidising and reducing agents, we used a 1% yeast autolysate (for which I am indebted to Dr J. Leibowitz) containing practically no maltase.

5 ml. samples of the yeast extract were mixed in different test-tubes, one with 1 ml. H_2O as control solution, one with 1 ml. 3% $\text{Na}_2\text{S}_2\text{O}_4$, and a third with 1 ml. 30% H_2O_2 . After 24 hr. at room temperature, the samples were dialysed until no $\text{Na}_2\text{S}_2\text{O}_4$ or H_2O_2 was detected.

Table 3. *Yeast sucrase*

Hours	mg. Cu		
	H_2O	$\text{Na}_2\text{S}_2\text{O}_4$	H_2O_2
0	0.00	0.00	0.00
3	4.46	12.80	0.00
6	7.74	23.20	0.00
24	37.00	60.00	0.00

Table 3 shows that yeast sucrase is completely inhibited by H_2O_2 but is strikingly activated by $\text{Na}_2\text{S}_2\text{O}_4$.

DISCUSSION

Taka-maltase, taka-sucrase and yeast sucrase were investigated as to the effect on them of treatment with H_2O_2 (as an oxidant) and $\text{Na}_2\text{S}_2\text{O}_4$ (as reductant), used separately or in succession. Each enzyme showed its own peculiar specific behaviour. Taka-maltase was markedly inhibited by H_2O_2 and slightly stimulated by $\text{Na}_2\text{S}_2\text{O}_4$. Taka-sucrase was strongly inhibited and sometimes completely inactivated by both reagents. Yeast sucrase was strongly stimulated by $\text{Na}_2\text{S}_2\text{O}_4$ and completely destroyed by H_2O_2 .

It is always a question whether a different response of two enzymes to the same physical or chemical agent is due to differences in the nature of the active principle of the enzyme molecule or of the accompanying substances ('carriers') which in most cases constitute the bulk of the substance in the active preparation. This uncertainty applies to a comparison of sucrase from *Aspergillus oryzae* and that from yeast. Since, however, taka-sucrase and taka-maltase belong to the same preparation, it follows clearly that the differences detected between them are due to the nature of these enzymes themselves.

The different behaviour of the taka-enzymes towards oxidizing and reducing agents was accentuated in successive treatment with $\text{Na}_2\text{S}_2\text{O}_4$ and H_2O_2 . In the case of sucrase, the second treatment simply added inhibiting effect to the inhibition already caused. The behaviour of maltase was quite different and very peculiar: inhibition through peroxide was not evoked or compensated by subsequent treatment with hydrosulphite, though the latter itself has a stimulating effect on the enzyme. If the enzyme was treated previously with $\text{Na}_2\text{S}_2\text{O}_4$, the stimulation caused was not diminished by the subsequent treatment with the inhibitor H_2O_2 . It appears that treatment by each of the two agents so affects the enzyme molecule as to render it insensitive to the effect of the other. It is difficult at the moment to give an explanation for this strange phenomenon.

Taken together, the facts obtained by the treatment of taka-diastrase by oxidants and reductants, confirm the theory that the maltose and sucrose splitting capacities of taka-diastrase must be attributed to two distinct and specific disaccharases.

SUMMARY

1. Taka-maltase is markedly inhibited by H_2O_2 and slightly stimulated by $\text{Na}_2\text{S}_2\text{O}_4$.
2. Taka-sucrase is strongly inactivated by both the oxidizing and reducing agents.
3. Yeast sucrase is strongly activated by hydrosulphite and completely inactivated by hydrogen peroxide.
4. In the event of successive treatment by both reagents in any order the inhibiting effects are cumulative in the case of taka-sucrase, while in taka-maltase only the effect of the initial treatment (whether stimulation by $\text{Na}_2\text{S}_2\text{O}_4$ or inhibition by H_2O_2) is manifested.
5. Support is lent to the theory that taka-maltase and taka-sucrase are two distinct and specific disaccharases.

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