ON THE INFLUENCE EXERCISED BY CERTAIN ACIDS ON THE INVERSION OF SACCHAROSE BY SUCRASE

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Among the chemical conditions which influence enzymic action is the degree of alkalinity or acidity of the medium in which the action takes place; that is to say, the rapidity with which a given enzymatic splitting proceeds is influenced by the addition to the reaction medium of small quantities of an acid or base. In general, enzyme action, if certain exceptional cases are excluded, is greatly retarded and ultimately arrested by the addition of comparatively small amounts of a base. The addition of acids to the reaction medium, on the other hand, in small but variable amounts, according to the particular acid employed, appears to favour enzyme activity in certain cases. Most enzymes are, however, remarkably sensitive to changes in the chemical reaction of the medium in which they ordinarily manifest their normal activity, and examples illustrative of this characteristic are not difficult to find.

Malt amylase, for example, as the investigations of Ford¹ demonstrate, evidently function best in a medium which is approximately neutral. Departure from this condition of relative neutrality on either side, provided by the addition of quite minute amounts of either an acid or alkali, leads, as his results indicate, to perceptible diminution of the activity of this enzyme.

The researches of G. Bertrand² afford ample evidence of the extreme sensitiveness of the oxidizing enzyme laccase towards a number of different acids, a very active yet dilute preparation of this enzyme being completely paralysed by quite minute amounts of such acids as sulphuric, hydrochloric, acetic, oxalic, etc.

While a relatively neutral medium favours amylolytic activity, as exhibited by malt amylase, and while the oxidising action of laccase is enormously retarded and eventually completely arrested by minute amounts of different acids, the inversion of cane sugar by sucrase is

1. *Journ. Soc. Chem. Ind.*, XXIII, 8, 1904.
2. *Ann. Institut Pasteur*, XXI p. 673, 1907.

favourably influenced by the presence of small but variable amounts of different acids. Thus, Kjeldahl¹ states that inversion of cane sugar by sucrase is favoured by the addition of very small quantities of sulphuric acid, while larger amounts exercise a marked retarding influence on the action.

The correctness of Kjeldahl's observations was confirmed by O'Sullivan and Tompson,² who state that the most favourable amount of acid varies with the preparation of enzyme employed and the temperature at which the experiment is made. They were, however, unable to explain the cause of the variations observed.

Fernbach's³ researches include a comparative study of the influence exercised by various acids, chiefly organic, on the inversive activity of sucrase. The fact is established that for each acid there exists a certain range of optimal concentration, varying considerably according to the acid employed, within the limits of which the enzyme manifests its maximal activity. The addition of still smaller amounts of acid than those indicated by these limits leads to a slight diminution of the action in most instances, while addition of progressively larger amounts produces great retardation, and ultimately complete arrest of the action, as shown in the case of sulphuric and oxalic acids.

The more recent work of Sørensen⁴ directs attention to the importance of the concentration of the hydrogen ions in enzymatic reactions. Results of experiments on the inversion of cane sugar by sucrase in the presence of sulphuric acid and of mixtures of citrates and phosphates in which solutions of sucrase differing in strength and mode of preparation were employed, tend to prove the statement that, in otherwise equal conditions, the optimal concentration of hydrogen ions in the inversion of cane sugar is *almost* independent of the kind and quantity of sucrase employed, and likewise of the acidifying reagent utilised.

Although the fact has been established, that in general the presence of small amounts of different acids exercises a favourable influence on the inversive function of sucrase, it nevertheless appeared to be of interest to investigate the subject anew by the employment of different acids either in molecular concentrations or in amounts proportional to these latter, and further to endeavour to ascertain whether highly ionised acids

1. *C. R. des travaux du Lab. de Carlsberg*, 1879 and 1881.

2. *Journ. Chem. S c.*, LVII, p. 854, 1890.

3. *Thèse doct. Sciences Paris*, 1890.

4. *C.R. des Travaux du Lab. de Carlsberg*, 1909.

(such as hydrochloric, etc.) markedly differentiate themselves from the less highly ionised acids (such as phosphoric and acetic acids) in the influence they exercise on the activity of this enzyme.

The present paper, therefore, contains a short account of the results furnished by this method of enquiry.

PREPARATION OF SUCRASE

While it is impossible to prepare a solution of sucrase completely exempt from substances which are capable of interacting with acids or bases, yet by repeatedly adhering to an identical method of procedure, preparations may be obtained which differ among themselves in a minor degree only, and, therefore, probably possess a sufficient degree of purity to permit of their being employed in experiments of a comparative nature.

Following the method indicated by Duclaux, and utilised by Fernbach¹, cultures of *Aspergillus niger* were established on Raulin's solution and continued for four to five days at a temperature of 30° C. The mycelium having attained maturity, generally indicated by the commencement of blackening over its entire upper surface, the culture liquid after careful decantation and thorough washing of its under surface with distilled water² (the operation being repeated several times), was replaced by a definite volume of distilled water. The plasmolysis of the mycelium was thus established, and shielded from the light was allowed to proceed for forty-eight to fifty hours at 30° C., and invariably yielded an almost colourless solution of sucrase possessing a satisfactory degree of activity. In this connection it is to be noted that during plasmolysis of the mycelium it must not be submerged; to obtain a satisfactory preparation of the enzyme by this means it is desirable that only the under surface of the tough mycelial growth shall be in contact with the plasmolysing liquid. In the preparation of the culture and the subsequent operations of decantation, washing and maceration of the mycelium, Fernbach's flasks are specially serviceable. Each of these flat-bottomed flasks, in which a volume of culture liquid having a depth of about 2 to 3 cm. is placed, has a lateral tubulure by means of which aeration of the mould during its growth may be conveniently carried

1. *Loc. cit.*

2. Throughout, in these cultural and also the inversion experiments, the water employed was prepared by re-distillation, under diminished pressure, of ordinary distilled water with the aid of a glass apparatus.

out. Under these conditions *Aspergillus* makes regular and vigorous growth. Repeated experience of this, and trial of other methods of culture, showed distinctly the superior advantages afforded by the employment of the Fernbach flasks in these cultural experiments.

Under the above conditions it is possible to prepare an active solution of sucrase entirely free from other disturbing organisms, but often containing minute mycelial fragments which may still possess tardy or limited powers of development. Such a solution exposed to free access or even a limited volume of air, as experience of such preparations retained under these conditions showed, leads more or less speedily to diminution either of the quantity of active enzyme or of its activity.

The experiments, to be presently described, were carried out in sections, each comprising a certain range of concentrations of the acid under investigation, and the period of time involved in passing from the initial to the final series of concentrations of acid covered several days. Hence, in order to render the results of one portion of an experiment comparable with those of another, a necessary condition imposed was that the enzyme solution should be conserved in such a way as to retain, as far as possible, its original activity. As it is almost impossible rapidly to prepare solutions of enzymes which are quite identical, the following method of procedure was followed. A number of pipettes were made, each of such a capacity that its contents sufficed for a single sectional series of experiments. After sterilisation, these pipettes were filled with the sucrase solution and at once hermetically sealed. The enzyme solution thus aseptically collected and protected from the action of diffuse daylight, was conserved without undergoing any very marked alteration even during considerable periods of time.

MODE OF OPERATION

To study the influence of the different acids investigated on the inversion of sucrose by sucrase, after a number of preliminary trials the following method of experiment was adopted:—

Into each of a series of tubes exactly 5 c.c. of a 20 per cent. solution of saccharose and definite volumes of acid and water were measured, which, after the addition of the sucrase solution, brought the total volume of the reaction medium to 10 c.c. Corresponding series of

controls were similarly prepared, with the addition of a solution of passive sucrase prepared by raising the enzyme solution to the boiling point and retaining it at that temperature for some minutes. The contents of each tube containing the solution of saccharose, together with the requisite volume of water, were first allowed to acquire the temperature of the latter. The requisite volume of acid was then added, well mixed, and a definite and equal interval of time permitted to elapse before the addition of the enzyme-solution, in order that inversion due to the acid itself might be identical in the experimental tube and in its corresponding control, and then, after addition of the enzyme solution, the experiment was continued for one hour at 56° C. and terminated by adding a very moderate excess of strong soda solution.

Finally, the contents of each tube were diluted to 50 c.c., and the amount of inversion measured by determining the copper-reducing power of a definite volume of this dilution.

Throughout the course of this work these determinations, carried out in duplicate, were effected by means of the method of Mohr, as modified by Bertrand.¹

Carefully standardised solution of acids have been employed, and in preparing the more dilute solutions for the weaker concentrations, quantities of not less than 200 c.c. of each dilution have been freshly prepared.

The volume of sucrase solution employed in each sectional series of experiments has been so chosen as to produce under the given conditions of time, temperature, etc., an amount of inversion of approximately the same order of magnitude.

In the tables which follow, the quantities of saccharose inverted are given in milligrams; these values representing the results obtained after deducting the amounts furnished by the corresponding control experiments with passive enzyme in the presence of similar quantities of acid.

Experiments were first undertaken with representatives of the 'strong' acids in the physico-chemical sense, namely, sulphuric, hydrochloric and nitric acids.

1. *Bull. Soc. Chim.*, XXXV, p. 1285, 1906.

Concentration of acid				H ₂ SO ₄		HCl Sucrose inverted		HNO ₃	
				(i)	(ii)	(i)	(ii)	(i)	(ii)
0·00	(Control) ¹	..	..	115·0	109·2	111·6	142·0	151·0	151·0
N·50	..	..	..	9·8	9·3	0·00	0·00	6·6	6·6
60	..	..	..	21·8	20·7	9·3	14·0	10·3	8·7
70	..	..	..	23·2	22·0	19·5	27·2	17·6	14·6
80	..	..	..	29·0	27·5	38·0	40·3	29·5	24·9
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0·00	(Control) ²	..	..	120·0	114·0	—	137·2	151·0	151·0
N·90	..	..	..	36·3	34·5	38·7	51·4	49·7	38·5
100	..	..	..	40·5	38·7	52·7	76·9	61·7	61·7
200	..	..	..	80·0	76·0	81·8	135·8	144·8	149·6
500	..	..	..	117·5	111·6	105·9	150·0	183·8	183·8
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0·00	(Control)	..	..	117·5	111·5	111·6	139·2	150·5	151·0
N·1000	..	..	..	119·0	113·5	117·5	155·5	181·9	176·2
2000	..	..	..	118·0	112·0	122·5	143·7	160·5	163·4
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0·00	(Control)	..	..	—	—	—	—	—	171·9
N·3000	..	..	..	114·5	108·7	118·7	145·8	165·7	180·5
4000	..	..	..	110·0	104·5	118·7	145·8	166·2	171·5
5000	..	..	..	115·0	109·2	97·8	142·5	159·6	172·0

These experimental results show that these acids in minute amounts favour, in a varying degree, the inversion of saccharose by sucrase. For each acid there exists a range of concentrations within the limits of which the action proceeds most rapidly, while the addition of gradually increasing quantities of acid leads to its progressive retardation, until ultimately when the concentration of the acid attains a value of about N/50 practically complete arrest of the action ensues. In general, amounts of these acids corresponding to concentrations ranging from N/1000 to N/5000 exercise a favourable influence on the action of the enzyme.

The method of measuring the amount of inversion by determining the copper-reducing power of the products of change, evidently does not permit of the defining of any one particular concentration as the optimal one, because the change in the amount of saccharose inverted in the

1. Control in this and succeeding experiments containing sucrose and enzyme without addition of acid.

2. Although solution of enzyme employed here perceptibly stronger, results are not quite comparable with those of first experiment.

passage from one dilute concentration of acid to the other of these optimal ranges of concentrations is relatively small. Moreover, it is probable that small amounts of substances capable of fixing a relatively large proportion of the added acid now make their presence felt, and thus somewhat obscure the actual influence exercised by the acid on the action of the enzyme.

The three acids so far examined are typically strong acids, and the experimental results adduced show that their behaviour is very similar.

The influence of phosphoric acid on the progress of the action affords a marked contrast to that exercised by either of the foregoing acids, as the following experiment shows:—

Concentration of acid		Phosphoric acid Saccharose inverted Milligrams	
0·00 (Control)	..	..	135·8
M/10	..	..	8·0
M/20	..	..	44·3
M/40	..	..	91·2
0·00 (Control)	..	..	164·3
M/60	..	..	124·9
M/80	..	..	140·6
M/100	..	..	152·4
M/200	..	..	166·3
0·00 (Control)	..	..	144·4
M/500	..	..	169·1
M/1000	..	..	163·4
M/2000	..	..	164·3
M/4000	..	..	158·6

To effect virtual arrest of the enzymatic action, a concentration of acid equivalent to that of an M/10 solution of the acid is required. The range of optimal concentrations evidently lies within the limits of M/200 and M/4000. The acid behaves somewhat similarly to a monobasic acid, apparently containing only one active hydrogen atom, a point already indicated by the work of G. Bertrand.¹

The following experiment with KH_2PO_4 lends support to this supposition, for in the experimental condition employed this salt is without any influence on the action of the enzyme.

1. *Loc. cit.*

Concentration of salt		Potassium dihydric phosphate Saccharose inverted Milligrams	
0·00 (Control)	..	..	159·6
M/5	..	..	160·5
M/10	..	..	159·6
M/20	..	..	159·6
M/40	..	..	159·6

The influence exercised by acetic acid on the action is peculiar. When comparison is made with the other acids examined, the principal feature exhibited is that relatively large amounts of this acid, as already evidenced by the investigations of Fernbach,¹ favour inversion instead of retarding it. This favourable influence is manifested, as the following experiment shows, over a wide range of concentrations. In the presence of amounts of acid corresponding to that of N/500 to N/2000 solutions, this favouring influence, however, declines somewhat.

Concentration of acid		Acetic acid Saccharose inverted Milligrams	
0·00 (Control)	..	..	121·6
N/1	..	..	71·2
N/2·5	..	..	119·7
N/5	..	..	128·2
N/10	..	..	133·9
N/20	..	..	137·7
0·00 (Control)	..	..	120·6
N/40	..	..	130·1
N/60	..	..	130·0
N/80	..	..	132·0
N/100	..	..	133·0
0·00 (Control)	..	..	151·0
N/200	..	..	167·2
N/500	..	..	159·6
N/1000	..	..	156·7
N/2000	..	..	155·8

1. *Loc. cit.*

SUMMARY

The results described in this paper show that within the limits represented by certain concentrations, each of the acids examined exercises a favourable influence on the inversion of saccharose by sucrase. The amounts of acid which exercise this favouring influence, as well as the extent of the latter, exhibit considerable variation, and apart from other considerations are apparently dependent on the nature of the acid and the proportion of enzyme employed.¹ Thus, while the action proceeds most rapidly in the presence of comparatively small quantities of sulphuric, hydrochloric, nitric and phosphoric acids, a similar result also ensues even where relatively much larger amounts of acetic acid are included in the reaction medium.

The retarding influence exhibited by these various acids also differs in a similar sense. It is most marked in the case of sulphuric, hydrochloric and nitric acids; amounts of these acids in the reaction medium corresponding to that of an N/50 solution being sufficient to almost completely arrest enzymatic action. Phosphoric acid behaves similarly when the quantity present is equivalent to that of an M/10 solution of this acid. The behaviour of acetic acid in this respect is singular; even when the amount present corresponds to that of an N/1 solution the action, instead of being completely arrested, is merely retarded to a pronounced extent.

In general the results demonstrate that the retarding and arresting influence exercised by the acids investigated on saccharose-inverting action of sucrase differs in a marked degree from that observed by

1. In view of the work of Sørensen, it appears to be not improbable that the observed changes in the progress of inversion of saccharose by sucrase in presence of varying quantities of the different acids examined actually turns, not on the degree of the acidity of the reaction medium, but on the concentration of the hydrogen ions present at each stage of the experiment.

On such an assumption the differences exhibited in the optimal range of concentrations possessed, for example, by acetic and phosphoric acids, as compared with the other acids investigated might perhaps be brought into line.

While the results of this enquiry confirm the favourable influence exercised, it is equally evident that the difficulty of determining the optimal range of concentrations for a given acid becomes a matter of difficulty on account of the disturbing influence of minute amounts of foreign substances unavoidably present in the enzyme solution, the influence of these being progressively more marked with diminution of the quantity of acid employed.

G. Bertrand¹ with these reagents in the case of laccase, the oxidising action of this enzyme being completely arrested by very minute amounts of sulphuric, hydrochloric, phosphoric and acetic acids.

In conclusion the writer desires to record his acknowledgements to M. Gabriel Bertrand for the laboratory facilities he so kindly placed at his disposal, and also for much critical and useful advice generally accorded during the progress of this enquiry.

1. *Loc. cit.*