CCI. INFLUENCE OF PROTEINS AND PROTEIN DERIVATIVES ON THE ENZYMIC HYDROLYSIS OF STARCH BY MALT DIASTASE.

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MUCH of the work published by earlier authors on the activation or inactivation of amylase by various substances, such as amino-acids, amines, amides, proteins, *etc.*, is vitiated by the fact that possible variations in the p_H of the medium due to the introduction of the substances examined were not taken into consideration. The same applies to other factors influencing the activity of this enzyme, such as purity, temperature, *etc.*

Euler and Josephson [1926] found that the addition of glucose or laevulose retarded the velocity of enzymic amylolysis whilst addition of amines or amino-acids to such systems accelerated the reaction. Sherman, Caldwell, and Adams [1926] considered that the activating effect of amino-acids was due to their inhibiting action on the autolysis of the enzyme. Przyłęcki and Majmin [1931] have shown that proteins can combine with, and consequently possibly activate the substrate, the combination between starch and proteins being dependent on the p_H of the medium. It follows that amylolysis in presence of protein should depend quantitatively on the p_H of the medium, and in the present paper work on this aspect of the problem will be described.

EXPERIMENTAL.

Methods.

Solutions of Merck's amylase (Diastase absolut) were prepared separately for each series of determinations by dissolving 0.1 g. of amylase in 100 cc. of 0.1 % NaCl, and filtering after 30 min. Solutions of Merck's soluble starch or dextrin were prepared by gradual addition of starch or dextrin paste to boiling water, boiling a further 15 min., and, after cooling, diluting to the requisite volume. Two buffer solutions were used: Walpole's acetate mixtures below p_H 4.5, and Sørensen's phosphates above p_H 4.5. p_H was determined colorimetrically in some cases but chiefly potentiometrically. Reducing sugars obtained from starch were determined by Bertrand's method, using 60 cc. of solution and 20 cc. each of Bertrand's reagents. Merck's ovalbumin (6 g.) was dissolved in 100 cc. of water, and filtered after 30 min.; the filtrate had

p_H 7.27. The solutions were freed from protein before determination of sugar by addition of 30 cc. of a mixture of 200 cc. of 4.5 % $ZnSO_4$ and 40 cc. of 0.1 N $NaOH$, heating for 3 min. at 100° , and filtering. 60 cc. of the filtrate were taken for determination of sugar. Peptone solutions (Poulenc) were prepared in an analogous manner; their p_H was 4.83. Gelatin (Coignet Père et Fils) was dissolved in water at 90° ; the solutions had p_H 5.89.

A. Enzymic hydrolysis of starch at varying p_H .

To a number of 200 cc. flasks 15 cc. of 3 % starch, 15 cc. of the appropriate buffer solutions, 1 cc. of 0.1 % amylase and 29 cc. of water were added. The flasks were then left for 1 hour at 30° , and maltose was determined. The results (Table I) indicate that the optimal p_H is, under the given conditions, 4-4.5. In more acid solutions the activity of amylase rapidly falls, being practically zero at p_H 3.0.

Table I. Enzymic hydrolysis of starch at varying p_H *.

p_H	mg. maltose	p_H	mg. maltose
3.0	5.1	6.0	59.5
3.5	57.4	6.5	54.1
4.0	65.8	7.0	48.2
4.5	64.0	7.5	37.5
5.0	62.2	8.0	25.3
5.5	59.8	—	—

* Results in all Tables are the mean of 4 observations.

B. Action of amylase in the presence of ethyl alcohol.

1. *Velocity of reaction in various concentrations of alcohol.* A series of flasks was prepared containing 30 cc. of 1.5 % starch, 10 cc. of buffer solutions p_H 5.6, 18 cc. of aqueous alcohol, so as to obtain concentrations of 1, 5, 10, 15 and 20 % of alcohol in the whole system, and 2 cc. of 0.1 % amylase. The systems containing 15 and 20 % of alcohol were turbid, owing to partial precipitation of starch. All flasks were kept 2 hours at 30° . The results (Table II) show that percentage retardation of reaction is directly proportional to the concentration of alcohol.

Table II. Influence of conc. of alcohol on amylolysis at p_H 5.6.

% alcohol	mg. maltose	% retardation
0	89.6	—
1	87.5	2.3
5	83.5	6.8
10	78.3	12.6
15	71.2	20.5
20	64.6	27.9

2. *Velocity of reaction in 5 % alcohol at varying p_H .* A number of flasks were prepared, containing 15 cc. of 3 % starch, 15 cc. of buffer solutions, 3.125 cc. of alcohol, 25.875 cc. of water, and 1 cc. of 0.1 % amylase. The systems were left 1 hour at 30° . The results (Table III) are similar to those

obtained in the absence of alcohol, maximum reaction taking place at p_H 4.5. Reaction is retarded at p_H above 4.5 by alcohol to an extent increasing with p_H .

Table III. *Amylolysis in 5 % alcohol at varying p_H .*

p_H	mg. maltose		% retardation due to alcohol
	With alcohol	Without alcohol	
3.5	51.5	52.8	2.6
4.5	58.4	60.6	3.7
6.0	48.5	55.3	12.5
7.5	23.2	32.3	28.7

3. *Reaction in alcoholic media with varying concentrations of starch.* Two series of flasks were prepared, one containing 0.5 and the other 1.5 % starch; in both series alcohol was present in concentrations of 0, 1, 5, 10, 15 and 20 %. The systems, consisting of 30 cc. of starch, 10 cc. of buffer solution (p_H 6.5), 19 cc. of aqueous alcohol or water, and 1 cc. of 0.1 % amylase, were kept at 30° for 2 hours. The results (Table IV) indicate that percentage retardation of reaction due to alcohol is practically independent of the concentration of starch taken. At the same time the yield of maltose is about 30 % greater using 1.5 than 0.5 % starch, both in the presence and absence of alcohol.

Table IV. *Amylolysis at various concentrations of alcohol and starch.*

% alcohol	0.5 % starch		1.5 % starch	
	mg. maltose	% retardation	mg. maltose	% retardation
0	41.0	—	54.7	—
1	39.5	3.6	53.8	1.6
5	36.2	11.7	51.1	6.5
10	34.7	15.3	47.1	13.9
15	31.9	22.2	41.7	23.7
20	28.7	30.0	35.4	35.2

4. *Comparison with dextrin.* The retarding action of alcohol may be due to partial precipitation of starch. Should this be the case, the retarding action of alcohol should be absent from systems containing dextrin, which is not precipitated by the concentration of alcohol used. The following experiment shows that this is not the case. A number of flasks each containing 30 cc. of 1.5 % starch or dextrin, 10 cc. of buffer solution (p_H 6.5), 12.5 cc. of 96 % alcohol or water, 5.5 cc. of water and 2 cc. of 0.1 % amylase were left 2 hours at 30°, after which maltose was determined. Since the dextrin used contained some reducing sugars, the value found for systems such as the above to which 2 cc. of water had been added in place of amylase, was deducted from the reducing sugar content of the above systems. The results obtained were substantially the same for starch as for dextrin; thus 86.5 mg. of maltose were produced from starch in the absence of alcohol and 62.9 mg. in its presence, a retardation of 26.8 %. The corresponding values for dextrin are 37.0 and 27.6 mg., and the retardation is 24.3 %.

5. *Action of alcohol on amylase.* 0.1 g. of amylase and 0.1 g. of NaCl were dissolved in 100 cc. of 20 % alcohol, and kept 1 hour at 30°, together with a

similar aqueous solution. A number of systems, containing 30 cc. of 1.5 % starch, 10 cc. of buffer solution (p_H 6.5), 12.5 cc. of alcohol or water, 2.5 cc. of water, and 5 cc. of aqueous or 20 % alcoholic amylase solution, were kept 1 hour at 30°, and reducing sugar was determined. The activity of amylase previously subjected to the action of alcohol was smaller than was that of amylase dissolved in water, in both alcoholic and aqueous solutions (81.5 and 84.7 mg. of maltose produced respectively, as compared with 109 and 111.5 mg. in the presence of aqueous amylase).

Four solutions of amylase were now prepared, as follows:

Amylase I. 0.4 g. of amylase and 0.4 g. of NaCl in 100 cc. of 20 % alcohol;

Amylase II. The same, in 5 % alcohol;

Amylase III. The same, in 1 % alcohol;

Amylase IV. The same, in water.

These 4 solutions were kept for 22 hours at 30°. Solutions I and II remained clear, whilst solutions III and IV contained a flocculent precipitate. Four series of flasks were prepared, each containing 30 cc. of 1.5 % starch, 10 cc. of buffer solution (p_H 6.5), 19.5 cc. of water, and 0.5 cc. of amylase solution I, II, III, or IV. The maltose produced after 1 hour at 30° was 89.5 mg. by amylase I, 85 mg. by amylase II, 88.3 by amylase III, and 74.6 mg. by amylase IV. Alcohol therefore inhibits the inactivation of amylase in solution.

Three volumes of water were now added to amylase I, II and IV, yielding amylase I' (0.1 %, in 5 % alcohol), II' (0.1 %, in 1.25 % alcohol), and IV' (0.1 % in water). These solutions were kept 2 hours at 30°, and 2 cc. of each were added to similar systems as in the preceding experiment. Maltose determined after 2 hours at 30°, amounted to 74.6 mg. with amylase I', 66.6 mg. with amylase II', and 59.2 mg. with amylase IV'.

The same experiment was repeated with amylase solutions I', II' and IV', kept a further 24 hours at 30° before addition to the systems, the maltose after 3 hours at 30° now being respectively 102, 68.1, and 50.1 mg.

It appears that the protective influence of alcohol on amylase becomes accentuated with duration of contact, the relative acceleration of action of amylase in 5 % alcohol amounting after 22 hours to 15 %, after 24 hours to 20 %, and after 48 hours to 100 %.

C. *Action of amylase in the presence of proteins and of their derivatives.*

1. *Influence of ovalbumin at varying p_H .* The action of amylase was examined in freshly prepared systems containing ovalbumin buffered to p_H 3.5, 4.5, 6 and 7.5. A number of 200 cc. flasks were prepared, containing 15 cc. each of 3 % starch, buffer solution, and 6 % ovalbumin solution or water, 14 cc. of water and 1 cc. of 0.1 % amylase. All systems were kept 1 hour at 30°, after which 20 cc. of Bertrand's solution I were added to the control flasks in order to interrupt the reaction, whilst 30 cc. of Hagedorn-Jensen solution

were added to the flasks containing albumin, which was then coagulated by the method described above. The systems were filtered, and 60 cc. of the filtrates were taken for determination. Maltose was determined by Bertrand's method, and the result was multiplied by 1.5 in order to obtain the value for the whole system. The results (Table V) indicate that the action of ovalbumin is a function of p_H ; thus at p_H 3.5 ovalbumin retards reaction by about 5 %, whilst at higher p_H it accelerates reaction progressively to 43.4 % at p_H 7.5.

Table V. *Action of ovalbumin at varying p_H .*

p_H	mg. maltose produced		% acceleration
	With protein	Without protein	
3.5	50.3	52.8	-4.9
4.5	63.8	60.6	+5.3
6.0	61.7	55.1	+11.9
7.5	46.1	32.1	+43.4

2. *Influence of ovalbumin in 5 % alcohol.* Similar systems to those described in the preceding experiment were set up, with the difference that 3.125 cc. of alcohol were added in place of a similar volume of water, so that these systems contained 5 % of alcohol. The p_H , temperature and duration of reaction were as before. The results (Table VI) were of the same order as in the absence of alcohol; the absolute yield of maltose was, however, smaller in all cases (retardation due to alcohol), whilst the retarding or accelerating influence of ovalbumin was relatively greater (12.3 % retardation at p_H 3.5, and 77 % acceleration at p_H 7.5).

Table VI. *Action of ovalbumin at varying p_H in 5 % alcohol.*

p_H	mg. maltose produced		% acceleration
	With protein	Without protein	
3.5	45.1	51.5	-12.3
4.5	63.1	58.4	+8.1
6.0	56.4	48.5	+16.3
7.5	41.8	23.2	+77.0

3. *Influence of gelatin at varying p_H .* A number of flasks were prepared, containing 15 cc. each of 3 % starch, phosphate buffers, and 4 % gelatin solution or water, 14 cc. of water and 1 cc. of 0.1 % amylase. The systems were buffered to p_H 5, 6, 7 and 8, and were kept 1 hour at 30°, after which maltose was determined as usual. The results obtained (Table VII) are not of the same

Table VII. *Action of gelatin at varying p_H .*

p_H	mg. maltose produced		% acceleration
	With gelatin	Without gelatin	
5	59.0	54.9	7.3
	80.2	77.9	3.0
6	65.6	59.3	10.8
7	49.3	38.4	29.0
	47.0	41.9	12.7
8	40.0	28.8	60.1

order of accuracy as in other experiments, owing to the unreliable values given by Bertrand's method in the presence of gelatin, and to the circumstance that experiments at each p_H were conducted separately. It is, however, obvious that gelatin accelerates reaction increasingly from p_H 5 to 8.

4. *Influence of gelatin in 20 % alcohol.* The systems consisted of 15 cc. each of 3 % starch, phosphate or acetate buffers, and 4 % gelatin or water, 12.5 cc. of alcohol, 1.5 cc. of water and 1 cc. of 0.1 % amylase. The systems were at p_H 3.5, 5, 5.8, 6.5 and 7, and were maintained at 30° for 1 hour. As in the preceding experiment, each value is strictly valid only for the corresponding control. Gelatin acts similarly in alcoholic and aqueous systems, its effect being however more marked in the former. In particular, very considerable retardation (64.6 %) is observed at p_H 3.5 (Table VIII), whilst at higher p_H acceleration increases from about 10 % at p_H 5 to about 70 % at p_H 7.

Table VIII. *Action of gelatin at varying p_H in 20 % alcohol.*

p_H	mg. maltose produced		% acceleration
	With gelatin	Without gelatin	
3.5	13.6	38.7	- 64.6
5.0	48.0	41.8	+ 13.3
5.8	47.7	37.6	+ 26.8
6.5	46.4	29.5	+ 57.3
7.0	30.0	17.2	+ 72.6

5. *Influence of peptone.* A number of systems consisting of 15 cc. each of 3 % starch, phosphate buffers to p_H 4.5, and 4 % Witte's peptone or water, 14 cc. of water and 1 cc. of 0.1 % amylase were kept 1 hour at 30°, and maltose was determined as usual. A mean value of 66.0 mg. was obtained in the presence of peptone, and of 60.3 mg. in its absence, representing an acceleration of 9.3 % due to peptone.

6. *Influence of glycine.* In the above experiments the effect on the action of amylase of addition of colloidal proteins or protein derivatives has been studied; the effects obtained have been ascribed by certain authors to protective colloidal action. In order to ascertain to what extent this view is justified, experiments were performed on the action of crystalloid protein derivatives.

(a) *Glycine.* A number of systems containing 15 cc. each of 3 % starch, buffer solutions, and 4 or 8 % glycine solution or water, 14 cc. of water and 1 cc. of 0.1 % amylase were kept 1 hour at 30° after which maltose was determined. The concentration of glycine in the systems was 0.133 *M* and 0.266 *M*. The results (Table IX) indicate that glycine has a similar action to that of the colloidal protein derivatives studied previously, and that the effect obtained increases with the concentration of glycine.

That the action of glycine is not exerted directly on the substrate was shown by experiments in which similar systems to the above, but without amylase, were taken; no production of maltose took place.

Table IX. *Action of glycine at varying p_H .*

Systems as in Table V, except that 4 or 8 % glycine is taken in place of 6 % ovalbumin.

p_H	1 % glycine			2 % glycine		
	mg. maltose produced		% acceleration	mg. maltose produced		% acceleration
	With glycine	Without glycine		With glycine	Without glycine	
3.5	51.1	54.2	- 6.4	—	—	—
4.5	69.4	61.0	+13.8	65.2	50.8	+28.4
6.0	65.5	54.3	+21.0	63.0	45.4	+38.8
7.5	48.3	35.1	+38.0	48.4	30.4	+59.2

(b) *Influence of temperature on action of glycine.* Systems of the same composition as in the above experiments, and at p_H 3.5, 4.5, 6 and 7.5, were studied at 18, 30, 38 and 50°. The time of reaction at 18° was 4 hours, and the yields of maltose were divided by 4 in order to obtain comparative results with systems kept 1 hour at 30, 38 and 50°. The results obtained (Table X) indicate that the absolute velocity of reaction increases up to 50°; at 60° the activity of amylase is smaller than at 50°. This is in accordance with Chrzyszcz's finding [1923] that at the optimal p_H amylase exhibits maximal activity at 55°. At lower p_H values the velocity of reaction appears to be independent of temperature. At higher p_H the accelerating effect due to glycine increases with temperature.

7. *Influence of sodium aspartate.* Similar experiments to those with glycine were performed, substituting 15 cc. of 9.4 % sodium aspartate for the same volume of 4 % glycine, so that the molar concentration (0.133) was the same as for glycine. At p_H 4.5, 97.6 mg. of maltose were produced in the presence of sodium aspartate, and 71.0 mg. in its absence, an acceleration of 37.4 %,

Table X. *Influence of temperature on enzymic hydrolysis of starch in the presence of glycine.*

Systems as in Table V, except that 4 % glycine is taken in place of 6 % ovalbumin. Time of reaction: 1 hour.

Temperature	p_H	mg. maltose produced		% acceleration
		With glycine	Without glycine	
18°	3.5	19.0	20.3	- 6.4
	4.5	28.0	23.6	+14.4
	6.0	24.2	20.0	+21.0
	7.5	17.6	12.6	+39.7
30°	3.5	54.9	58.5	- 6.2
	4.5	74.6	65.7	+13.7
	6.0	69.4	56.7	+22.5
	7.5	53.6	38.6	+38.8
38°	3.5	60.9	65.8	- 7.4
	4.5	89.5	78.5	+14.0
	6.0	84.1	66.9	+25.7
	7.5	64.6	35.5	+82.0
50°	4.5	125.0	107.0	+16.8
	7.5	81.0	31.0	+161.0

whilst at p_H 6.0 the corresponding figures are 63.8 and 51.0 mg., and 21.8 % acceleration.

D. *The active group of amino-acids.*

1. *The carboxylic group.* Sherman and co-workers [1927], who investigated the influence of acids on the action of pancreatic amylase, found that at the optimal p_H the presence of acetic, phosphoric, boric and citric acids did not affect the velocity of hydrolysis. The effects of addition of oxalic and acetic acids will now be described. Systems containing 15 cc. each of 3 % starch, phosphate buffer p_H 7.5, and 9.36 % potassium oxalate solution or water, 14 cc. of water and 1 cc. of 0.1 % amylase were kept 1 hour at 30°, and reducing sugar was determined. Oxalate retards reaction under these conditions by about 8 %, the yield of maltose being 45.2 mg. in the absence of oxalic acid, and 41.6 mg. in its presence. The molar concentration of potassium oxalate was 0.133.

The concentration of sodium acetate taken in similar systems was 0.266 M , so that the concentration of carboxylic groups was the same in both cases. Acetate produces a relatively greater retardation of reaction, viz. 29 %, the yield of maltose falling from 45.2 to 32.1 mg.

2. *The amino-group.* The influence of amino-groups has been investigated by Caujolle and Molinier [1930], who found that at 50° aliphatic amines inactivated pancreatic, salivary and malt amylases. The hydrochlorides of these amines, on the other hand, activated pancreatic and salivary amylases, but were without effect on malt amylase. These authors do not, however, state whether the p_H of their systems was affected by the addition of amines, so that it is possible that the effects observed were due to change in p_H . Sherman and Naylor [1922] also report an inactivation of amylase by aliphatic and aromatic amines.

(a) *Guanidine carbonate.* This was taken at concentrations of 0.013 and 0.133 M , in systems consisting of 15 cc. of 3 % starch, 15 cc. of hydrochloric acid solution, 7.5 cc. of 0.54 or 5.4 % guanidine carbonate solution or water, 21.5 cc. of water and 1 cc. of 0.1 % amylase solution. The p_H of the systems was adjusted to 4.5 and 6 by addition of hydrochloric acid of the requisite strength, and maltose was determined after 1 hour at 30°. The yield of maltose in the control systems was 76 mg., whilst in the presence of guanidine only traces of sugar were found.

(b) *Diethylamine.* Diethylamine in concentrations of 0.133 M similarly totally inhibits reaction at p_H 7.5.

(c) *Asparagine.* Asparagine (1 %) in similar systems totally inhibits reaction at p_H 4.5.

(d) *Methylamine hydrochloride.* This salt (0.133 M) totally inhibits reaction at p_H 7.5, whilst at p_H 4.5 the yield of maltose is 58.0 mg. in its presence, as compared with 71.1 mg. in its absence, a retardation of 18.4 %. Sørensen's phosphate buffers were used in these experiments.

(e) *Urea*. 0.133 *M* urea has only a small retarding influence, the yield of maltose at p_H 4.5 being 71.0 mg. in its presence, and 75 mg. in its absence, a retardation of 5.4 %.

(f) *Choline hydrochloride*. This salt, at concentrations of 0.133 *M*, exerts a slight activating influence, the yield of maltose being 63.6 mg. in its presence, and 61.0 mg. in its absence, an activation of 4.3 %.

DISCUSSION.

The above results indicate that proteins and their derivatives are capable of exerting a profound influence on the velocity of enzymic hydrolysis of starch, to an extent dependent on the p_H of the medium. At p_H lower than the optimal (p_H 4.5) the reaction is in all cases retarded, whilst at and above p_H 4.5 it is accelerated to an extent proportional to the alkalinity of the system.

These results appear to be connected with Przyłęcki and Majmin's [1931] work on the combination of proteins and their derivatives with starch. These authors found that the stability of such complexes varied inversely with the p_H of the medium, combination being irreversible in acid solutions. The starch in such complexes is possibly more resistant to the action of amylase than is free or loosely combined starch, and this might explain the inhibitory action of the substances examined in systems at p_H 3.5.

Activation of reaction at higher p_H may be due to activation of the enzyme or of the substrate, or to protection of the enzyme from some anticatalyst formed during the reaction; which of these factors is actually responsible for the effect observed it is not possible, on the basis of the experimental material available, to state. Since, however, the action of protein substances on the enzyme itself has not been examined, these explanations must be regarded as being purely speculative.

The magnitude of the effect observed appears to be proportional to the active concentration of amino-acid units present, as is shown by Table XI.

Table XI.

Substance	% concentration	p_H	% acceleration
Ovalbumin... ..	1.5	4.5	5.3
Gelatin	1.0	5.0	6.6
Peptone	1.0	4.5	9.0
Glycine 0.133 <i>M</i>	1.0	4.5	13.8
Glycine 0.266 <i>M</i>	2.0	4.5	28.4
Sodium aspartate 0.133 <i>M</i>	2.3	4.5	37.4

Percentage acceleration is twice as great at p_H 4.5 in presence of 2 % as in that of 1 % glycine. At other p_H the results are qualitatively of the same order, but the proportionality between concentration and activation is not so exact.

The action of amino-acid groups is not due to either the amino- or the carboxyl groups taken separately; the addition of carboxylic acids is either without effect or exerts a retarding action, whilst amines inhibit reaction to an

extent proportional to the basic character of the amino-group. These findings are in accordance with those of earlier workers in this field.

The action of proteins and of their derivatives appears to be due to their amino-acid units, and not, as has been supposed by certain authors, to their protective colloidal character. The results obtained possess a considerable biological significance, in that they indicate that the action of amylase within the living organism will be modified by the protein substances which are present in abundance, and this applies in particular to its action at varying p_H . Thus, in the intestinal digestion of carbohydrates it would follow that the velocity of hydrolysis should be greater when proteins are simultaneously undergoing digestion than when these are absent.

In the presence of ethyl alcohol, retardation of reaction is observed both in the presence and in the absence of protein substances, and this retardation is, at a given concentration of starch, roughly proportional to the concentration of alcohol, and is greater in alkaline than in acid media. On the whole, the action of alcohol is greater in more dilute than in more concentrated solutions of starch.

Were the inhibitory action of alcohol to be due to denaturation of the enzyme, it might be expected that this effect would be obtained in quite low concentrations of alcohol, as the concentration of enzyme is small. Experiment showed, however, that this is not the case, as the activity of the enzyme was little affected by 1 hour's contact with 20 % alcohol, and after 48 hours of contact alcoholic enzyme solutions were more active than were similar aqueous solutions.

The inhibitory action of alcohol may be due to some extent to diminution in the degree of dispersion of starch, solutions of which exhibit a marked turbidity on the addition of alcohol. This explanation would agree well with the observed proportionality between the concentration of alcohol and the percentage inhibition of reaction, as well as with the observation that the yield of maltose, at the same concentration of enzyme, is proportional to the concentration of the substrate only with small concentrations of starch. On the other hand, experiments with dextrin show that a practically identical inhibition of reaction is obtained as with starch, in spite of the fact that dextrin is aggregated by alcohol to a far less extent.

The possibility remains that alcohol acts on the enzyme-substrate complex, by eluting particles of starch from the surface of the enzyme, which is consequently isolated from contact with the substrate to a greater or less extent.

The effect of the addition of protein to systems containing alcohol is of the same order as in non-alcoholic systems, but is numerically greater, both at p_H at which protein retards and at which it accelerates reaction. The absolute values are, however, in all cases lower than in the absence of alcohol.

SUMMARY.

1. Ethyl alcohol retards the reaction of hydrolysis of starch by malt diastase to an extent proportional at p_H 5.6 to the concentration of alcohol. The optimal p_H in the presence of alcohol is the same as in its absence, viz. 4.5. Percentage retardation of reaction due to alcohol is greater the more the p_H of the system exceeds 4.5.

2. Similar results are obtained taking dextrin in place of starch.

3. The activity of 20 % alcoholic amylase solutions is greater than is that of similar solutions not containing alcohol, after keeping up to 48 hours.

4. Ovalbumin, gelatin, peptone, glycine and aspartic acid retard at p_H below the optimal (p_H 4.5), and accelerate reaction at p_H higher than 4.5, to an extent increasing with the alkalinity of the medium. In the case of glycine, the relative acceleration is greater the higher the temperature.

5. The action of protein substances is not due either to the carboxylic or to the amino-group separately, as both carboxylic acids and amines retard the reaction.

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