LXIV. STRUCTURE AND ENZYME REACTIONS.

XI. ACTION OF SALTS IN THE SYSTEMS: STARCH—AMYLASE—PROTEINS.

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IN Part X of this series [Chrempińska, 1931] the effect of the addition of electrolytes to systems containing protein, starch and amylase of vegetable origin was studied. The action of the enzyme was examined by determining the amount of residual starch, whilst in the present paper the same problem was studied, determining reducing sugars produced in the presence of taka-diastase and of ptyalin.

EXPERIMENTAL.

Materials used.

Enzyme. Merck's "Diastase absolut" (0.03–0.1 % solutions in 1 % NaCl.). Ptyalin: 10 cc. of filtered saliva diluted to 50 cc. with water or 1 % NaCl.

Substrate. Merck's sol. starch, solutions of which were prepared as in Part X.

Protein. Fresh or heat-coagulated egg-white was taken.

METHODS.

The p_H of solutions was adjusted by addition of 0.1 *N* HCl or NaOH. The reaction temperature was 25°. Solutions were freed from protein by adding 10 cc. of Hagedorn-Jensen reagent (40 cc. 4.5 % $ZnSO_4$, 80 cc. *N* NaOH, and 120 cc. H_2O) to 20 cc. of the solution under examination. Starch is unaffected by this reagent, and maltose can be determined in the filtrate by Bertrand's method with fair accuracy (this was tested by heating 20 cc. of 0.2 % maltose (40 mg.) for 6 minutes with 10 cc. of reagent, and determining maltose in the filtrate; 39.1 and 39.4 mg. were found). In view of the fact that reducing substances pass from egg-white or ovalbumin into the solution during the reaction, a control system containing the same constituents as the experimental systems, but with water in place of amylase, was taken in all cases, and its apparent maltose content was deducted from the values obtained for the experimental systems. All results are the mean of 4–6 determinations.

RESULTS.

A. *Taka-diastase*.

(1) p_H 8.85. Mixtures of 5 cc. of egg-white, 10 cc. of 1 % starch, and 4 cc. of water or of solutions of NaCl, Na_2SO_4 or $CaCl_2$ were thoroughly shaken, and kept 15 minutes at 25°, after which 1 cc. of water or of 0.1 % amylase in 1 % NaCl solution was added, and the reducing sugar content was determined after 4.3 hours at 25°. The results (Table I) indicate that reaction is accelerated by

Table I. *Influence of $CaCl_2$ or NaCl on the action of taka-diastase.*

Salt taken	Without NaCl or KCl							
	p_H 8.85 time 4.3 hr.		p_H 8.1 time 3.75 hr.		p_H 6.5 time 1.5 hr.		p_H 4.7 time 1 hr.	
	% accelera- tion		% accelera- tion		% accelera- tion		% accelera- tion	
	mg. maltose		mg. maltose		mg. maltose		mg. maltose	
—	4.33	—	32.6	—	31.25	—	33.3	—
0.005 <i>M</i> $CaCl_2$	7.77	79.4	33.3	2.1	30.35	—	32.1	-3.6
0.01 <i>M</i> $CaCl_2$	19.37	347.3	35.6	9.1	31.0	—	29.7	-10.8
0.02 <i>M</i> $CaCl_2$	25.18	481.3	38.3	17.5	31.45	—	27.25	-18.2
0.05 <i>M</i> $CaCl_2$	32.94	660.7	39.25	20.4	31.15	—	25.8	-22.5
—	4.15	—	32.6	—	31.25	—	33.3	—
0.01 <i>M</i> NaCl	5.67	36.6	33.58	3.0	32.72	4.7	34.10	+2.4
0.02 <i>M</i> NaCl	8.53	105.6	36.0	10.3	34.0	8.7	35.14	+5.5
0.05 <i>M</i> NaCl	12.28	196.0	36.76	12.8	34.9	11.6	35.6	+6.9
0.1 <i>M</i> NaCl	16.05	287.0	37.2	14.1	35.1	12.3	36.25	+8.9

	With NaCl				With KCl			
	p_H 8.85 time 6.75 hr.		p_H 8.1 time 2.5 hr.		p_H 8.85 time 7 hr.		p_H 8.1 time 2.5 hr.	
	% accelera- tion		% retarda- tion		% accelera- tion		% retarda- tion	
	mg. maltose		mg. maltose		mg. maltose		mg. maltose	
—	8.1	—	31.35	—	7.5	—	31.7	—
0.005 <i>M</i> $CaCl_2$	11.48	41.7	30.0	4.2	10.5	40.0	30.85	2.7
0.01 <i>M</i> $CaCl_2$	17.54	116.5	29.8	5.0	17.15	129.0	29.45	7.1
0.02 <i>M</i> $CaCl_2$	31.06	283.5	28.65	8.6	30.15	302.0	27.6	12.9
0.05 <i>M</i> $CaCl_2$	35.23	335.0	27.47	12.4	32.85	338.0	25.77	18.7

from 79.4 % with 0.005 *M* $CaCl_2$ to 660.7 % with 0.05 *M* $CaCl_2$. The p_H of the systems containing 0.05 *M* $CaCl_2$ was reduced to 8.1. Na_2SO_4 has no influence on the action of Merck's amylase at all p_H values studied (4.7, 6.5, 8.1 and 8.85), and at concentrations of 0–0.5 *M*. The activating effect of NaCl is less marked than is that of $CaCl_2$, viz. from 36.6 to 287 % acceleration with 0.01 to 0.1 *M* NaCl, the p_H being reduced in 0.1 *M* NaCl to 8.8.

(2) p_H 8.1. Similar experiments were performed on systems at p_H 8.1, reducing the time of reaction to 3.75 hours. The action of $CaCl_2$ is much less at this p_H , acceleration varying from 2.1 % with 0.005 *M* $CaCl_2$ to 20.4 % with 0.05 *M* $CaCl_2$; the p_H of the systems was reduced to 7.85 by 0.05 *M* $CaCl_2$. The action of NaCl is similarly smaller, varying from 3 to 14 % over the same range of concentrations.

(3) p_H 6.5. At this p_H CaCl_2 has no appreciable action. With NaCl, an acceleration of 4.7 to 12.3 % is obtained over the range 0–0.1 M NaCl.

(4) p_H 4.7. The time of reaction was in this case 1 hour. Reaction is retarded by 3.6 % with 0.005 M CaCl_2 and by 22.5 % with 0.05 M CaCl_2 ; with NaCl an acceleration of 2.4 to 8.9 % was observed.

B. Action of CaCl_2 in the presence of NaCl or KCl.

(1) p_H 8.85. Na_2SO_4 has no influence on the velocity of reaction in the presence of 0.9 % NaCl or KCl. The acceleration due to CaCl_2 is much smaller in the presence of these salts than in their absence (Table I), varying from 41.7 to 335 % in the case of NaCl, and from 40.0 to 338 % in that of KCl.

(2) p_H 8.1. CaCl_2 exerts an inhibitory action at this p_H , which varies from 4.2 to 12.4 % in the presence of NaCl, and from 2.7 to 18.7 % in that of KCl.

C. Salivary amylase.

A number of systems at p_H 8.1 were set up, containing 5 cc. of egg-white, 10 cc. of 1 % starch, 4.5 cc. of water, CaCl_2 or NaCl solution and 0.5 cc. of a 20 % solution of filtered saliva or water, and maltose was determined after 1 hour at 22°. The results (Table II) show that the reaction is retarded by

Table II. Influence of CaCl_2 or NaCl on the action of salivary amylase at p_H 8.1.

Salt	Without NaCl or KCl		With NaCl		With KCl	
	mg.	% retarda- tion	mg.	% retarda- tion	mg.	% retarda- tion
—	44.0	—	43.05	—	48.65	—
0.005 M CaCl_2	42.85	2.6	41.95	2.55	47.12	3.15
0.01 M CaCl_2	41.72	5.2	40.32	6.34	45.63	6.20
0.02 M CaCl_2	39.94	11.5	39.07	9.25	43.62	10.34
0.05 M CaCl_2	36.30	17.5	38.12	11.45	41.81	14.06
0.01 M NaCl	47.30	— 7.5				
0.02 M NaCl	49.60	— 12.7				
0.05 M NaCl	47.55	— 8.1				
0.1 M NaCl	41.15	+ 6.5				

Time of reaction 1 hr.

CaCl_2 , both in the presence and absence of NaCl or KCl. The percentage retardation over the range 0.005–0.05 M CaCl_2 varies from 2.6 to 17.5, with CaCl_2 and NaCl from 2.55 to 11.45, and with CaCl_2 and KCl from 3.15 to 14.06. NaCl accelerates reaction in concentrations of less than 0.05 M (maximal acceleration of 12.7 % with 0.02 M NaCl), and retards reaction in concentrations of 0.1 M (6.5 % retardation).

The action of CaCl_2 in systems containing egg-white in solution and heat-coagulated was next studied. The systems contained 5 cc. of egg-white, 10 cc. of starch, 9 cc. of water or CaCl_2 solution, and 1 cc. of 20 % saliva. The concentration of CaCl_2 was 0.05 M in the whole system. Maltose was determined after 30 minutes at 22°; 55 mg. were found in the absence of protein, 57.7 and 58.8 mg. respectively in the presence of soluble and of heat-coagulated egg-

white; in the presence of 0.05 *M* CaCl₂ the latter values fell to 49.3 and 49.5 mg. respectively, corresponding to retardations of 14.56 and 15.82 %. These results indicate that the action of CaCl₂ is the same irrespective of the state of dispersion of the protein.

DISCUSSION.

Of the three salts studied, Na₂SO₄ is without action at all the *p_H* values and concentrations taken, whilst NaCl and CaCl₂ act differently according to the type of amylase taken, to the *p_H*, and to their concentration. In the case of taka-diaxase, considerable activation was observed with CaCl₂ at *p_H* 8.85; this effect was considerably weaker in the presence of NaCl or KCl. At *p_H* 8.1 the action of CaCl₂ was also accelerative, although the percentage acceleration amounted to only 20, as compared with 660 at *p_H* 8.85; at *p_H* 6.5 the presence of CaCl₂ had no effect on the velocity, whilst at *p_H* 4.7 the reaction was retarded, as was also the case at *p_H* 8.1 in the presence of NaCl or KCl. NaCl accelerated reaction at all *p_H* values taken; at *p_H* 8.85 and 8.1 its action was, however, much feebler than that of CaCl₂. With salivary amylase its action may be accelerating or retarding according to its concentration.

A comparison of these results with those of Chrempieńska shows that the augmented production of reducing sugars in the presence of CaCl₂ at *p_H* 8.85 is relatively much greater than is the augmented hydrolysis of starch under analogous conditions.

The accelerating action of CaCl₂ is in all probability due to reduction of the *p_H* of the medium where this is higher than the optimal, whilst retardation of reaction in acid media might be ascribed to the action of CaCl₂ on the enzyme. As was shown by Giedroyc and Przyłęcki [1931] the reduction of the *p_H* of alkaline solutions by CaCl₂ may be quite considerable (in this case from *p_H* 8.85 to 8.1), and, as will be shown in a succeeding paper, the production of reducing sugars is relatively considerably more increased than is that of non-reducing dextrans.

With salivary amylase the action of CaCl₂ is one of retardation, in the presence of either soluble or heat-coagulated egg-white.

The above observations on the whole indicate that the formation of compounds between the enzyme and substrate is not, at least in the presence of high concentrations of enzyme, of any great importance. As to the rôle of structure, this would probably only come into account in cases in which parts of the system containing enzyme are mechanically separated from others containing substrate, either by occlusion or by the interposition of difficultly permeable partitions.

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