29. On Amylosynthase.¹⁾ III.

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(1) *Reactivation of amylosynthase.*

Amylosynthase is completely precipitated from its aqueous solution by adding cadmium chloride to the extent of M/160.

When the yellowish white precipitate thus produced is suspended in water and saturated with ammonium sulphate, the enzyme can be obtained as a white insoluble residue, which, when washed with glycerine and dissolved in water, gives clear solution having strong polymerizing power.

Amylase is neither inhibited nor precipitated by cadmium chloride at the same concentration.

Amylosynthase is also precipitated by M/200 solution of mercuric chloride, but it can never be reactivated by any treatment, while amylase inactivated by the same reagent can be easily recovered by treating the precipitate with hydrogen sulphide.

Amylosynthase inactivated by acid, by alkali or by heat never recovers its activity by either neutralization or cooling.

We see thus, that there is a remarkable difference between amylase and amylosynthase in the behavior towards enzyme poisons and other reagents.

(2) *Isolation of amylosynthase from polished glutinous rice.*

Polished glutinous rice is finely powdered, macerated with 3-4 volumes of cold water, filtered through cloth and then through paper.

When the filtrate thus obtained is treated with the M/100-M/160 solution of cadmium chloride, a yellowish white precipitate is produced which carries down amylosynthase leaving amylase in solution. The precipitate is now suspended in water and saturated with ammonium sulphate, whereby an active amylosynthase is obtained.

This preparation has the same properties with those of the amylosynthase prepared from potato and non-glutinous rice. Its activity is com-

1) Proc. **7** (1931), 258-260. **8** (1932), 244-246.

pletely destroyed by heating at 50°C for 10 minutes. The optimum temperature lies between 20–25°C. The activity is also retarded by the addition of buffer solution, while this is not the case with yeast amylosynthase.

(3) *Action of pancreatic tryptase on amylosynthase.*

When 1–2% aqueous solution of pancreatine (Japanese Pharmacopoea) is added with ammonium sulphate up to 24–25%, a white flocculent precipitate is obtained. This precipitate is collected on a filter, dissolved in 30–50 times of water after drying in vacuum and treated with acetone up to 80%, whereby tryptase is again precipitated. By repeating this process, tryptase quite free from amylase can be obtained.

Now, on adding this tryptase to the solution of amylosynthase, the power of polymerising dextrine gradually decreases and finally the activity is completely lost when a definite quantity of the tryptase is added.

From this inactivated solution, the both enzymes can be fractionally precipitated by ammonium sulphate, i.e. by adding the latter up to 24–25% the tryptase is completely thrown down, while the active amylosynthase is precipitated from the filtrate by saturating with the same salt.

From such an experiment, it can be presumed that the amylosynthase is not of protein nature, although it is easily precipitated by ammonium sulphate in its impure state.

(4) *Polymerisation of glycogen.*

The glycogen used in this experiment, is prepared by digesting commercially purified glycogen with cold 7.5% hydrochloric acid and precipitating with alcohol after 2 or 3 days. The preparation thus obtained dissolves clearly in water and gives a brownish red colour with iodine. Now by adding a little amylosynthase to the above solution, the polymerisation proceeds at once and the colour changes to red, violet and finally to blue in the course of time.

It is interesting to note that genuine glycogen which can scarcely be acted by amylosynthase, becomes polymerisable after treating it with cold hydrochloric acid as above stated.

The degradation products of glycogen, obtained by the action of amylase can also be polymerised.

(5) *Polymerisation of dextrine.*

Soluble starches are prepared by treating the starches of the glutinous varieties of true millet (*Panicum miliaceum*) and foxtail millet

(*Setaria italica*) with cold 7.5% hydrochloric acid solution. The aqueous solutions of these samples give, like that of glutinous rice, a violet and afterwards bluish violet colour with iodine after being added with amylosynthase.

It is interesting that the polymerised products both from millets and from rice form insoluble precipitates, which resemble closely each other in their properties.

The author has also observed that the dextrine prepared from potato starch by amylase can easily be polymerised by amylosynthase, while those prepared by simply heating or by treating with acids can not be polymerised. So, it seems that these dextrines are chemically different, though the both give red colour with iodine.

(6) *Properties of synthetic starches.*

The author has prepared 4 kinds of synthetic starches from different sources by amylosynthase, i.e. from soluble starches of glutinous rice and millets (A), from dextrine prepared by amylase (B), from hexahexosan (C), and from soluble glycogen (D).

The viscosities of these 4 synthetic starches are found to be far smaller than those of the natural ones, resembling rather to those of soluble starches.

Further, these 4 synthetic starches give clear solution in boiling water. On cooling with ice, however, the synthetic starch A alone quickly separates white coagulum which can be collected on filter by suction. This property disappears, however, when it is treated with cold dilute hydrochloric acid.

The chemical and physical properties of these 4 synthetic starches are shown in the following tables.

(a) Analysis of the synthetic starches and their original materials.

	Water %	Ash %	Phosphoric acid %	Nitrogen %
{ Synthetic starch A	4.21	0.56	0.16	0.08
{ Soluble starch	4.37	0.18	0.04	0.08
{ Synthetic starch B	3.35	0.24	0.09	0.07
{ Dextrine	4.51	0.10	0.04	0.04
{ Synthetic starch C.....	4.80	0.15	0.05	0.06
{ Hexahexosan	5.21	—	—	—
{ Synthetic starch D	3.56	0.22	0.07	—
{ Glycogen treated with Hcl. ...	3.87	0.17	0.05	—
{ Synthetic starch A treated with Hcl.	4.23	0.12	0.02	—

(b) Saponification value,¹⁾ viscosity, rotatory power
and $\text{CH}_3\text{CO}\%$ of acetylated products.

	Saponification value	Viscosity (2.5% sol. 20° C)	Rotatory power	$\text{CH}_3\text{CO}\%$ ²⁾ of acetylated products.
Synthetic starch A	4.63	38.5 min.	246.0	53.8
Soluble starch	1.26	27.3 „	180.0	56.2
Synthetic starch B	1.71	34.5 „	228.0	52.0
Dextrine	0.73	21.7 „	174.0	53.1
Synthetic starch C	1.27	36.5 „	—	44.1
Hexahexosan	—	21.5 „	—	47.2
Synthetic starch D	1.64	24.0 „	169.0	45.3
Glycogen treated with Hcl.	1.03	20.5 „	—	46.6
Potato starch.....	6.20	∞ „	—	—
Solube starch (Merk)	—	48.4 „	—	—
Water	—	19.8 „	—	—

1) Saponification value was determined by Rosenthaler's method.

2) The above samples were acetylated according to Rosenthaler and the percentage of CH_3CO group was determined by the Pregl's micromethod.