24. *Purified Invertase.*

I. Its Purification, Action and Properties.

By Toyosaku MINAGAWA.

Biochemical Laboratory, Institute of Physical and Chemical Research, Tokyo.

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It was one of the most difficult tasks to isolate pure invertases of strong power, free from diastase, protease and other enzymes.

Recently, the present author succeeded to purify mould and yeast invertases from Takadiastase and beer yeast, and found that those two enzymes had similar constitutions and were both pentosides in nature.

Especially, the configurations of these two enzymes were considered to be very analogous to these of pectase and amylosynthase, already described by the present author, as shown in the following formulae:—

Invertase (Pentoside of minaliny'l-tyrosine)

Pectase (Pentoside||of oxyminaliny|l-tyrosine)

Amylosynthase (Pentoside of oxyprolinyl-tyrosine)

It is important to note that a new amino acid, named "Minaline" by the present author, has been found as one of the important components of invertase.

"Minaline" [Isolated from invertase] and "oxyminaline" [Isolated from pectase] were both similarly reduced to proline and oxyproline by H_2 gas with PtO_2 as catalyser as shown in the following lines:—

It is the purpose of these papers to describe briefly the entire data:—

Purification of invertase.

The method of purification according to the present author is described briefly in the following scheme:—

Takadiastase or solid culture of mould [2 kg]

Dried beer yeast [2 kg]

4.5 times of cold water added and filtered.

Extract

4 times of saturated solution with ammonium sulphate added and filtered.

Filtrate

The precipitates, obtained by saturating with ammonium sulphate collected and washed with the saturated solution of the same salt.

Precipitates

Dissolved in cold water, added with barium hydroxide to remove sulphuric acid and filtered.

Filtrate

The cold filtrate added with cold acetone in the ratio of 50 cc filtrate to 35 cc acetone, and the precipitates therefrom collected.

Precipitates

Dissolved in cold water, filtered and precipitated again by the same treatment with acetone.

Precipitates

Dissolved in a small quantity of water, concentrated in a vacuum desiccator and the crystals of inorganic salts filtered.

Syrup

Treated with cold acetone.

Precipitates

Washed with alcohol and ether and dried in vacuum.

Purified invertases [3 g]

Purified invertase contained almost no ash when burned in crucibles and by analysis showed it to contain *C*: 50.2 %, *H*: 6.6 %, *N*: 6.1 %.

The pure preparation of invertases has the strongest inverting power, but no power of diastase, lactase, maltase, pectase and other enzymes.

It is important to note that mould and yeast invertases are completely separated from diastase and other enzymes only by the following simple methods, and these treatments form the skeleton of the purification method described by the author.

Beer yeast

Macerated with 3-4 times of water and filtered.

Extract

The cold extract added with cold acetone (in the ratio of extract 50 T and acetone 35 T) and the precipitates collected, leaving diastase and other enzymes in solution.

Precipitates

Dissolved in cold water, filtered, and the insoluble residue removed.

Clear water solution

Precipitated by adding cold acetone washed with alcohol and ether, and dried in vacuum.

Purified yeast invertase (free from diastase)**Takadiastase**

Dissolved in water and filtered

Clear extract

Added with 4 times the volume of saturated solution of ammonium sulphate and the precipitates which contained diastase and other enzymes removed.

Clear filtrate

Added with sufficient ammonium sulphate and completely saturated.
The precipitates, therefrom collected.

Purified invertase (free from diastase and pectase)

Action of invertase.

The purified preparations of invertases had the strongest power of inverting saccharose into glucose and fructose.

The powers of invertases were measured by the following method:—

0.001 g of purified invertase was mixed with sugar solution which contained 0.1 saccharose in it and kept at 34 C in the thermostat for 5 hours, after which the invert sugar was measured by Bertrand's method.

Sample	Sugar	Invert sugar
0.001 g Invertases (yeast and mould)		0%
	0.1 g saccharose	0%
0.001 g Invertase (yeast) + 0.1 g saccharose		100%
0.001 g Invertase (mould) + 0.1 g saccharose		95%
0.001 g Takadiastase + 0.1 g saccharose		18%

Chart 1.
Power of purified invertase
[0.1 g Saccharose
[at 34°C, for 5 hours]

The destruction temperatures of purified invertases are much higher than those of impure preparations.

Destruction temperature

Sample	Pure	Impure
Mould invertase	70°C	45 — 50°C
Yeast invertase	70°C	45 — 50°C

Purified invertases behaved powerfully in a fairly weak acidic medium, i. e. at 34°C in 0.01% acetic acid medium.

The destruction point falls practically along the vertical axis as shown in Chart 2.

Chart 2.
Destruction temperature curve.

Invertases were easily destroyed by alkali, i. e. sodium hydroxide, potassium hydroxide, etc., but never by ammonium hydroxide. When ammonium hydroxide, already added in invertase solution was neutralized by acetic acid, invertases began to act again as before without receiving any harm.

Physical and chemical properties of invertase.

The purified invertases either from mould or from yeast are yellowish amorphous powder, which dissolve quickly in cold water and give clear solution of yellow colour.

Invertase is one of the most easily precipitable enzymes by organic solvents, i. e. when 50 cc invertase solution was added with 35 cc acetone,

invertase was completely precipitated out of the solution, leaving diastase, protease, pectase, etc. in solution.

By ethyl and methyl alcohol, invertases were similarly precipitated out of the solution at fairly low percent, leaving diastase and other enzymes in solution in the same manner.

Therefore, these properties may be most convenient to use for the separation of invertase from other enzymes.

Up to the present, invertase was considered to be hardly salted out of solution by saturation with ammonium sulphate. Nevertheless, it has become clear that invertase is completely salted out by complete saturation with ammonium sulphate.

When the enzyme solution, which contained invertase was added with 4 times the volume of saturated solution of ammonium sulphate, diastase, protease and other enzymes were salted out at first and from the filtrate invertase free from those enzymes was easily precipitated by complete saturation with the same salt.

This property may be also profitable for the separation of invertase from other enzymes.

The aqueous solution of purified invertases gave no reduction of Fehling's solution, but gave typical α -naphtol and Bial's pentose reactions, exhibiting their true pentoside nature.

When invertase was hydrolysed by boiling with 1-2 % hydrochloric acid or sulphuric acid for 1 hour, 38 % pentose was estimated by Bertrand's method and the aglycone which contained nitrogen was precipitated out of the solution by adding with ethyl alcohol.

Reaction	Invertase	Diastase	Pectase	Synthesease	Protease
Reduction of Fehling's solution	—	—	+	—	—
α -naphtol reaction	+	+	+	+	—
Bial's reaction	+	$\pm$	+	+	—
Organic sulphur reaction	—	—	—	—	+
Buiet reaction	+	+	+	+	+
Ninhydrine reaction	+	+	+	+	+
Millon's reaction	—(Reduced)	+	—(Reduced)	+	+
Liebermann's pirrole reaction	+	—	+	+	+

From these experimental results, it is clear that invertases are new saccharides, just like diastase, pectase and amylosynthese, but not protein.

The important colour reactions of invertase are briefly summarized in the above table, comparing with that of diastase, protease, pectase and amylosynthese.