E2-03: Novel inhibitors for invertase (from *Saccharomyces cerevisiae*)

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Invertase is widely found in nature and highly abundant in Baker's yeast, *Saccharomyces cerevisiae*. This enzyme plays a prominent role in the confectionery industry and is responsible for the hydrolysis of sucrose. It is

also used for bulk inversion of sucrose as an alternative to acid hydrolysis. Invertase catalyses the hydrolysis or 1,2-glycosidic linkage of sucrose giving rise to equivalent amounts of glucose and fructose.

This enzyme has been purified and characterised. Several inhibitors for invertase have been found. They are iodine, mercury, silver and other metal ions, urea, raffinose and sucrose. Since this enzyme is highly beneficial in commercial sphere, further knowledge of inhibitors will be important. Objective of this study is to investigate more inhibitors for invertase.

Materials: *Saccharomyces cerevisiae*, Ammonium sulphate, Sodium citrate, Nelson's Reagent, Sucrose, Acetamide, Semicarbazide hydrochloride, Uric acid.

Saccharomyces cerevisiae (10g) in citrate buffer (0.5M, pH 5, 10ml) was ground with sand and centrifuged at 15,000 rpm for 15 min at 1°C. Supernatant was brought 25% ammonium sulphate saturation and pellets were prepared. Enzyme activity was assayed using Nelson's procedure.

Enzyme (0.1 ml, 1.475 mg/ml) in citrate buffer (pH 5, 0.5 M) was incubated with sucrose (1.00 ml, 0.5M) for 10 min at 25°C. Produced glucose was assayed using Nelson's procedure. Control experiment was performed without the substrate.

Enzyme was assayed under the standard assay conditions at the following concentrations of each compound (1.0 ml) which was to be investigated as an inhibitor (semicarbazide hydrochloride, acetamide and uric acid). Control experiments were performed without adding any of the above compounds.

Enzyme was assayed under the standard assay conditions at the following concentration of sucrose in the presence of each inhibitor (1.00 ml, acetamide 5 M, Semicarbazide hydrochloride (2.5M). Concentrations of sucrose (M): 0.04, 0.1, 0.2, 0.4 and 0.5.

Experimental evidence indicated that acetamide and semicarbazide hydrochloride are inhibitors for invertase whereas uric acid is not.

Table 1 : Effect of acetamide on invertase activity

Concentration of acetamide mol/l	A_{630} min^{-1}	Activity x 10^{-5} ml^{-1}
0	5.52	4.535
2	4.34	3.751
3	3.75	3.220
4	3.50	3.010
5	3.55	3.050
6	2.36	2.033
7	0.87	0.753

Table 2: Effect of semicarbazide hydrochloride on invertase activity

Concentration of semicarb-azide hydrochloride mol/l	A_{630} min^{-1}	Activity x 10^{-5} ml^{-1}
0	5.60	4.589
0.5	5.13	4.250
1.0	4.43	3.871
2.0	3.92	3.363
2.5	3.56	3.058
3.0	2.19	1.844
3.5	1.62	1.396
4.0	1.72	1.037

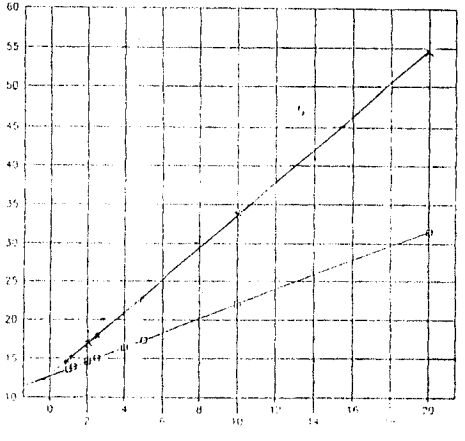
Table 3: Effect of uric acid on invertase activity

Concentration of uric acid mol/1	A_{630} → Activity x min^{-1}	→ 10^{-5} ml^{-1}
1	5.49	4.432
2	5.50	4.435
3	5.52	4.540
4	5.51	4.538
5	5.53	4.542

Nature of inhibition of acetamide on invertase activity

x-with inhibitor

o-without inhibitor

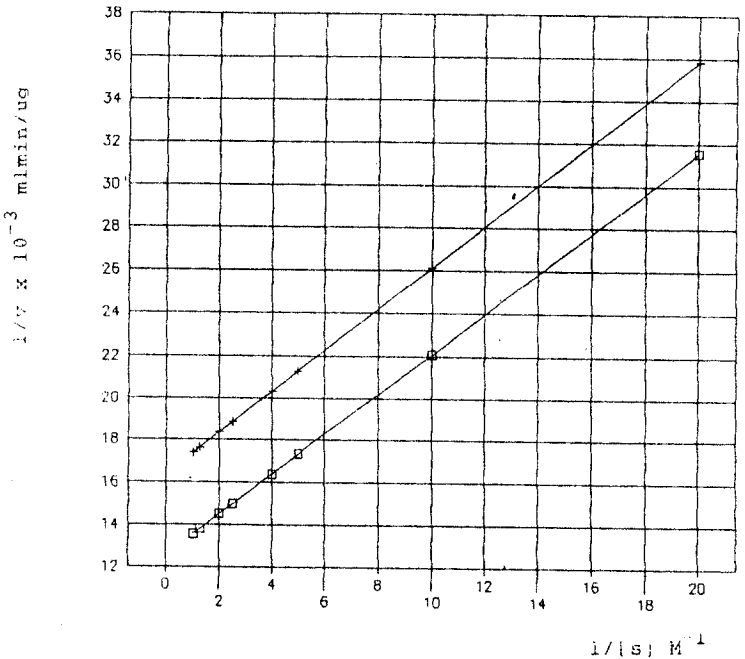


Product concentration

Incubation time

Substrate concentration

Nature of Inhibition of semicarbazide hydrochloride on invertase activity



$$v = \frac{\text{Product concentration}}{\text{Incubation time}}$$

s = Substrate concentration