CHEMICAL ACTIVATION OF α-GALACTOSIDASE FROM TRIHODERMA HARZIANUM

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The treatment of the α-galactosidase with hydrogen peroxide leads to the increase of the enzyme activity. The peroxidatic oxidation of the prosthaetic -SH -group seems bring to the stabilisated sulphenic-SOH group. The carbohydrolase activity of the sulphenic prosthenic group is revealed for the first time.

The enzyme was purified from cultural medium of the *trihoderma harzianum* in two stages by means of ion-exchange chromatogrphy,the HPLC being used. The enzyme homogenity was proved by electrophoresis and isofocusing. The molecular weight is *53.5 kDa*, the activity on the α-paranitrophenilgalactopiranoside ~*25* unit per *mg* at 35° C. The increase of the activity after the peroxide treatment is up to 800%.

EXPERIMENTS

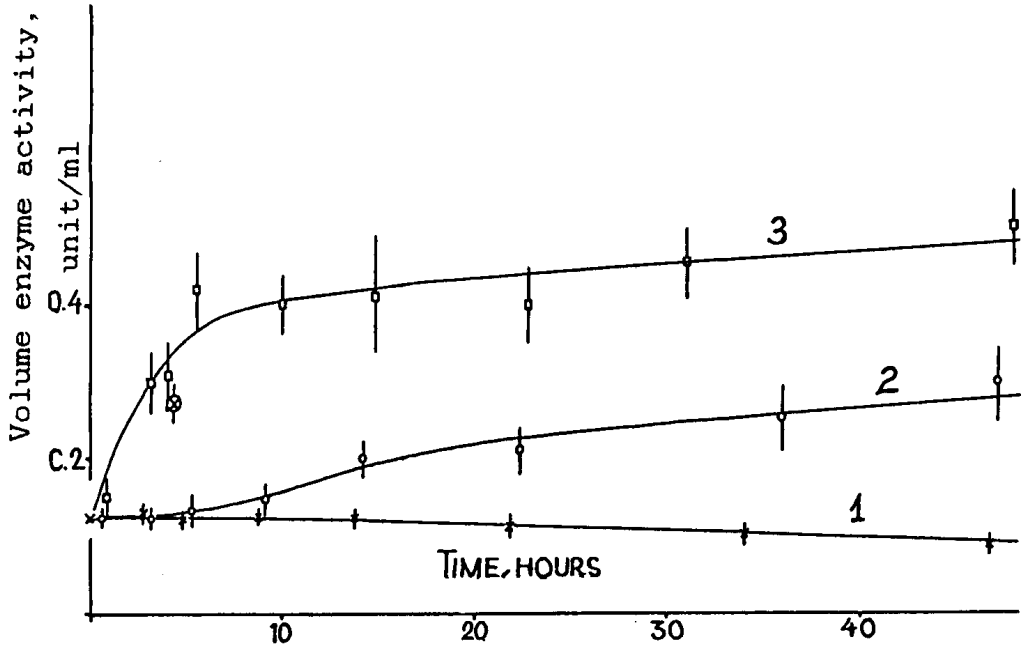

Fig.1. Activation of α-galactosidase by hydrogen peroxide.

The solution of the enzyme (0.15 *mg/ml*) was incubated at pH = 6.2 and room temperature (sample *1*, lower curve). Sample *2* (middle curve) contained also 0.05 *M* peroxide and 0.02 *M* galactose (being competitive inhibitor). Sample *3* (upper curve) contained peroxide and glucose instead of galactose. Before the activity test all the tested portions were accomplished with the lacking substances (for example, peroxide, glucose and galactose into the sample *1*), the identical solution being composed

Not only the activity rise could be seen, but also the protective effect of the galactose, shielding the active center. The distringuished points ⊗ and ⊙ are the activity in the samples *1* and *2* without galactose.

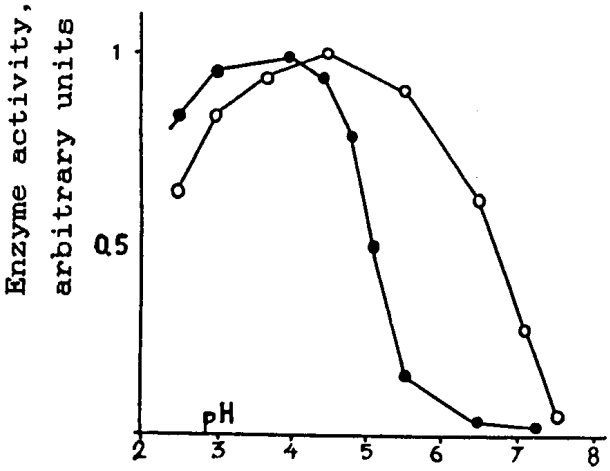

Fig.2. pH-dependence of α-galactosidase activity. o — native enzyme, o — activated one. Shift of the enzyme activity is connected with lower sulphenic pK value compared to the one of sulfhydryl group

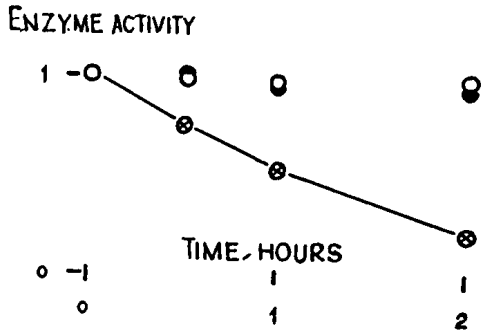

Fig.3. Inactivation of peroxide-activated α-galactosidase by thiol.

The enzyme was incubated anaerobically in presence of 0.2 *M* cisteamine at pH = 5.6 and room temperature. o — native enzyme, o — activated one, o — one activated, in presence of 0.02 *M* galactose. Thiol reacts with sulphenic group, producing bysulphide: R1SH + R2SH → R1S-SR2 + H2O Protection by galactose is obvious