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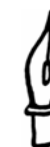

STABILIZATION OF CATALASE IN THE PRESENCE OF ADDITIVES

Madalina Nita, Adina Raducan*, Mihaela Puiu and D. Oancea

abstract The stability of catalase in the reaction of hydrogen peroxide decomposition was studied in the presence of some potential stabilizers: ethylene glycol, glycerol, fructose, sucrose, fucose, ribitol and 2 nitro phenyl-β-D-galacto-pyranosid (niphegal). The sucrose, ethylene glycol and glycerol showed the best performance for long-term storage at 30°C. The stability of catalase increases with the amount of additive used.

Introduction

Enzymes are inherently labile; therefore their operational stability is of great importance for any bioprocess. The problem of enzyme stability has been approached from different perspectives. Systematic efforts are done in the search of new biocatalysts, including enzymes extracted from extremophiles, cloning some termophilic genes into more suitable mesophilic hosts or using site-directed mutagenesis to code for more stable enzymes [1]. Operational stabilization of enzymes is an alternative. Immobilized and crystallized enzymes are stable forms already in use [2]. Also engineering the reaction media can contribute to enzyme stabilization. This is a key factor for using enzymes in organic synthesis where nonaqueous media are mandatory or at least highly desirable, or in industrial or medical purposes. Different agents, like temperature and chemicals, promote enzyme inactivation. Inactivation by chemicals can often be avoided rather easily by keeping them out of the reaction medium. Temperature however produces opposed effects on enzyme activity and stability and is therefore a key variable in any biocatalytic process [3]. Enzyme stability is the limiting factor in most bioprocesses, biocatalyst stabilization being then a central issue of biotechnology. Beside the production of intrinsically stable biocatalysts, enzyme stabilization involves studies regarding strategies for operational stabilization and mathematical modeling of the biocatalyst inactivation during operation. Increasing of the thermostability and operational stability of the enzymes can be made, with low costs, by the addition of salts, polyols (polyethylene glycol, glycerol), dextran, bovine serum albumine, polyethylenimine, polyelectrolytes, organic osmolytes (e.g. betaine), organic solvents (isoctane, cyclohexane, chloroform, benzene), sugars (e.g. lactiol, mannitol, sorbitol, xylitol, inositol, erythritol) and other modulators [4,5]. The selection of the appropriate additive depends on the nature of the enzyme [4].

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Catalase is a component of the anti-oxidative defence system acting in human and animal tissues against hydrogen peroxide, which is a potentially deleterious reactive species that destroys (oxidizes) cellular molecules. Catalase is one of the most studied enzyme since the beginning of 20th century [6]. The mechanism of hydrogen peroxide decomposition depends, among other variables, on the initial concentration of the substrate. At high concentrations of H_2O_2, an inactive complex is formed and catalase suffers a process of irreversible inactivation [7]. Catalase is used in medicine for prevent and control oxidative damages associated with some pathophysiological situations, including inflammatory diseases, ischemia-reperfusion injury, aging, and cancer [8] by scavenging the excess of hydrogen peroxide which is toxic for cells [9]. Also catalase is used in several industrial processes like textile industry for degrading the remaining peroxide after the bleaching treatment of fabrics, manufacturing industry for cleaning up the residual H_2O_2 during semiconductor/circuit chip manufacturing [10], cosmetic industry as an oxygenator for skin rejuvenation [10] and food industry to improve the freshness preservation of shrimp and fish [11]. It is suggested that the active oxygen species generated by oxidoreductases can act as bactericides. Even the stability of commercial catalases is quite good, several studies regarding the stabilization and thermostabilization of enzyme using additives were done. Stabilization of catalase ca be made either chemically by immobilization on polysaccharides [12] or by addition of several additives like polyethylene glycol, glycerol, glutaraldehyde and bovine serum albumin [13,14]. Another interesting approach for catalase stabilization is the use of nonaqueous solvents, like methanol [15] or acetonitrile [16] or anionic surfactants like sodium n-dodecyl sulphate [17].

Thus, the objective of this study was to improve stability of an aqueous solution of catalase obtained from bovine liver by addition of some polyols: ethylene glycol, glycerol, fructose, sucrose, fucose, ribitol and 2 nitro phenyl-β-D-galacto-pyranosid (niphegal). For the classic additives ethylene glycol and glycerol several concentrations were used, while for the others, the concentration was 1M.

Experimental part

The decomposition of hydrogen peroxide (Merck) 0.4077M in the presence of bovine liver catalase (E.C. 1.11.1.6) provided by Flucka, $7\cdot10^{-7}$M (in protein) was investigated in aqueous media in the presence of several additives by a gas-volumetric displacement method in isobaric-isothermal conditions (p_0 = 1atm; T = 303 K). The exact initial concentration of H_2O_2 was determined by titration with potassium permanganate. The initial H_2O_2 concentration was chosen to allow both a detectable inactivation of enzyme and a large conversion of substrate. The additives chosen as potential stabilizers were polyols (ethylene glycol, glycerol, fructose, sucrose, fucose, ribitol and 2 nitro phenyl-β-D-galacto-pyranosid (niphegal)). For each additive, the solution was kept at 30°C and the decomposition of H_2O_2 was measured for 10 minutes at several storage times, until the inactivation of catalase was significant.

For each kinetic set, the substrate concentration was calculated as:

$$[S]\ = [S]_0 - \frac{2p_0V_g}{V_sRT} = [S]_0 - 1.339\cdot10^{-2}\cdot V_g \qquad (1)$$

where $[S]$ and $[S]_0$ are substrate concentrations at time t and $t = 0$ respectively, V_g (in cm^3) is the volume of water displaced by the evolved oxygen, V_s (in cm^3) is the volume of the solution and $R = 82.01\ cm^3\ atm/\ mol\ K$.

The data for the reference solution (with no additives) and for sucrose are presented in Figs. 1 and 2.

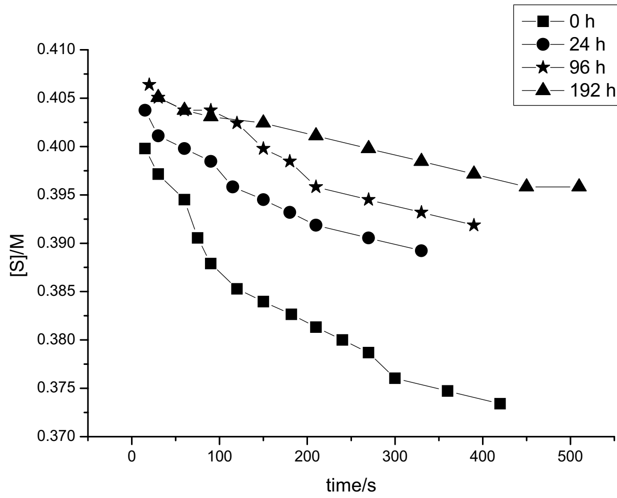

Fig. 1 *Kinetic data at several storage times in water*

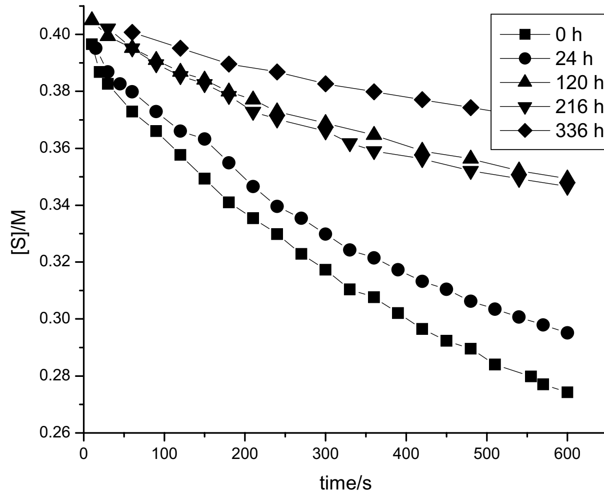

Fig. 2 *Kinetic data at several storage times in the presence of sucrose*

Results and Discussions

For each kinetic set, the initial rate was estimated from the kinetic cuves [S] = f(time), as the derivative at t=0 of the function [S] = a + b·exp(-ct) + d·exp(-et), where a,b,c,d and e are the best fit parameters of this function on the experimental data. The variation of initial rates (V_r^0) with the storage time (t_{st}) for all additives are presented in Fig. 3.

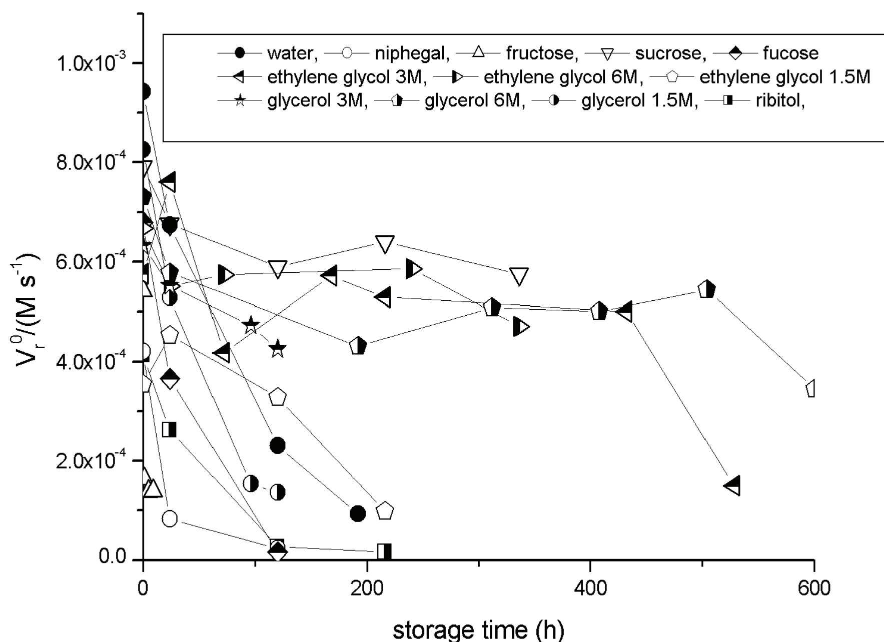

Fig. 3 *Variation of initial rate with the storage time*

The inactivation of catalase in time can be explained by the denaturation of its tertiary structure, leading to an inactive form [18]. This process can be assumed to follow a first order kinetics:

$$[E] = [E]_0 \cdot \exp\left(-k^* \cdot t_{st}\right) \qquad (2)$$

where $[E]$ and $[E]_0$ are the active enzyme concentrations at time t and $t = 0$ respectively, t_{st} is the storage time and k^* is the stability constant.

Since the initial rate depends linearly on the initial enzyme concentration, the equation (2) can be expressed as a function of the storage time. The results of nonlinear fit of equation $V_r^0 = A \exp(-k^* t_{st})$ on the experimental data are presented in Table 1.

The results showed that in media containing ethylene glycol, glycerol at concentration over 1.5M, fructose and sucrose the enzyme is stabilized as compared with water, while in media containing glycerol 1.5M, ribitol, fucose and niphegal, catalase is inactivated as compared with water.

Table 1 Parameters of equation (2)

additive	C_{add}/M	A 10^4/(Ms^{-1})	k^* 10^3/s^{-1}	correlation coeff.
	0.0	9.29 ± 0.30	12.1 ± 1.0	0.9982
	1.5	4.84 ± 0.57	3.37 ± 0.67	0.9632
ethylene glycol	3.0	5.59 ± 0.24	2.32 ± 0.28	0.9638
	6.0	6.27 ± 0.25	0.758 ± 0.213	0.9681
	1.5	8.16 ± 0.23	16.8 ± 1.1	0.9961
glycerol	3.0	6.17 ± 0.16	3.03 ± 0.41	0.9857
	6.0	6.59 ± 0.30	0.866 ± 0.097	0.9863
ribitol	1	4.15 ± 0.11	20.1 ± 1.7	0.9667
niphegal	1	4.19 ± 0.23	67.2 ± 11.8	0.9951
fructose	1	5.06 ± 0.56	9.16 ± 1.77	0.9597
sucrose	1	7.55 ± 0.31	1.91 ± 0.30	0.9813
fucose	1	6.81 ± 0.13	26.5 ± 1.6	0.9991

For ethylene glycol and glycerol the stability constant decrease with the concentration of additive (the stability of catalase increases with the amount of additive used). In the case of glycerol the effect of additive is very important, while for ethylene glycol, the variation of the stability constant with the concentration of additive is quite small.

These data indicate that the stability of catalase can be improved by the addition of some polyols; among the examined additives, the best results were obtained for sucrose, ethylene glycol and glycerol.

Conclusions

This study evaluates the stabilization efficiency of various additives on the activity of catalase. The experiments revealed that some polyols (ethylene glycol, glycerol, sucrose and fructose) provided better long-term storage stability at 30°C. The stability of catalase increases drastically with the concentration of glycerol, while for ethylene glycol, the effect is not very important. From these results, it could be suggested that the stabilized catalase could be potentially applied in textile and cosmetic industries.

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