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Stability of Invertase in Reverse Micelles

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ABSTRACT

The stability of invertase was studied under various conditions, including at 75°C, in presence of stabilizers (sorbitol and glycerol) at 75°C, and in the presence of denaturants (urea and trichloroacetic acid) at 37°C in reverse micelles. Stability of the invertase in reverse micelles was found to be improved over that of the enzyme in bulk aqueous solution. Sorbitol could enhance enzyme stability as it does in the bulk aqueous system. The stabilizing effect of glycerol was reduced in reverse micelles. The denaturation pattern of urea remains unaltered. However, the denaturation effect of trichloroacetic acid has been reduced in reverse micelles.

Index Entries: Invertase; stability; stabilizers; denaturants; reverse micelles; microenvironment.

INTRODUCTION

Invertase catalyzes the hydrolysis of sucrose to glucose and fructose. This enzyme is active in organic solvents, such as water-miscible solvents with low water content (*1*) and reverse micelles (*2*) under different reaction conditions.

Activity of many enzymes in reverse micelles has been investigated by various research groups to obtain a better understanding of structure-function relationships, as well as their industrial applications (*3–8*). Most

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of the work deals with kinetics in bis(2-ethylhexyl) sodium sulfosuccinate (AOT) reverse micelles, but very few studies on stability in this system have appeared (*9–11*).

Enzymes are expected to work differently, if they are isolated from their natural environment (in vivo). They become unstable and are rapidly inactivated. The increased stability of enzyme results from multipoint interaction with cell components, like lipids, polysaccharides, and proteins, and so on in supramolecular structure. Structured water in the cytoplasmic gel also plays an important role. As a result of natural immobilization, catalytically-active conformation of its active center is fixed. It is known that in thermophilic microorganisms such a matrix mechanism of stabilization functions more efficiently than in a mesophilic cell (*12*).

In much the same way, reverse micelles possess a microenvironment in which multilayers of structured water exist. If this is the case, the reverse micelles should be able to offer a better microenvironment for enzyme stability. This speculation caused us to study the stability of invertase in reverse micelles. The phenomenon of gradual inactivation at elevated temperature is undesirable in industrial processes. To test the generality of enzyme stability in two unrelated systems, i.e., bulk aqueous medium and reverse micelles, these experiments were performed.

In this article, we report on the various parameters which govern the stability of invertase in reverse micelles stabilized by nonionic surfactant Triton X-100 in xylene, in the presence of stabilizers, such as sorbitol and glycerol, and denaturants, such as urea and trichloroacetic acid (TCA).

MATERIALS AND METHODS

Chemicals

Invertase (β-D-fructofuranosidase, EC 3.2.1.26) from yeast was obtained from Sigma (St. Louis, MO), and used without further purification. Sucrose, Triton X-100, urea, sorbitol, glycerol, xylene, and all other chemicals used were of analytical grade. Doubly Pyrex distilled water was used in all reagents preparation.

Reagents

One molar Triton X-100 was prepared in xylene. Invertase (4 mg/1.4 mL) and sucrose (0.3*M*), was prepared in sodium acetate buffer of pH 4.5 (0.03*M*). Sorbitol and glycerol used were of 1*M* in 0.03*M* sodium acetate buffer. Urea 10*M* and 12.5% trichloroacetic acid (TCA) were also prepared in buffer.

Stability in Reverse Micelles

The solubility of buffer through reverse micelles was predetermined at 37°C and 75°C. Stability was analyzed at 75°C with and without stabilizers and at 37°C with denaturants.

Reverse micelles was prepared by injecting 20 μL of enzyme and the required amount of buffer and additives (sorbitol 30 μL, glycerol 50μL, TCA 80 μL, and urea 80 μL) into 1 mL organic solution using a micropipet. The contents were treated as required in the presence and absence of additives for different time intervals at 75°C and 37°C, respectively. The set of tubes treated at 75°C were cooled immediately in an ice bath to arrest the thermal inactivation, whereas additional buffers were added to dilute the denaturants in other sets of tubes. Retained activity was assayed by adding 20 μL sucrose at 37°C. Reaction was arrested by adding 1 mL dinitrosalicylic acid reagent, followed by immediate extraction of the aqueous content by addition of 3 mL chloroform. A known amount of aqueous supernatant was estimated for reducing sugars according to the 3, 5-dinitrosalicyclic acid (DNSA) method (*13*).

RESULTS AND DISCUSSION

Thermal Stability of Invertase in Reverse Micelles

When the temperature rises, enzymes in aqueous solutions are unfolded by heat-induced disruption of the balance of non-covalent interactions (*14*) which maintain the active conformation. This process leads to disintegration of the active center. Upon longer heating, a fraction of the enzymatic activity is regained; upon cooling, the process of irreversible denaturation is shown (*15*).

Incubation of invertase in aqueous solution at pH 4.5 (which is the optimum pH) and 75°C results in progressive inactivation as assayed at 37°C. Inactivation in reverse micelles, even for a prolonged time of 30 min, has not shown complete inactivation as it occurred in bulk aqueous medium (Fig. 1). However, the heat inactivation depicted in Fig. 1 is not accompanied by a precipitation of the enzyme. We thus tested whether the use of stabilizers like glycerol and sorbitol has an effect on the stability in both aqueous and reverse micelles at 75°C. Indeed, addition of glycerol has not been able to enhance the stability in reverse micelles (Fig. 2), as it has in bulk aqueous medium. The same trend has been observed in lipase stability in reverse micelles (*16*). Nevertheless, sorbitol improved the stability to a considerable extent in reverse micelles over the bulk aqueous system (Fig. 3). Nonspecific ligands, such as polyhydric alcohols and salts, are known to influence enzyme stability in organic solvents (*17*). Similarly, drastic enhancement of invertase thermostability was noticed in organic solvents (*17*).

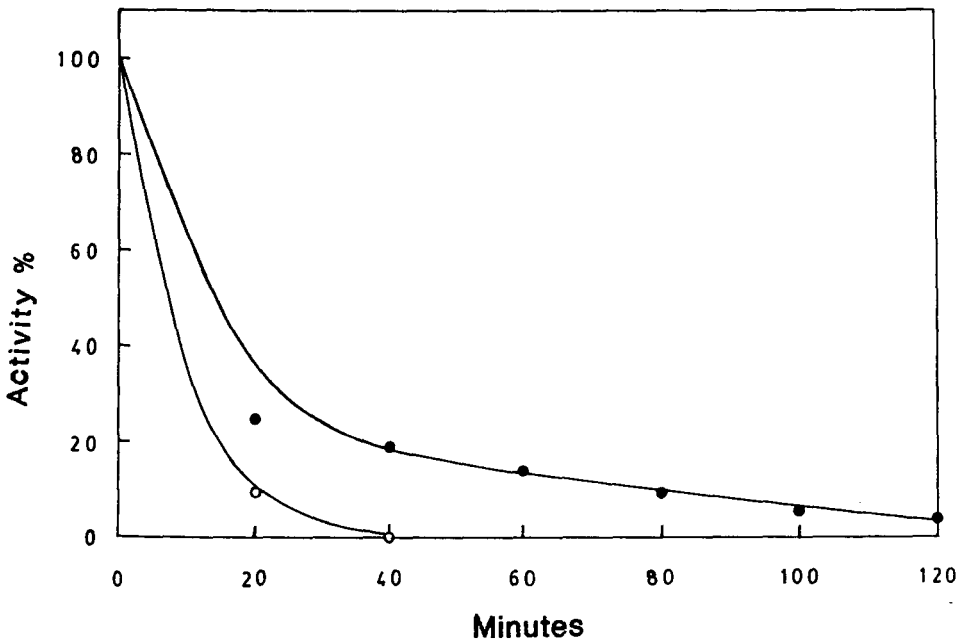

Fig. 1. Stability of invertase in reverse micelles (—●—), and in aqueous system (—○—) at 75°C.

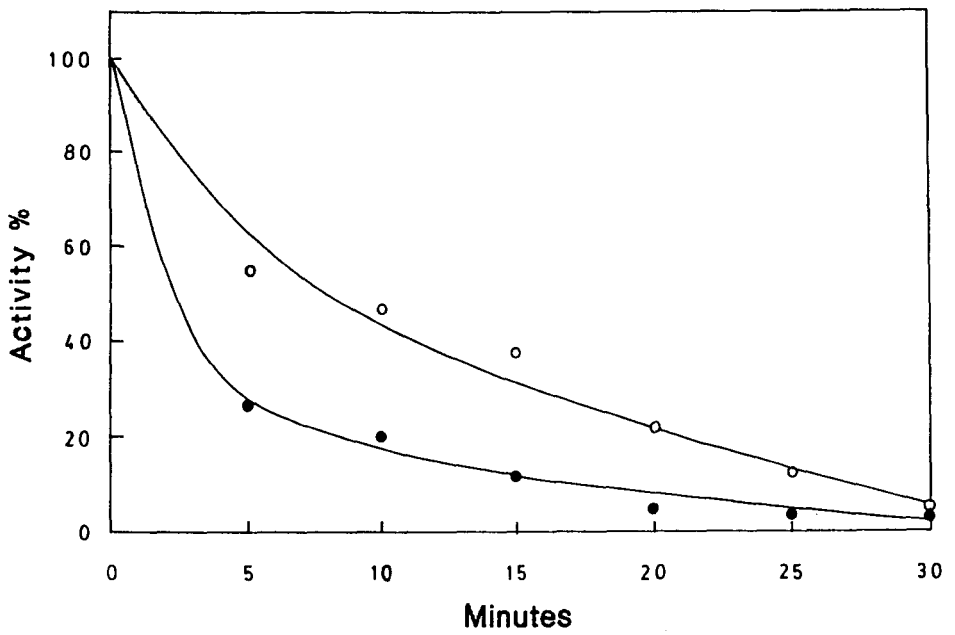

Fig. 2. Stability of invertase in presence of glycerol (0.41*M*) in reverse micelles (—●—) and in aqueous system (—○—) at 75°C.

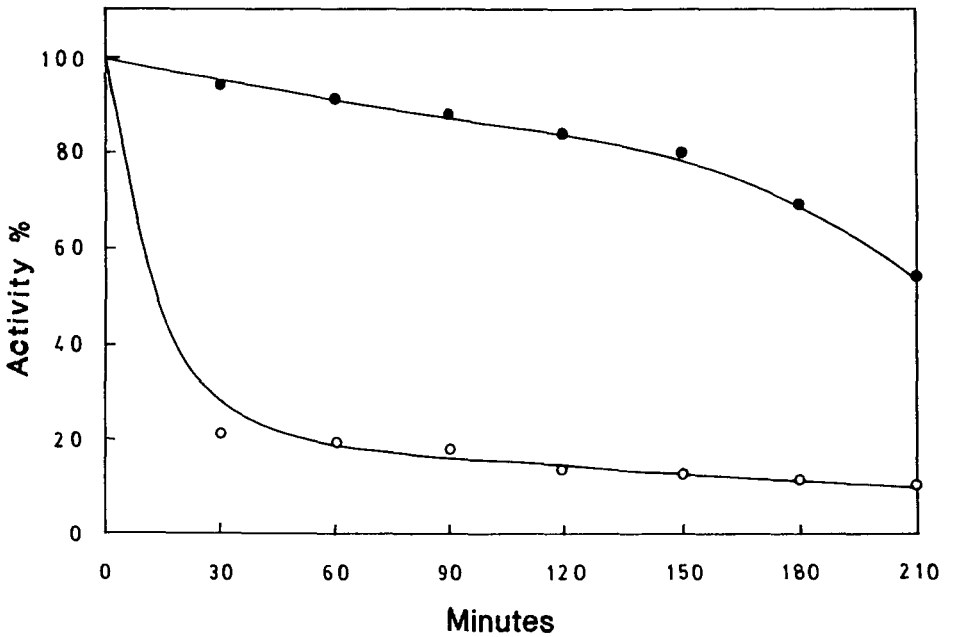

Fig. 3. Stability of invertase in presence of sorbitol (0.6*M*) in reverse micelles (—●—) and in aqueous system (—○—) at 75°C.

Stability in Presence of Urea and TCA

Experiments performed in presence of urea and TCA at 37°C show a considerable variation in the enzyme stability. The time course of inactivation of invertase at pH 4.5 in reverse micelles is comparable to that in aqueous system. Inactivation resulted in the appearance of progressive decrease in enzyme activity. However, the denaturation could be terminated after desired time intervals by diluting the aqueous solution nearly 7–10 times by the addition of excessive buffer. TCA afforded 60% residual activity after incubation for 30 min in reverse micelles (Fig. 4). This is attributed to the microenvironment inside the reverse micelles that enhances the enzyme's stability and restores the active conformation even in the presence of a denaturant. However, this cannot be generalized. The enzymes cannot be protected from all denaturants in reverse micelles, as evidenced in the presence of urea, where inactivation was similar to that in bulk aqueous solution (Fig. 5). The disappearance of activity did not occur simultaneously in both systems, implying a multistate denaturation mechanism, along with structured water for the active conformation of invertase.

The data presented above unequivocally indicate a much greater stability against irreversible thermoinactivation of invertase at 75°C compared to its dissoluation in in aqueous solution. The microenvironment of reverse micelles stabilizes the enzyme molecule to a considerable extent.

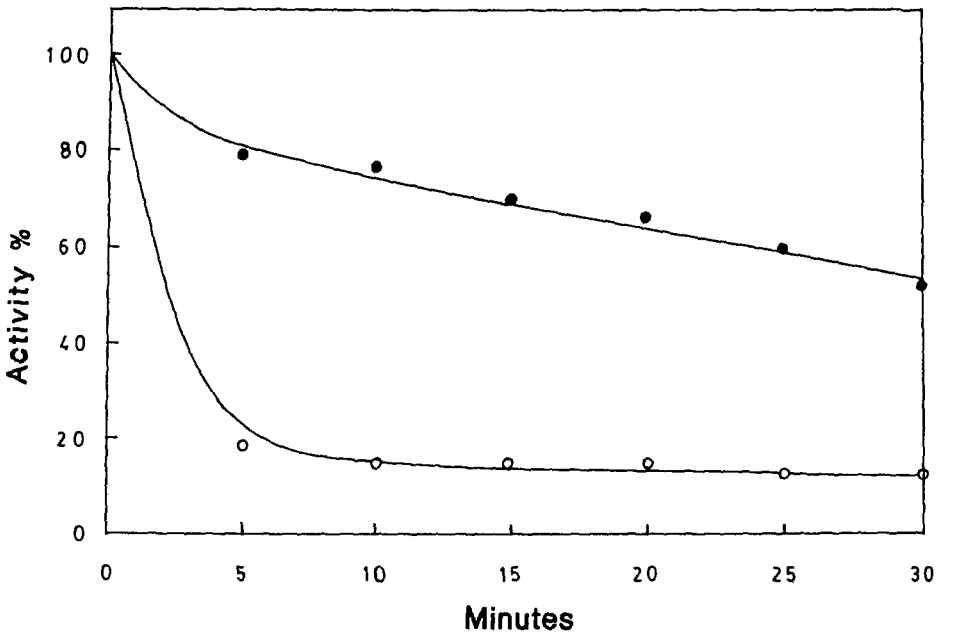

Fig. 4. Stability of invertase in presence of tricholoroacetic acid (10%) in reverse micelles (—●—) and in aqueous system (—○—) at 37°C.

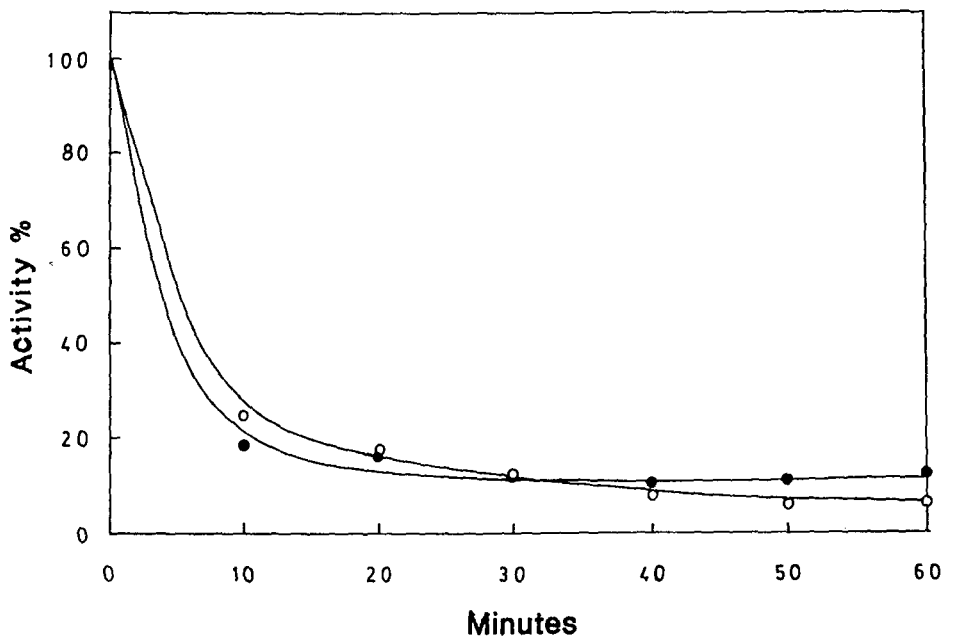

Fig. 5. Stability of invertase in presence of urea (8*M*) in reverse micelles (—●—) and in aqueous system (—○—) at 37°C.

This may be because structured water is present in the reverse micelle; hence, nature might be employing the same mechanism using structured water for stabilization of enzymes in the cell, along with other factors, such as a more favorable microenvironment than in conventional in vitro aqueous reaction environment.

Our findings with sorbitol, glycerol, urea, and TCA also explain the diverse role of stabilizers and denaturants in reverse micelles. It is well-observed that microenvironment inside the reverse micelle most often shows more enhanced stability than in bulk aqueous system.

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