Stability of Invertase in Reverse Micelles

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Received July 8, 1995; Accepted July 27, 1995

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 (0.3M), was prepared in sodium acetate buffer of pH 4.5 (0.03M). Sorbitol and glycerol used were of 1M in 0.03M sodium acetate buffer. Urea 10M and 12.5% trichloroacetic acid (TCA) were also prepared in buffer.