SHORT COMMUNICATIONS

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Reactivation by organic solvents of an alcohol dehydrogenase from *Candida lipolytica* grown on *n*-hexadecane

The alcohol dehydrogenase (alcohol:NAD oxidoreductase, EC 1.1.1.1) of *Candida lipolytica*, like a similar enzyme in *Pseudomonas aeruginosa*[1], loses its activity very rapidly in crude extracts[2]. The addition of 10% (v/v) ethanol, methanol or acetone strongly reduces the rate of inactivation[2]. Water-soluble organic solvents are known to protect several other dehydrogenase activities against inactivation[3,4].

However, it has hitherto never been shown that, once inactivated, the enzyme could be reactivated. We wish to report that water-soluble organic solvents are also able to restore most of the enzymatic activity previously lost in crude extracts.

C. lipolytica, obtained from the C.B.S. (Delft), was cultured on 1% *n*-hexadecane and harvested as described earlier[5,6]. The cell crop was suspended in bidistilled water, broken by ultrasonication and the soluble crude extract obtained by centrifugation at 105 000 × g^2. The specific enzymatic activity was computed from the initial rate of NAD^+ reduction, measured spectrophotometrically at 340 nm and at 23°, using the following assay mixture: 0.05 M pyrophosphate buffer (pH 8.0), 1.0 M ethanol, 5.0 mM NAD^+, and enough crude extract to give a change in absorbance of

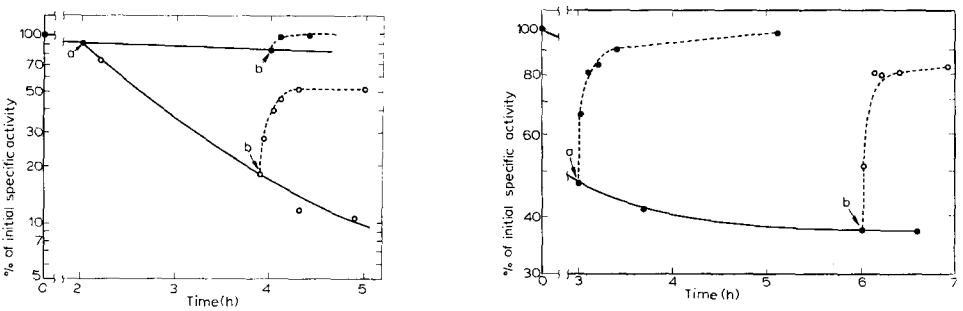

Fig. 1. Reactivation of the alcohol dehydrogenase of *C. lipolytica* by ethanol: influence of protein dilution. The crude extract contained 9.0 mg protein per ml, pH 6.6. Its specific activity, which initially amounted to 1100 m.I.U., was followed as a function of time (●———●) and is expressed in per cent of the specific activity at zero time. At the time indicated by a, a portion of the crude extract was diluted 1 : 2 with bidistilled water. The specific activity of the latter portion was followed as a function of time (○———○). At the time indicated by b, 10% (v/v) ice-cold ethanol was added to portions of the diluted and undiluted crude extracts. The specific activity of these solutions were followed as a function of time (○– – –○; ●– – –●). All enzyme solutions were kept at 0° throughout the experiment.

Fig. 2. Reactivation of the alcohol dehydrogenase of *C. lipolytica*: influence of organic solvents. The crude extract contained 8.3 mg protein per ml and was at pH 6.6. Its specific activity which initially amounted to 700 m.I.U., was followed as a function of time and expressed in per cent of the specific activity at zero time (●———●). At the time indicated by a, 10% (v/v) ethanol was added to a portion of the crude extract. At the time indicated by b, 10% (v/v) acetone was added to another portion of the crude extract. The specific activities of both portions were followed as a function of time (●– – –●; ○– – –○). All enzyme solutions were kept at 0° throughout the experiment.

0.1–0.5 per min. Under these conditions, a linear correlation was observed between the initial rate of NAD^+ reduction and the enzyme concentration, though the concentration of ethanol was sufficiently low to avoid any noticeable enzyme reactivation. The specific activity is expressed in milli-international units (m.I.U.): nmoles of NAD^+ reduced per min and per mg protein. Protein concentrations were determined by a conventional biuret method.

The activity of the alcohol dehydrogenase was measured in a crude extract kept at 0° as a function of time (Fig. 1). The inactivation was more pronounced when the crude extract was diluted. At various intervals of time, 10% (v/v) ethanol was added to portions of the crude extract. In all cases, most of the lost enzymatic activity was restored within a few minutes.

The amount of reactivation was low when little enzyme had been inactivated, so that the total recovered specific activity never exceeded the specific activity of the crude extract at zero time. However, a relatively large amount of enzymatic activity could still be restored when most of the enzyme had been inactivated. As a matter of fact, up to 50% of the specific activity could be restored when 92% of that activity had been lost.

Acetone was able to replace ethanol as the reactivating agent (Fig. 2). This excludes a mere protection of the enzyme by its substrate. It should be stressed that the reactivating compounds were only present in the storage medium and were absent from the assay mixture. This excludes a mere activation of the residual active molecules or a protection against a drastic loss of activity through protein dilution in the assay mixture.

Water-soluble solvents are known to act on protein structure by rupturing hydrophobic bonds or by interacting with hydrogen bonds[7]. The relatively large concentrations of the solvents required for reactivation and their apparent lack of specificity suggests that the reactivation of the alcohol dehydrogenase of *C. lipolytica* by water-soluble organic solvents is mediated by a transconformation[7] of the protein structure from the inactive to an active form. In an alternative hypothesis the inactivation would be due to a slow interaction of the enzyme with a hydrophobic substance present in the extract, which is removed by organic solvents.

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