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CONFORMATIONAL DRIFT OF LACTATE DEHYDROGENASE

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The loss and recovery of the enzymic activity of lactate dehydrogenase (LDH) subjected to pressure have been studied by Jaenicke and co-workers (1, 2). On the assumption that the fraction of enzyme activity after decompression accurately represents the degree of dissociation of the tetramer into monomers at the incubation pressure, they calculated a standard change in volume on dissociation of −500 ml/mol. They ascribe the slow reactivation at atmospheric pressure to the reassociation of the monomers. We have used fluorescence polarization methods (3) to monitor the degree of dissociation of LDH under pressures of 1 atmosphere to 3 kbar. When degree of dissociation and enzymic activity are separately measured they reveal a more complex situation than that postulated by Jaenicke et al.

RESULTS

The product of the dissociation under pressure is shown to be the monomer by its 30 ns rotational relaxation time and by the uniform appearance in time of the three mixed isozymes when the M_4 and H_4 lactate dehydrogenases are jointly subjected to high pressure (4). Fig 1 shows the degree of dissociation, calculated from the tryptophan fluorescence polarization, observed at pressures of up to 2.5 kbar, at three protein concentrations. The apparent change in volume upon dissociation is about −300 ml/mol. The initial polarization is recovered "immediately" after decompression, indicating prompt reassociation of the monomers to form an aggregate of the volume of original tetramer. This is confirmed by observations of electrophoretic mobility and rotational relaxation time of the decompressed preparations. However, these newly associated tetramers have both diminished catalytic activity (1, 4) and subunit affinity (4). The original enzyme activity gradually returns after a time that increases with the magnitude of the applied pressure and the time of pressure incubation. From the above information we conclude that the dissociated monomers undergo a progressive loss in conformation, i.e., a "conformational drift," whose extent depends on both magnitude and time of pressure application, and that these monomers reassociate into inactive tetramers after decompression. Fig. 1 shows that measurable degrees of dissociation require pressures in excess of 1 kbar. However, appreciable amounts of inactivation, as well as hybridization of mixed M_4 and H_4 isoenzymes, are seen after long incubation (3–24 h) at pressures <1 kbar (1, 4). We conclude that in these conditions a microscopic cycle of associations and dissociations takes place, and that because of the very short lifetime of the monomer in these circumstances (5) a great many such cycles are necessary to produce drifted monomers that result in inactive tetramers. As pressure and degree of dissociation increase so does the lifetime of the monomer and a proportionally faster conformational drift follows. At 2 kbar the enzyme is

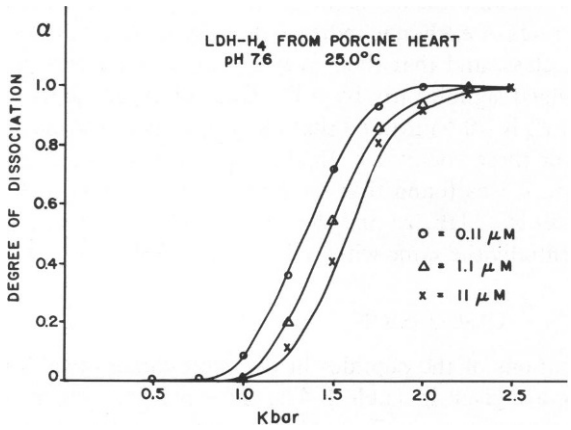

FIGURE 1 Plots of degree of dissociation from tryprophan polarization at three different protein concentrations against pressure, at 25°C. Solvent: 50 mM Tris-HCl with 1 mM EDTA and 1 mM DTE, pH 7.6. Excitation: 280 nm, through additional 7–54 Corning filter. Emission filter: WG320. Protein concentrations (micromolar): 0.11(o), 1.1 (Δ), 11 (×).

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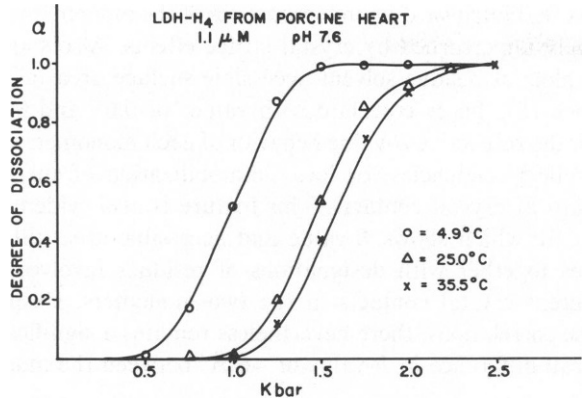

FIGURE 2 Plots of degree of dissociation of 1.1 micromolar LDH against pressure, at three different temperatures. Conditions as in Fig. 1. 4.9°C (o), 25.0°C (Δ), 35.5°C (×).

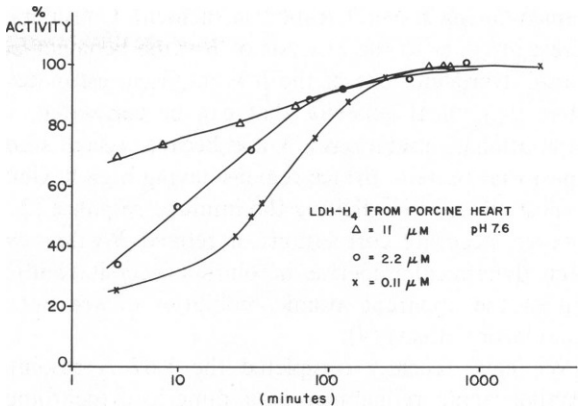

FIGURE 3 The course of the activity recovery after the cold samples (6°C) are stored at room temperature. Protein concentrations (micromolar): 0.11 (×), 2.2 (o), 11 (Δ).

completely dissociated and rapid conformational drift and inactivation take place.

The experiments described, and those in Fig. 1, were carried out at 25°C. Fig. 2 shows the effect of varying the temperature at which pressure is applied. The results clearly indicate that pressure dissociation is facilitated by lowering the temperature. The pressure for half dissociation, $p_{1/2}$, is 1470 bar at 25°C and 970 bar at 4°C. The effect diminishes at temperatures higher than 25°C: at 35°C $p_{1/2}$ = 1590 bar with no further change at higher temperatures.

The repeated cycles of association and dissociation that lead to inactivation at low pressures can be shown to take place also at atmospheric pressure at low temperature. Hybridization of ~26% of mixed H_4 and M_4 (7 μM) takes place after six days in the refrigerator (4.3°C) and this amount doubles in 12 d. Fig. 3 shows the course of the activity recovery with time after the cold samples are stored at room temperature. It takes several hours for the return of full activity and the time for recovery is concentration-dependent; faster recovery occurs at the higher concentrations. This observation is fully consistent with the concept of a conformational drift proportional to the fraction of the time that the protein spends as monomer. We expect that speed of recovery will increase with the fraction of the time that the drifted subunits spend as tetramer.

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ANOMALOUS TEMPERATURE FACTOR BEHAVIOR AND CRYSTAL LATTICE MOBILITY IN CYTOCHROME C′

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Protein structures obtained by x-ray crystallography represent a time and space average of the large ensemble of molecules incorporated in the crystal lattice. The effects of averaging the scattering from molecules that may have slightly differing local conformations or orientations in the crystal lattice, or that undergo thermally induced motions, are reflected in the atomic temperature factors (*B*-values)

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