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

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 H₄ and M₄ (7 \(\mu\)M) 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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REFERENCES

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).