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The Allosteric Properties of the Reduced Nicotinamide-Adenine Dinucleotide Phosphate Dehydrogenase of *Azotobacter vinelandii* Respiratory Membranes

By B. A. C. ACKRELL, S. K. ERICKSON and C. W. JONES (*Department of Biochemistry, School of Biological Sciences, University of Leicester, Leicester LE1 7RH, U.K.*)

Phosphorylating respiratory membranes of *Azotobacter vinelandii* oxidize NADPH via a rate-limiting NADPH dehydrogenase that exhibits only very weak oxidative phosphorylation ($P/2e$ ratio ≤ 0.05) and sigmoidal saturations kinetics ($s_{0.5}$ 0.44 mM-NADPH; Hill coefficient, $n_{\text{NADPH}} \approx 2$) when assayed at pH 6.8 (Erickson *et al.*, 1972).

NAD⁺ acted as an allosteric inhibitor (partially competitive kinetics) of the dehydrogenase (no change in V_{max} , K_m 1.28 mM-NADPH, K_i 7 μ M-NAD⁺) and changed the sigmoidal saturation plot to a rectangular hyperbola ($n_{\text{NADPH}} \approx 1$). The dehydrogenase was inhibited in a purely competitive (dead-end) manner by AMP (K_i 0.73 mM), ADP (K_i 1.2 mM) and ATP (K_i 4.5 mM), and was therefore subject to control by the adenylate energy charge (as defined by Atkinson, 1968). No activators were found for the dehydrogenase. When assayed at pH 8.5 the general regulatory properties of the dehydrogenase were largely unchanged, but at pH 5.5 the saturation curve was hyperbolic ($n_{\text{NADPH}} \approx 1$) and NAD⁺ no longer had any inhibitory effect.

These results suggest that the respiratory-chain NADPH dehydrogenase of *A. vinelandii* contains a catalytic site, which binds NADPH or adenine nucleotides, and an acid-labile regulatory site, which binds NADPH (positive homotropic effector) or NAD⁺ (negative heterotropic effector). The control properties of NADPH dehydrogenase are thus clearly different from those of NADH dehydrogenase and malate dehydrogenase, which are subject to classical ADP-dependent respiratory control and product inhibition by oxaloacetate respectively.

The ability of low energy charge or very low concentrations of NAD⁺ to inhibit NADPH dehydrogenase, together with the presence in this organism of a highly active NADPH-NAD⁺ transhydrogenase (Chung, 1970; Van den Broeck & Veeger, 1968), suggest that at the whole-cell level NADPH may be preferentially oxidized indirectly via NADH and the more efficiently phosphorylating NADH dehydrogenase. The homotropic kinetics suggest that significant direct oxidation of NADPH via NADPH dehydrogenase may occur only at relatively high NADPH concentrations, e.g. under highly aerobic conditions when nitrogen fixation, a major consumer of NADPH (Benemann *et al.*, 1971), is switched off (Yates, 1970). Under these conditions NADPH oxidation may contribute to the respiratory protection of nitrogenase (Dalton & Postgate, 1969) and to the maintenance of the requisite intracellular NADPH/NADP⁺ concentration ratio.

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Determination of the Molecular Weights of Actins from Different Species by Gel Chromatography

By F. B. PRESTON and G. N. GRAHAM (*Astbury Department of Biophysics, University of Leeds, Leeds LS2 9JT, U.K.*)

Recent studies (Spudich & Watt, 1971; Adelstein & Kuehl, 1970; Rees & Young, 1967) on rabbit actin indicate a molecular weight of 45000–48000. In all cases a purified protein was necessary for molecular-weight determination, gel chromatography often being used for purification.

Gel chromatography separates substances according to molecular size; Andrews (1965) has shown that, on Sephadex G-200, for many globular proteins over a considerable molecular-weight range (25000–200000) elution volumes are a linear function of the logarithm of the molecular weight.

Our studies show that actins from different sources obey this relationship, and that gel chromatography, as well as being a useful preparative tool,