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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,

is also a reliable means of molecular-weight determinations.

A column (98cm $\times$ 2.5cm) packed with Sephadex G-200 (Pharmacia, Uppsala, Sweden) was used for these studies. All separations were carried out at 4°C. Calibration of the gel bed was with proteins of known molecular weight, and the void volume, V_0 , was determined with Blue Dextran. Calibration standards (15–20mg) dissolved in 3–4ml of 0.2M-KCl and 0.02% NaN_3 were applied individually to the column, equilibrated with same solution, by ascending flow at 10ml/h.

Aqueous extracts of acetone-dried muscle protein powders (Barany *et al.*, 1957) from dogfish, trout, chicken and rabbit were used as sources of actin. To maintain actin in the monomeric form (G-actin), the column was equilibrated with 0.5mM-ATP in 2.5mM-tris buffer, pH 8.1, plus 0.02% NaN_3 . Up to 20ml of 5mg/ml muscle extracts was applied to the column as described. Column effluents were collected in 5ml (calibration standards) or 10ml (muscle extracts) fractions.

Proteins were measured either by light-extinction at appropriate wavelengths or by the method of Lowry *et al.* (1951). Muscle extracts from each species separated into several peaks showing similar distribution and elution to those found by Adelstein *et al.* (1963) for the rabbit and Weihing & Korn (1971) for *Acanthamoeba*.

The location of the G-actin peak was established by its development of flow birefringence in 0.1M-KCl–10mM-MgCl₂. The purity of the actin was determined from gel electrophoresis, amino acid analysis and sedimentation analysis.

Our estimates of the molecular weight of rabbit actin (i.e. about 45000) do not differ significantly from other findings, and show that there is virtually no difference in this respect between the species studied.

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Sequence Studies on the Milk Protein α -Lactalbumin by using Mass Spectrometry

By J. R. BACON and G. N. GRAHAM (*Astbury Department of Biophysics, University of Leeds, Leeds LS2 9JT, U.K.*)

The rate-governing step in the determination of the amino acid sequences of proteins is often the separation and purification of peptides rather than the actual determination of the sequences of the peptides. An approach has been made to decrease considerably the number of separation stages by applying the technique of mass spectrometry to the determination of the sequences of proteins. The use of mass spectrometry to the determination of the sequences of peptides was first suggested by Biemann *et al.* (1959) and was subsequently developed by several workers, with Morris (1970) showing that the sequences of peptides of unknown composition obtained from proteins and also mixtures of peptides could be determined by mass spectrometry.

Bovine α -lactalbumin is a milk protein whose sequence was fully determined by Brew *et al.* (1970). Tryptic and chymotryptic digests of the protein were partially separated on a Sephadex G-25 column, and the fractions taken were analysed by mass spectrometry. Although the separation stage does not yield a good separation, it does separate the peptides in the digest into groups of approximately the same molecular weight; and the weight of the sample to be analysed is the main factor governing the volatility of a sample, which itself determines whether a sample can be analysed by mass spectrometry. The volatility of each component of a mixture, however, will not be exactly the same, and this can be exploited by applying a temperature gradient to the sample inside the mass spectrometer. Samples of molecular weight up to about 1000 can be analysed completely, whereas those of larger size can have their sequences partially determined or be further digested to yield smaller peptides.

Although the peptides had been obtained from a protein source and had been converted into derivatives, no difficulties were experienced with impurities, for these tend to be more volatile than the peptides themselves and so can be removed at a low temperature. All the samples taken from the digests of α -lactalbumin were of completely unknown amino acid composition and therefore of unknown sequence, and the results were found to compare very favourably with the sequence published by Brew *et al.* (1970).

Three consecutive low-molecular-weight fractions from a Sephadex G-25 separation of a chymotryptic digest of α -lactalbumin yielded the sequence of 30 amino acid residues, i.e. a quarter of the whole molecule, illustrating the relative rapidity with which