ATP HYDROLYSIS AND ENERGY TRANSDUCTION BY NITROGENASE


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1. INTRODUCTION:

We have, in the past, described the functioning of molybdenum nitrogenase in terms of a kinetic model that describes the interactions between the two component proteins (the Fe-protein and the MoFe-protein), reductant dithionite, substrates and products (Lowe, Thorneley, 1984a, b; Thorneley, Lowe, 1983, 1984a, b). In this cycle ATP hydrolysis is associated with electron transfer from Kp2 to Kp1 followed by obligate, rate-limiting, dissociation of the protein complex to give reduced Kp1 and free oxidised Kp2ox(MgADP)2. Phosphate release was arbitrarily assigned to the protein complex dissociation step. Eight Fe-protein cycles, each involving the transfer of an electron to the MoFe-protein, combine to give a single MoFe-protein cycle in which a molecule of dinitrogen and eight protons are reduced to two molecules of ammonia and one of dihydrogen. The rates of all the partial reactions involved in these reactions have been independently measured.

In this paper we wish to address the hydrolysis of ATP by nitrogenase in terms of our Fe-protein cycle (Figure 1, cycle (a)). Under steady-state conditions the enzyme hydrolyses at least two molecules of ATP in a complex with a divalent metal ion, usually Mg²⁺, per electron transferred to reducible substrate. In addition to the Fe-protein cycle of nitrogenase Figure 1 also shows, in cycle (b), reactions that occur in the absence of reductant which also hydrolyse ATP rationalising the observations that, under a number of non-optimal conditions, the efficiency of coupling of ATP hydrolysis to electron transfer can be much higher (Jeng et al., 1970; Imam and Eady, 1980; Watt et al., 1975; Hageman and Burris, 1978). Note that in both cycles (a) and (b) ATPase activity is a property of a complex involving both proteins and not of either protein when isolated. The two putative ATP binding sites identified on the Azotobacter vinelandii Fe-protein by X-ray crystallography (Georgiadis et al., 1990) have been generally assumed to be the sites of MgATP hydrolysis. The simulations of the dependence of the rate of dihydrogen evolution on dithionite concentration support this assignment since they depend critically

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Figure 1. The Fe-Protein cycle of Nitrogenase. Kp1 and Kp2 are the MoFe- and Fe-proteins; cycles (a) and (b) show reductant dependent and independent ATP hydrolysis.

The overall process of ATP hydrolysis by nitrogenase has been studied using a number of techniques including pre-steady-state rapid quench of turning over enzyme to monitor formation of phosphate (Eady et al, 1978; Hageman et al, 1980), pre-steady-state proton production using pH indicators (Mensink et al, 1992), mass spectrometry (McKenna et al, 1989), stopped-flow calorimetry (Thorneley et al, 1989) and directed mutagenesis (Seefeld et al, 1992). There are problems with unambiguous interpretation of these data: rapid acid quench cannot distinguish between on-enzyme cleavage of ATP and subsequent phosphate release whereas heat and pH changes are difficult to assign to a particular partial reaction. Our original formulation of the Fe-protein cycle rationalised...