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Kinetic Analysis of Cooperative Interactions Induced by Mn^{2+} Binding to the Chloroplast H^+-ATPase†

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ABSTRACT: The kinetics of Mn^{2+} binding to three cooperatively interacting sites in chloroplast H^+-ATPase (CF_1) were measured by EPR following rapid mixing of the enzyme with $MnCl_2$ with a time resolution of 8 ms. Mixing of the enzyme-bound Mn^{2+} with $MgCl_2$ gave a measure of the rate of exchange. The data could be best fitted to a kinetic model assuming three sequential, positively cooperative binding sites. (1) In the latent CF_1, the binding to all three sites had a similar on-rate constants of $(1.1 \pm 0.04) \times 10^4$ M^{-1} s^{-1}. (2) Site segregation was found in the release of ions with off-rate constants of 0.69 ± 0.04 s^{-1} for the first two and 0.055 ± 0.003 s^{-1} for the third. (3) Addition of one ADP per CF_1 caused a decrease in the off-rate constants to 0.31 ± 0.02 and 0.033 ± 0.008 s^{-1} for the first two and the third sites, respectively. (4) Heat activation of CF_1 increased the on-rate constant to $(4.2 \pm 0.92) \times 10^4$ M^{-1} s^{-1} and the off-rate constants of the first two and the third site to 1.34 ± 0.08 and 0.16 ± 0.07 s^{-1}, respectively. (5) The calculated thermodynamic dissociation constants were similar to those previously obtained from equilibrium binding studies. These findings were correlated to the rate constants obtained from studies of the catalysis and regulation of the H^+-ATPase. The data support the suggestion that regulation induces sequential progress of catalysis through the three active sites of the enzyme.

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Characteristics of the Combination of Inhibitory Mg^{2+} and Azide with the F_1 ATPase from Chloroplasts†

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ABSTRACT: The interactions between ADP, Mg^{2+}, and azide that result in the inhibition of the chloroplast F_1 ATPase (CF_1) have been explored further. The binding of the inhibitory Mg^{2+} with low K_d is shown to occur only when tightly bound ADP is present at a catalytic site. Either the tightly bound ADP forms part of the Mg^{2+}-binding site or it induces conformational changes creating the high-affinity site for inhibitory Mg^{2+}. Kinetic studies show that CF_1 forms two catalytically inactive complexes with Mg^{2+}. The first complex results from Mg^{2+} binding with a K_d for Mg^{2+} dissociation of about 10–15 μM, followed by a slow conversion to a complex with a K_d of about 4 μM. The rate-limiting step of the CF_1 inactivation by Mg^{2+} is the initial Mg^{2+} binding. When medium Mg^{2+} is chelated with EDTA, the two complexes dissociate with half-times of about 1 and 7 min, respectively. Azide enhances the extent of Mg^{2+}-dependent inactivation by increasing the affinity of the enzyme for Mg^{2+} 3–4 times and prevents the reactivation of both complexes of CF_1 with ADP and Mg^{2+}. This results from decreasing the rate of Mg^{2+} release; neither the rate of Mg^{2+} binding to CF_1 nor the rate of isomerization of the first inactive complex to the more stable form is affected by azide. This suggests that the tight-binding site for the inhibitory azide requires prior binding of both ADP and Mg^{2+}.