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Asp50, and Val51 in Lys82 mutants of the chaperone Hsp70. Mutations of the highly conserved residues Asp50, Val51, and Glu166 in the ATP-binding domain of the Escherichia coli chaperone Hsp70 result in a slowing down of the ATPase activity and in a decrease of the binding affinity for the nucleotide triphosphate.

Previous studies have shown that this effect results from a decrease in the intrinsic ATPase activity of the mutant protein [Brosch et al., 1995: Biochemistry 34, 5885-5890]. In the present study, we have used the paramagnetic relaxation agent Ni(III)-octaethylporphyrin (OEP) to investigate the accessibility of Asp50, Val51, and Glu166 of the apo and ATP-bound Hsp70 N domain in the wild type and Lys82 variants. Glu166 is accessible to Ni(III)OEP both in apo and ATP-bound Hsp70, whereas Asp50 is accessible only in the apo protein. The quenching rate constants for Val51 are approximately 12-fold higher in the apo protein than in the ATP-bound protein. The increase in Val51 quenching rate in the apo protein can be explained by the formation of a covalent bond between Val51 and a cationic Ni(III)OEP in the ATP-bound protein. The rate of quenching of Asp50 does not change appreciably upon

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