

P013 Kinetic analysis of two mutants of the FAD/NADPH binding domain of human methionine synthase reductase

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Human methionine synthase reductase (EC 2.1.1.35) is a key eukaryotic enzyme in folate and methionine metabolism, where it plays a role in maintaining the activity of cobalamin-dependent methionine synthase.

From the 1.9 Å crystal structure of the FAD/NADPH binding domain of MSR, Asp652 was suggested to destabilize the binding of NADPH. To further investigate the role of Asp652, the residue was mutated to Alanine, Arginine and Asparagine. These three mutations, which are expected to change the electrostatic surface of the MSR NADPH binding pocket, were generated in the full length enzyme and the isolated FAD/NADPH binding domain. Stopped-flow kinetic experiments showed that the rate of flavin reduction decreased 137-fold for D692A and 400-fold for D692R compared to wild-type. Product inhibition experiments with NADP⁺ showed weaker binding for the oxidized coenzyme in both mutants. These results demonstrate that Asp652 plays a key role in NADPH binding and hydride transfer in MSR.