

Pig Liver Phosphomevalonate Kinase. 2. Participation of Cysteinyl and Lysyl Groups in Catalysis

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Phosphomevalonate kinase from pig liver is inactivated by 5,5'-dithiobis(2-nitrobenzoate) and pyridoxal 5'-phosphate. The substrate phosphomevalonate protects the enzyme against inactivation by these reagents. Inactivation by 5,5'-dithiobis(2-nitrobenzoate) is complete and may be reverted by 2-mercaptoethanol or dithiothreitol. Experiments carried out with partially inactivated enzyme show no change in the k_{cat} or in the apparent K_m for the Substrates, as compared with the native enzyme, indicating the existence of two populations of molecules, one intact and the other totally inactive. These results suggest that 5,5'-dithiobis(2-nitrobenzoate) reacts with the only cysteinyl residue of the enzyme and that this residue is located in or near the active site. Inhibition by pyridoxal 5'-phosphate can be reverted, either by dialysis or by the addition of lysine, but not if the partially inactivated enzyme is treated previously with $NaBH_4$, in agreement with the formation of a Schiff base be