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Purification and Characterization of Ascorbic Acid Kinase

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Maximum activity for phosphorylating C_2-OH of the ascorbic acid was observed at the time of 16hr incubation from the culture of *Flavobacterium devorance* ATCC 10829. The enzyme was purified 30-fold, via ammonium sulfate fractionation, Fast Q anion exchange and phenyl agarose chromatography. Gel chromatography and SDS-polyacrylamide electropheresis experiments showed that enzyme is tetramer with subnit MW of 29kDa. Among available substrates, pyrophosphate showed the highest activity. Optimum temperature and pH were 45℃ and 5.5, respectively. The enzyme was chemically modified only by diethylpyrocarbonate and EDC, indicating that histidine and carboxylate are in the active site. pH studies showed that 2 hisitidines are involved in binding of the substrates and a carboxylate in catalysis. Therefore, the chemical mechanism of the enzyme is likely that 2 histidines binds to pyrophosphate and carboxylate, respectively, and a carboxylate acts as a general base