INTERACTIONS OF THE PHOSPHORUS ANALOGUE OF OXALOACETATE AND THE ARSENIC ANALOGUE OF MALATE WITH MALATE DEHYDROGENASE

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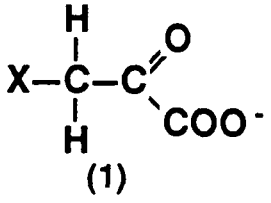
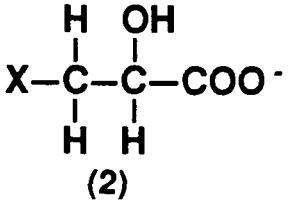
Malate Dehydrogenase (MDH), a constitutive enzyme of the citric acid cycle catalyses the reduction of oxaloacetate (1, X=CO_2^-) to malate (2, X=CO_2^-) as NADH is converted to NAD+. In an attempt to gain information about the active site, we have investigated the interaction of the enzyme with phosphorus and arsenic analogues of malate and oxaloacetate. Phosphonopyruvate (1, X=PO_3^{2-}) is a substrate for MDH, albeit a poor one with a K_m of 10.6 mM, and a pH optimum of <6.5. Arsonolactate (2, X=AsO_3^{2-}) is neither a substrate nor an inhibitor of MDH, as determined first by the inability to detect NADH formation, and second by the lack of influence of this compound on the rate of reaction of malate with MDH, measured by spectrophotometry at 340 nm.



(1)



(2)

These results, together with the published data on the sulphur analogues, Weinstein and Griffith (1986) (1, X=SO_3^- is a substrate, K_m 6.3 mM; 2, X=SO_3^- is an inhibitor), have been rationalised using theoretical calculations, paying particular attention to the shape, size, and charge density of each molecule. "Docking" the natural substrate (1, X=CO_2^-) into the crystal structure, Birktoft et al (1989), of MDH suggests that the two carboxylate groups form hydrogen bonds with the arginine-161 and asparagine-130 residues on the active site. On substituting any of the above analogues for oxaloacetate, the planar carboxylate anion becomes a tetrahedral group, which affects the hydrogen bonding to these residues. The van der Waals volumes of the sulphur and phosphorus analogues are both 10 A^3 greater than that of the malate, and this increase can just be accommodated by the active site of MDH. The arsenic analogue is 8 A^3 larger still, which is likely to prevent access of this molecule to the active site. The natural substrates and sulphur analogues have the same overall charge. In contrast, the phosphorus and arsenic analogues are triionic having an extra negative charge which makes charge stabilisation at the active site more difficult. It is likely that this charge differential is reflected in the pH optimum of <6.5 for the reaction with phosphonopyruvate, suggesting that the dianion is the active form.

Weinstein, C L and Griffith, O W (1986). Ann. Biochem. 156: 154-160.
Birktoft, J J et al (1989). Biochemistry 28: 6065-6081.