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THE REGULATION OF SOME GLYCOLYTIC ENZYMES IN *STREPTOCOCCUS LACTIS*

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Vaughan Leslie CROW

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ABSTRACT

Certain aspects of the control of carbohydrate metabolism have been studied primarily in *S. lactis* C_{10}. The kinetic and regulatory properties of two enzymes, lactate dehydrogenase and pyruvate kinase were investigated in some detail whereas a third enzyme, 6-phosphogluconate dehydrogenase, was subjected to a preliminary investigation only. A brief investigation was made of the *in vivo* concentrations of some metabolites in exponentially growing cells in batch culture.

The *S. lactis* lactate dehydrogenase (LDH) was purified about 100 fold. The mobility pattern of the purified enzyme on polyacrylamide disc gel electrophoresis was a complex function of pH and ionic strength. From sodium dodecylsulphate-gel electrophoresis the LDH appeared to have a subunit molecular weight of 37,000. A tentative model indicating a pH dependent association/dissociation has been suggested on the basis of the gel results and heat stability studies. At acid and neutral pH values a tetrameric species is favoured. At alkaline pH values (pH 8.0) a dimeric species is favoured. The tetrameric protein is more stable to heat than the dimeric species. The purified LDH requires fructose-1,6-diphosphate (FDP) for catalytic activity at acid and neutral pHs. For pyruvate reduction, in the presence of FDP the pH optimum was 6.9 whereas in the absence of FDP only very low activity was found and the pH optimum was 8.0 to 8.2. The pH optimum for lactate oxidation in the presence or absence of FDP was 8.0 to 8.2 and the activation by FDP was very much less than the FDP activation of pyruvate reduction. The kinetics of lactate oxidation suggested that only the pyruvate reduction direction was significant *in vivo*.

A significant finding was the effect of different buffers on the FDP activation of LDH. The concentration of FDP required for 50% maximal activity was 0.002 mM when determined in triethanolamine/HCl buffer, 0.2 mM in tris/maleate buffer and 4.4 mM in phosphate buffer; a 2,000 fold difference depending on the choice of the assay buffer. At the pH optimum (pH 6.9) there appeared to be at least two FDP binding sites which interact with each other in a co-operative manner. The choice of buffer was shown to affect other properties of LDH, such as the pH effect on FDP binding, the heat stability of the enzyme at 55°C, the binding of NADH and pyruvate and the effect of the inhibitor, oxamate. Stopped-flow analysis of the LDH showed that a lag period was present at pH 6.9. This lag period could be eliminated by pre-incubation with FDP. No

such lag period was demonstrated at pH 8.2. It is suggested that this lag period is due to a conformational change in the tetrameric species induced by FDP. The properties of the S. lactis LDH, taking into account the buffer effects, have been discussed in terms of the carbohydrate metabolism and related to other FDP-activated streptococcal LDH's. A brief comparative study of the S. faecalis ATCC 8043 LDH was made. The two major findings were its insensitivity to phosphate inhibition and its activation by manganese ions.

Pyruvate kinase was purified to near homogeneity as determined by polyacrylamide gel electrophoresis, with and without SDS. With SDS, a subunit molecular weight of 60,750 was determined. From equilibrium sedimentation studies the molecular weight of the native protein is 235,000. The enzyme is therefore a tetrameric protein. The kinetic properties of the pyruvate kinase were more complex than those of LDH, for as well as requiring FDP as an activator, the enzyme had an essential requirement for both a monovalent and divalent cation. FDP under most conditions bound to the enzyme in a co-operative manner. Phosphoenolpyruvate (PEP), and to a lesser extent, ADP, showed co-operative binding to the enzyme only at unsaturating FDP concentrations. Both the monovalent and divalent cations showed co-operative binding to the enzyme in the presence of saturating FDP concentrations. The activation properties of the enzyme were considerably different when Mn^{++} was substituted for Mg^{++} as the divalent cation. Like LDH, the pyruvate kinase was also affected by the nature of the buffer components. Pyruvate kinase was inhibited by lower concentrations of phosphate than were required to inhibit LDH. In addition the pyruvate kinase activity was inhibited by high concentrations of Mg^{++} and ADP. The properties of the S. lactis pyruvate kinase have been discussed in relation to other pyruvate kinases and to carbohydrate metabolism in S. lactis.

The S. lactis 6-phosphogluconate dehydrogenase (6-PGDH) did not appear to be inhibited by FDP, nor did the enzyme from S. faecalis ATCC 8043. This is contrary to published findings by other workers. Because of the preliminary nature of this investigation, further work is required on the S. lactis 6-PGDH to establish whether or not its activity is regulated by FDP.

The in vivo concentration of several metabolites were determined in exponentially growing cells and related to the in vitro kinetic properties of the two enzymes, LDH and pyruvate kinase. The metabolites

studied were; FDP, PEP, triose phosphates, ADP, ATP, glucose-6-phosphate and pyruvate. The *in vivo* FDP concentration was at a sufficiently high level (12.7 to 14.9 mM) to fully activate the two enzymes as indicated by *in vitro* determinations under a number of different assay conditions. The *in vivo* studies have suggested further *in vitro* kinetic studies which may be useful to investigate to gain a fuller understanding of the regulation of carbohydrate metabolism in *S. lactis*.

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