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Divergent Binding Sites in Pyruvate Kinases I and II from *Escherichia coli*

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Summary: Pyridoxal 5′-phosphate incorporation into pyruvate kinase II from *E. coli* was decreased by the substrate phospho*enol*pyruvate and increased by the allosteric activator ribose 5-phosphate, the total incorporation being linearly related to inactivation. Four lysyl residues were substantially modified, whatever the incubation conditions were while two additional residues became reactive only in the presence of the allosteric activator. Six tryptic peptides containing modified lysines were purified and sequenced. They defined five regions of pyruvate kinase II, since one of them contained two labelled lysines and included a peptide which also appeared independently. Sequence comparison with *E. coli* type I, yeast and cat muscle pyruvate kinases shows that the binding regions of pyruvate kinase II are clearly divergent from those of pyruvate kinase I and of the eukaryotic enzymes.

Key terms: Pyruvate kinase, binding sites, *E. coli*.

Pyruvate kinase (EC 2.7.1.40), an important glycolytic enzyme, is a tetramer of identical subunits of approximately 500 amino-acid residues, which, in the presence of $K^{\oplus}$ and $Mg^{2\oplus}$, catalyses the transphosphorylation from phospho*enol*pyruvate to ADP, yielding pyruvate and ATP. Most pyruvate kinases are subjected to allosteric control and, being members of a multigene family, exist in the same organism as differently regulated isozymes[1,2]. The comparison of several known pyruvate kinase sequences reveals highly conserved regions, which, on the basis of the correlation between structure and function, available for the cat muscle M1 isozyme, have been recognised as structurally similar active sites[3].

Two isoforms are expressed in *E. coli*: the fructose 1,6-bisphosphate-activated type I (Pyk I) and the AMP/ribose 5-phosphate-activated type II (Pyk II) pyruvate kinases[4,5]. The genes coding for both of them have been recently sequenced[6,7].

In order to identify regions of pyruvate kinase II involved in the binding of ligands, we treated the enzyme with pyridoxal 5′-phosphate, an affinity reagent for phosphate-binding sites[8], since both substrates and effectors of pyruvate kinases are phosphorylated compounds.

We isolated and sequenced six tryptic peptides containing lysines reactive towards pyridoxal 5′-phosphate. Peculiarities of the pyruvate kinase II sequenced regions are discussed.

Enzymes:
Lactate dehydrogenase, (*S*)-lactate:$NAD^{\oplus}$ oxidoreductase (EC 1.1.1.27);
Pyruvate kinase; ATP:pyruvate O^2-phosphotransferase (EC 2.7.1.40);
Trypsin (EC 3.4.21.4).

Abbreviations:
Hepes, 4-(2-hydroxyethyl)-1-piperazinethane sulphonic acid; HPLC, high-performance liquid chromatography; i.d., inner diameter; a.u.f.s., absorbance units full scale; TosPheCH$_2$Cl, tosylphenylalanylchloromethane.