The ability of mammals to catalyse the phosphorylation of glucose to glucose 6-phosphate utilizing MgATP as phosphoryl donor is provided by four distinct isoenzymes. The three hexokinases, types I, II and III (EC 2.7.1.1) are monomeric, have a broad hexose substrate specificity, a low \( K_\text{m} \) for glucose (0.01–0.2mM) and a molecular weight of about 100000. The fourth isozyme, hepatic glucokinase (EC 2.7.1.2), also known as type IV hexokinase, is also monomeric, but has a molecular weight of 50000 and a high \( K_\text{m} \) for glucose, which is its primary substrate.

When the amino acid compositions of these enzymes are normalized to the same molecular weight they are strikingly similar, and this has led to speculation that the hexokinases have arisen by gene duplication from glucokinase (Easterby, 1971; Colowick, 1973; Holroyde & Trayer, 1976). Although the isoenzymes may be prepared from the appropriate tissue in good yield (Holroyde et al., 1976; Holroyde & Trayer, 1976; Wright et al., 1978), they are present only in small amounts in any given species, and thus any sequence comparison has not been possible. We have therefore investigated the possibility of sequence homology between the hexokinases by the use of a sensitive peptide-‘mapping’ procedure capable of separating picomole quantities of enzyme digest.

Problems of detection of very small amounts of material and interpretation of peptide ‘maps’ of such large proteins (the 100000-mol. wt. isoenzymes could potentially give 110 peptides from a tryptic digest) are overcome by labelling the protein tyrosine residues with I\(^{125}\). Thus the numbers of peptides are decreased to the number of tyrosine residues in the protein, and sensitivity is enhanced, since the ‘maps’ may be observed by radioautography. The manipulation of the protein is made easier by labelling within a polyacrylamide-gel slice and digesting the protein out of the gel by the method of Elder et al. (1977) and modified by Zweig & Singer (1979).

Peptide ‘maps’ of hexokinase II, glucokinase and yeast hexokinase B have been prepared by separating the peptide, trypic and V8-protease (\textit{Staphylococcus aureus}) digests on high-performance thin-layer silica-gel plates (Nano-Plates-SIL 20; Macherey Nagel & Co., Duren, Germany) by electrophoresis at pH 2 in one direction and chromatography at right angles. Of the two mammalian hexokinases analysed by this method, hexokinase type II resembles the yeast isoenzyme more closely than does glucokinase. Tryptic digests gave 31 peptides for the yeast enzyme, 9 for glucokinase and 19 for hexokinase type II; these values are comparable with the total tyrosine contents, of 14, 8 and 16 residues respectively (Holroyde et al., 1976). Internal sequence duplication in the high-molecular-weight hexokinase is therefore not obvious from this particular labelling method. Of these peptides, four appear to be common to all three enzymes, with a further five being unique to yeast hexokinase and hexokinase type II, and two unique to glucokinase and yeast hexokinase. No peptides were found to be unique to hexokinase type II and glucokinase. Peptic digests confirm these results; in this case, however, three unique peptides to hexokinase type II and glucokinase are found.

These studies suggest that, if gene duplication has occurred during evolution of the hexokinases, glucokinase has diverged from some presumptive hexokinase ancestor before the low-\( K_\text{m} \) mammalian and yeast isoenzymes. As to the nature of the common peptides, they may reflect the conservation of particular sequences to maintain some aspect of the structure. For example, although the active sites are different with respect to essential residues (Connolly & Trayer, 1979a,b; Otieno et al., 1975), they may be involved in the creation of a cleft within the globular structure of the enzymes.

The ‘mapping’ technique has been extended to investigate the proposed conformational change that the yeast enzyme undergoes on binding glucose. In this case the soluble native enzyme was labelled at tyrosine residues by using Sepharose-immobilized lactoperoxidase and \( {\text{I}}^{125}\) in the presence and absence of substrates. Differences in intensity of labelling are visible, though not as dramatic as one might expect considering the extent of the conformational change involved (McDonald et al., 1979). One peptide is labelled with \( {\text{I}}^{125}\) when glucose is removed from the ‘cold-labelled’ enzyme. This single peptide may be one at the active site of the enzyme which is inaccessible to I\(^{125}\) when glucose is present. No differences were seen between ‘maps’ obtained in the presence and absence of MgATP\(^{2-}\), which supports this conclusion.

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Isotope-exchange evidence for allosteric regulation of hexokinase II by glucose 6-phosphate and for an obligatory addition of substrates

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The mechanism of rat skeletal-muscle hexokinase II has been shown by several initial-rate studies carried out by Fromm and his colleagues, using partially purified enzyme preparations, to proceed by a ternary-complex mechanism (e.g. Hanson & Fromm, 1967). Furthermore, these authors interpreted alternative-substrate studies as evidence for a rapid random addition of glucose and ATP, and these conclusions were supported by product-inhibition studies of ascites-tumour mitochondrial hexokinase II by Kosow & Rose (1968). However, this type of kinetic evidence could be given alternative interpretations, and does not provide unambiguous evidence for a rapid random addition of substrates.