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Distribution of isomerases for free and phosphorylated sugars *

by

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A simple micromethod for the semiquantitative assay of isomerases in tissue extracts has been developed. The reaction is carried out as a spot test in porcelain plates, allowing to stand at room temperature mixtures of each of a series of free and phosphorylated aldoses, the extract, and a pH 7 borate buffer (ALVARADO and SOLS, 1). After appropriate time intervals ketose accumulation can be observed by means of a micro-adaptation of DISCHE's cysteine-carbazol method. Purple colour appears when any ketose is formed.

As a check of the method we tested it with respect to the phosphohexose isomerases activities in extracts of kidney and testicle of the rat. The Kidney extract showed greater phosphoglucose isomerase activity than that of testicle, while the activity of phosphomannose isomerase was greater in testicle than in Kidney. The semiquantitative results of this experiment are in agreement with the quantitative results obtained in a parallel experiment with the conventional method.

With the aid of this micromethod a number of tissues, including microorganisms, vegetal and animal organs have been examined towards a dozen odd presumptive substrates.

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MICROORGANISMS

<i>Aspergillus orizae</i> <i>Penicillium notatum</i>	}	Grown in	{	L-Arabinose Glucose Mannose Xilose
<i>Neurospora crassa</i>	»	»	{	L-Arabinose Mannose Sacarose Xilose
<i>Aspergillus niger</i> <i>Bacillus subtilis</i> <i>Proteus OX-19</i> <i>Saccharomyces fragilis</i>	}	»	»	Glucose
<i>Saccharomyces cerevisiae</i>	»	»		Sacarose
<i>Escherichia coli</i>	»		{	L-Arabinose Galactose Glucose Lixose Mannose L-Rhamnose d-Ribose Xilose

PLANTS

GERMINATED SEEDS	{	<i>Hordeum vulgare</i>
		<i>Medicago sativa</i>
		<i>Secale cereale</i>
		<i>Triticum sativum</i>
HIGHER PLANTS	{	<i>Allium cepa</i>
		<i>Allium porrum</i>
		<i>Apium graveolens</i>
		<i>Beta rapa rubra</i>
		<i>Beta vulgaris cycla</i>
		<i>Brassica oleracea capitata</i>
		<i>Brassica rapa esculenta</i>
		<i>Cichorium endivia</i>
		<i>Citrus sinensi sanguinea</i>
		<i>Cynara scolymus</i>
		<i>Daucus carota</i>
		<i>Lactuca sativa</i>
		<i>Malus domestica</i>
		<i>Musa paradisiaca</i>
		<i>Petroselinum hortense</i>
		<i>Pisum sativum</i>
<i>Raphanus sativa</i>		
<i>Solanum lycopersicum</i>		
<i>Solanum melongena</i>		
<i>Spinacia oleracea</i>		

		ANIMALS	
		INSECTS	$\left\{ \begin{array}{l} \textit{Apis mellifica} \\ \textit{Blatta orientalis} \\ \textit{Drosophila melanogaster} \\ \textit{Grillus campestris} \end{array} \right.$
INVERTEBRATES		WORMS	<i>Ascaris suis</i>
		RAT	
VERTEBRATES			

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In mammal organs phosphoglucoseisomerase is from ten to one hundred times more active than phosphomannoseisomerase. This is also the rule for invertebrates *ascaris* and some insects: *bees*, *vinegar flies*, *cockroaches*) and plants. Phosphohexose isomerases are less active in invertebrates and plants than in mammal organs, presumably in relation with the higher amount of inert substances like water, chitin, or cellulose.

In some microorganisms, and fungus, we have found that only phosphohexose-isomerases are present in the extracts, when they were grown in a medium with glucose as the only carbon source.

Growth in other sugars can in some cases induce the formation of adaptive isomerases. With *Escherichia coli* grown in a medium with l-arabinose we found l-arabinose-isomerase. We studied the enzymatic activity of the extract, towards: glucose-6-phosphate, d-arabinose, l-arabinose and galactose. The higher activity is that of l-arabinose-isomerase, more strong even than the phosphoglucoseisomerase. There was no activity with d-arabinose and galactose.

Along the last few years a number of other isomerases for phosphorylated and free sugars have been found. With our micromethod we indeed detected the l-arabinose-isomerase; but adaptive isomerases in general do not seem to be easy to be found. In some cases it might depend on an enzyme being inactive in our standard assay conditions. Nevertheless, our results, on the whole, indicate that the variety and frequency of appearance of isomerases for free sugars is considerably smaller than it appeared likely from the recent individual observations above mentioned.

As a conclusion we could say that only phosphohexose isomerases are universally distributed while isomerases for free sugars are not common.

Bibliography

- (1) ALVARADO, F., and A., SOLS: *Biochim. Biophys. Acta*, 25, 77, 1957.

