TRANSIENT AND STEADY STATE CONCENTRATIONS OF THE INTERMEDIATES OF THE PENTOSE PHOSPHATE PATHWAY IN KREBS ASCITES TUMOUR CELLS METABOLISING GLUCOSE

K.A.GUMAA, Patricia McLEAN and J.G.BENNETTE
Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London W. 1, U.K.

Received 25 July 1968

1. Introduction

The rate of glucose consumption by ascites tumour cells following the addition of glucose to cells incubated *in vitro* is subject to rapidly acting control mechanisms. There is evidence for an important regulatory site at hexokinase. Three phases have been distinguished: (1) an initially rapid rate of glucose uptake and phosphorylation with apparently maximal hexokinase activity; (2) a marked inhibition of hexokinase; (3) a slow steady rate which is only 10-20% of the initial rate [1-4]. While the concentrations of certain glycolytic intermediates during these different phases are known [1-4], relatively little is known about the concentration changes of intermediates of the pentose phosphate pathway under these varied rates of glucose-6-phosphate formation. Measurements have therefore been made of the concentrations of glucose-6-phosphate (G6-P), 6-phosphogluconate, the pentose phosphates, sedoheptulose 7-phosphate, erythrose 4-phosphate, glyceraldehyde 3-phosphate and ATP during the transient initial stage and the later steady state conditions of glucose metabolism. It was found that while 6-phosphogluconate, the pentose phosphates and triose phosphate changed in parallel with alterations in G6-P, the concentration of both sedoheptulose 7-phosphate and erythrose 4-phosphate behaved differently. Sedoheptulose 7-phosphate, in particular, was sustained at a high and relatively constant concentration and showed a tendency to be inversely related to the G6-P concentration. These results are discussed in relation to the control of the pentose phosphate pathway of glucose metabolism.

2. Materials and methods

The substrates and purified enzymes used in the assay of the metabolites were all purchased from Boehringer and Soehne, Mannheim, Germany, with the following exceptions: transaldolase was a gift from Dr. B.L.Horecker; spleen enzymes (a mixture of ribulose 5-phosphate epimerase and ribose 5-phosphate isomerase) were prepared by the method of Ashwell and Hickman [5]; transketolase was prepared by the method of Simpson [6] as modified by Novello and McLean [7] but the purification was stopped short of the treatment with lead acetate. Transketolase prepared by the above procedure was free from enzymes oxidising NADH but was contaminated with aldolase.

Krebs 2 ascites tumour cells were harvested 7 days after implantation, and were then washed free from ascites plasma and red blood cells by differential centrifugation in 0.154 M–NaCl with heparin (1 unit/ml) and their packed weight determined. The cells were suspended in Krebs-Ringer phosphate buffer, without calcium, the final suspension contained 1 g cells/5 ml. After withdrawal of a zero time sample, glucose was added to make a final concentration of 10 mM and the cells were incubated at 37° in a large beaker with shaking at a rate of 90 cycles/min. Subsequent samples were withdrawn at the stated time intervals, de-

proteinised with ice cold perchloric acid and neutralised with KOH. The supernatant after removal of potassium perchlorate was diluted to make the final extract equivalent to 1 g cells/10 ml.

Metabolites were estimated by standard methods described by Bergmeyer [8]. Sedoheptulose 7-phosphate and erythrose 4-phosphate were estimated using transaldolase as described by Racker [9]. Dihydroxyacetone phosphate, glyceraldehyde 3-phosphate, fructose 1:6 diphosphate and pentose phosphates were estimated in the same cuvette by the sequential addition of α-glycerophosphate dehydrogenase, triose isomerase, aldolase, spleen enzymes and transketolase and by following the oxidation of NADH.

All results are expressed as μmoles substrate/g packed cells.

3. Results and discussion

The results are summarized in fig. 1, the upper part

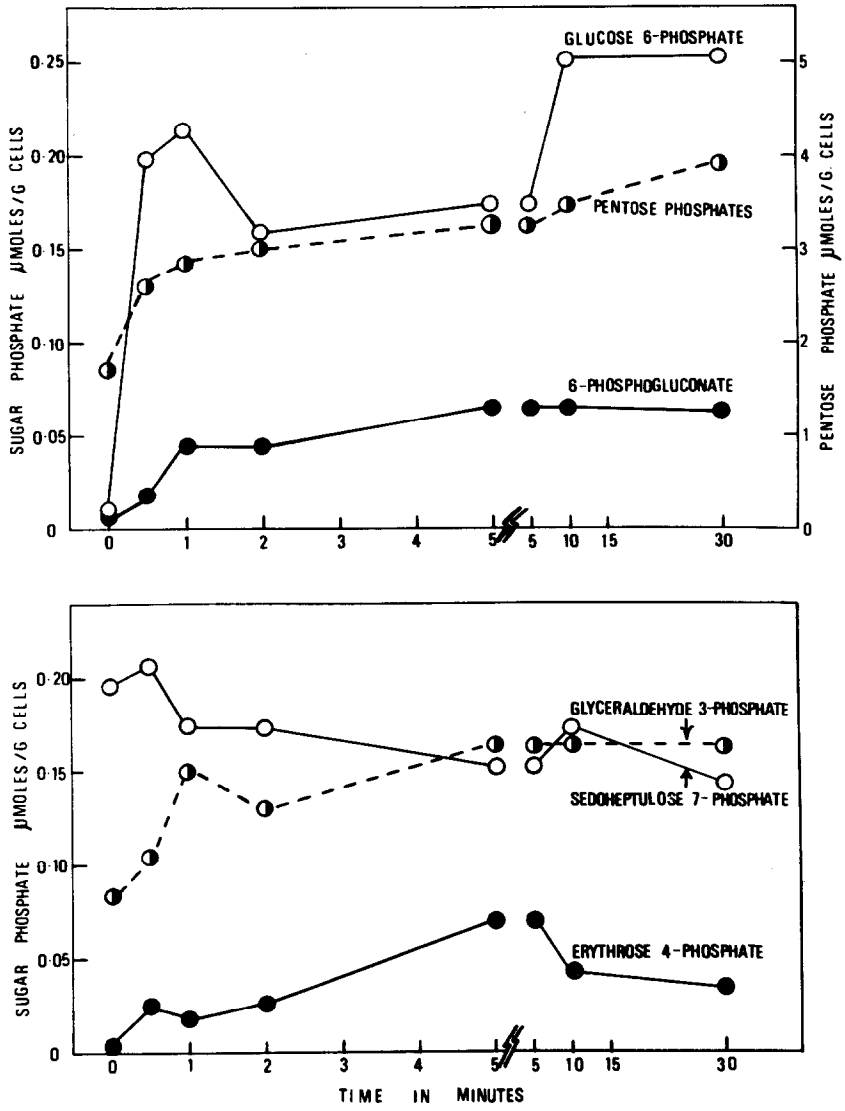

Fig. 1. Concentration of intermediates of the pentose phosphate pathway in Krebs ascites cells metabolising glucose. The incubation procedures and assay of metabolites were as described in the Methods. The initial glucose concentration was 10 mM, the glucose was added after withdrawal of the zero time sample. The results are given as μmoles substrate/g packed cells.

of which shows the concentration changes of G6-P, 6-phosphogluconate and the combined values for the pentose phosphates (the sum of ribose 5-phosphate, ribulose 5-phosphate and xylulose 5-phosphate). The pattern of change of these metabolites is very similar, an initial rise over the first minute being followed by a short period of inhibition and then by a slow increase to the steady state levels. The G6-P concentration changes are similar to those reported by Sauer [1] for Ehrlich-Lettré ascites cells. During the first 30 sec after the addition of glucose there is some 25-fold increase in the G6-P concentration. This is followed by a period of inhibition and then, at 5-10 min, by a slow increase in the G6-P concentration at about 5% of the apparent initial rate. It has been shown that the ATP/G6-P concentration is a key factor in the control of hexokinase [1,2,4]. A 90-95% inhibition of hexokinase was found when the ratio ATP/G6-P approached 3 at 1 mM-ATP concentration and with 3 mM-Pi [1]. In the present experiments this quotient was over 200 before the addition of glucose to the cells (1.7/0.008 μmoles/g cells respectively) and fell to a value of 5, 30 sec after glucose was added. The steady state quotient ATP/G6-P after a 30 min period of incubation was 5(1.25/0.25 μmoles/g cells respectively). A very marked inhibition of G6-P formation 1-2 min after addition of glucose may be due to factors other than the ATP/G6-P quotient since this value was not markedly different from that found at 30 sec and in the steady state at 30 min. The quotient ATP/G6-P was approximately 4 at both 1 and 2 min (0.87/0.21 and 0.65/0.16 respectively). More significant may be the ratio and the absolute values of ATP/ADP. It is known that ADP is a competitive inhibitor of brain, skeletal muscle and heart hexokinase and that the K_I is similar to the K_m for ATP that is 1.3×10^{-4} M [10]. At 1 and 2 min after addition of glucose the ADP concentration in the ascites tumour cells reached a maximum value and the quotient ATP/ADP fell to less than 1; the values as μmoles/g cells are 0.87/1.03 and 0.65/0.71 respectively. This quotient contrasts with all the other values found in the present experiments which are all greater than 1 and which reach a value of approximately 3.5 during the steady state period 30 min after addition of glucose.

The changes in sedoheptulose 7-phosphate are quite dissimilar from those of G6-P and 6-phosphogluconate (fig. 1). There is initially a relatively high concentration of sedoheptulose 7-phosphate in the absence of glucose which decreases 1 min after the addition of glucose to the medium and thereafter shows a slow decline; that is, a change tending in the opposite direction from that of G6-P. The concentration changes of glyceraldehyde 3-phosphate show that there is a rise in the concentration of the triose phosphates at a time when sedoheptulose 7-phosphate decreases and this may be correlated with the requirement for glyceraldehyde 3-phosphate in the further metabolism of sedoheptulose 7-phosphate by the transaldolase reaction. It may be postulated that at low glyceraldehyde 3-phosphate concentrations, such as exist in the basal state before the addition of glucose, the sedoheptulose 7-phosphate cannot be metabolised further by the transaldolase reaction. As the concentration of glyceraldehyde 3-phosphate rises with the flow of glucose through the glycolytic pathway, so sedoheptulose 7-phosphate is utilised by the transaldolase reaction. This is supported by the late rise found in the concentration of erythrose 4-phosphate which reaches a maximum value 5 min after addition of glucose (fig. 1).

Horecker et al. [11] first drew attention to the importance of competing reactions for triose phosphate in controlling the products when liver preparations were incubated with ribose 5-phosphate *in vitro*. The draining of triose phosphate by the formation of fructose 1:6 diphosphate and fructose 6-phosphate was shown to lead to an accumulation of sedoheptulose 7-phosphate. The present results with Krebs ascites cells also point to the importance of such factors in the accumulation of sedoheptulose 7-phosphate. In intact ascites cells where the full complement of coenzymes and cofactors is available, glyceraldehyde 3-phosphate may be metabolised to yield 1:3 diphosphoglycerate, or it may be converted to α-glycerophosphate or fructose 1:6 diphosphate. The results reported here point to a close integration of the pentose phosphate pathway and the glycolytic route at the level of triose phosphate.

Another important control mechanism relating the two pathways of glucose metabolism may be found in the competitive inhibition of phosphoglucose isomerase by sedoheptulose 7-phosphate. The reported K_i for this enzyme is 8.6×10^{-6} M [12], well below the concentration found in ascites tumour cells, which is 0.2 mM; the authors state that this inhibition may

well be modified by the concentration of intracellular glutathione and cysteine.

Acknowledgements

The authors are indebted to Mr. B.C.Teo for skilled technical assistance. This work was supported in part by a grant to the Medical School from the British Empire Cancer Campaign.

References

[1] L.A.Sauer, J. Biol. Chem. 243 (1968) 2429.
[2] P.K.Maitra and B.Chance, in: Control of Energy Metabolism, eds. B.Chance, R.W.Estabrook and J.R.Williamson (Academic Press, New York, 1965) p. 157.
[3] I.A.Rose, in: Control of Energy Metabolism, p. 177.
[4] R.Wu, Biochem. Biophys. Res. Commun. 18 (1965) 402.
[5] G.Ashwell and J.Hickman, J. Biol. Chem. 226 (1957) 65.
[6] F.J.Simpson, Canad. J. Biochem. Physiol. 38 (1960) 115.
[7] F.Novello and P.McLean, Biochem. J. 107 (1968) 775.
[8] H-U. Bergmeyer, Methods in Enzymatic Analysis (Verlag Chemie and Academic Press, New York and London, 1965).
[9] E.Racker, in: Methods in Enzymatic Analysis, ed. H-U. Bergmeyer (Verlag Chemie and Academic Press, 1965): (a) p. 107, (b) p. 205.
[10] R.K.Crane and A.Sols, in: Methods in Enzymology, Vol. 1, eds. S.P.Colowich and N.O.Kaplan (Academic Press, New York, 1955) p. 277.
[11] B.L.Horecker, M.Gibbs, H.Klenow and P.Z.Smyrniotis, J. Biol. Chem. 207 (1954) 393.
[12] R.Venkataraman and E.Racker, J. Biol. Chem. 236 (1961) 1876.