BBA 65934

COMPARISON OF THE ACTIVITY OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE IN RABBIT TISSUES

ELLIOTT BONDI, JAMES WATKINS AND MARY E KIRTLEY

Department of Biological Chemistry, University of Maryland, School of Medicine, Baltimore, Md 21201 (U S A)

(Received March 14th, 1969)

SUMMARY

The pH–activity profiles of pure glyceraldehyde-3-phosphate dehydrogenase (EC 1 2 1 12) from rabbit muscle, liver and heart are compared with the profiles obtained in crude extracts of these tissues No significant differences were found among the pure enzymes and the crude extracts with the exception of unusual activity of the crude heart extract at a low pH These findings suggest that there are no significant differences in the properties of this enzyme in different rabbit tissues and that there are no readily detectable alterations of the enzymatic activity in the physiological pH range as the result of interaction with other intracellular components These findings are discussed in relation to the behavior of other glycolytic enzymes

INTRODUCTION

This study was carried out to determine if there are functional differences in the activity of glyceraldehyde-3-phosphate dehydrogenases (EC 1 2 1 12) in the complex environments of different mammalian tissues The approach used here was to measure the activity of the enzyme in crude extracts of various rabbit tissues under standard conditions and over a wide range of pH values and to compare this activity with that of the purified enzymes The situations which we wanted to evaluate were (1) whether the intrinsic activity of glyceraldehyde-3-phosphate dehydrogenase from various rabbit tissues was identical with respect to the parameter chosen, and (2) whether the activity of the enzyme in each crude tissue extract was modified by interactions with other small molecules or proteins

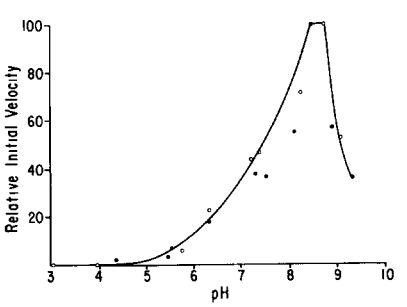
METHODS

Frozen tissues of New Zealand white rabbits were obtained from Pel-Freez Biologicals Each tissue was thawed, minced and suspended in a volume of buffer (0.02 M phosphate, 1 mM EDTA, 1 mM dithiothreitol, pH 7 5) equal to the weight of

the tissue The suspension was homogenized for 2 min in a Sorvall Omni-Mixer at top speed and was centrifuged at 27 000 × *g* for 20 min Then the supernatant solution was filtered through glass wool to give the crude enzyme preparation

Spectrophotometric enzyme assays were carried out in solutions of 2 0 mM NAD, 10 mM sodium arsenate, 2 0 mM DL-phosphoglyceraldehyde and 0 066 M buffer The buffers utilized were citrate, acetate, phosphate, pyrophosphate and glycine covering the pH range 3 0–10 0

Purified rabbit muscle glyceraldehyde-3-phosphate dehydrogenase was purchased from Sigma The purified enzyme from rabbit heart and liver were obtained by chromatography of the crude tissue preparations on cellulose phosphate in a buffer of 0 02 M phosphate, 1 mM EDTA and 1 mM dithiothreitol (pH 6 5) The enzyme was eluted by KCl in the same buffer In typical experiments the enzyme was eluted between 0 15 and 0 2 M KCl The enzyme is routinely eluted just before a peak containing a red protein or proteins In liver preparations the glyceraldehyde-3-phosphate dehydrogenase is often contaminated with a yellow protein A repeat of the chromatographic procedure is usually sufficient to obtain liver glyceraldehyde-3-phosphate dehydrogenase which shows a single band on disc-gel electrophoresis This chromatographic procedure can also be used to purify the enzyme from rabbit muscle



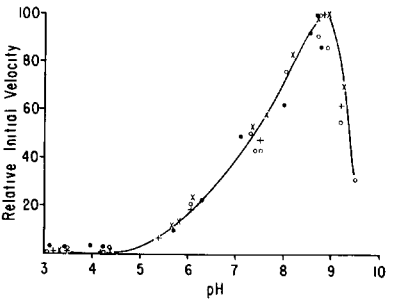


Fig 1 pH–activity profiles of rabbit muscle glyceraldehyde-3-phosphate dehydrogenase Pure rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (○), crude homogenate of rabbit muscle (●)

Fig 2 pH–activity profiles of glyceraldehyde-3-phosphate dehydrogenase from rabbit liver, brain and kidney Crude homogenates of rabbit liver (○), brain (●), kidney (×), pure rabbit liver glyceraldehyde-3-phosphate dehydrogenase (+)

RESULTS AND DISCUSSION

In Fig 1 are shown the pH–activity profiles for pure rabbit muscle glyceraldehyde-3-phosphate dehydrogenase and the crude muscle extract The pH optimum of 8 7 for the pure enzyme was first reported by CORI *et al* [1], and a sharp decrease in enzymatic activity at higher and lower pH values is apparent both in the crude and purified enzyme preparations The pH–activity curves obtained with crude extracts of liver, kidney and brain and pure liver glyceraldehyde-3-phosphate dehydrogenase are shown in Fig 2 These curves are virtually identical and differ only slightly from those of muscle

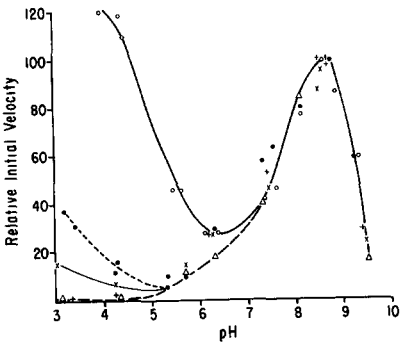


Fig 3 pH–activity profiles of glyceraldehyde-3-phosphate dehydrogenase from rabbit heart. Crude homogenate of frozen heart (○), fresh rabbit heart (●), frozen rabbit heart before (×) and after (+) ultracentrifugation, pure rabbit heart glyceraldehyde-3-phosphate dehydrogenase (△)

The pH–activity profiles of three crude heart extracts and pure heart glyceraldehyde-3-phosphate dehydrogenase are shown in Fig 3 From pH 5 to 10, these curves are virtually identical with those in Fig 2 However at lower pH values, there is an increase in the apparent activity of the enzyme in crude heart extracts This effect has been quite variable from preparation to preparation for as yet unexplained reasons Controls for turbidity or endogenous interfering enzymatic activities, such as alcohol dehydrogenase, have been tested and cannot account for the observed activity The pure heart enzyme shows no corresponding enzymatic activity at a low pH The activity at a low pH is retained by the crude heart extract on storage over a period of several days In one experiment a crude heart extract was centrifuged at 30 000 rev / min for 30 min in a Spinco Model L ultracentrifuge Almost all activity at a low pH was lost during the procedure which suggests that particulate fractions are involved in the unusual activity

The results of this work show that there is very little difference in the pH–activity profiles of pure glyceraldehyde-3-phosphate dehydrogenase isolated from rabbit muscle, liver or heart In addition, there is no difference in the mobility on disc-gel electrophoresis of the purified enzymes as they are obtained by the procedure described above Although these observations in themselves are insufficient to establish that these enzymes are identical, they do indicate that if structural differences do exist, they do not affect the gross structural and functional properties examined here.

Perhaps the more interesting question in a physiological context is whether these enzymes in the more complex intracellular environment are altered in function either by the familiar allosteric mechanisms or by other mechanisms perhaps involving protein–protein or membrane–protein interactions Although the crude homogenate procedures used in this study may not reproduce the intracellular environment, they do permit a rapid survey for such possible modifications of enzyme activity within the cell The experiments reported here indicate that in muscle, liver, brain and kidney, no such interactions occur which affect the activity of the enzymes with respect to the parameters investigated The single exception noted is that of the crude heart extract Although the activity detected at a low pH in these extracts is undoubtedly not physiologically significant in itself, the observation shows that the glyceraldehyde-3-phosphate dehydrogenase activity in the crude extract in this respect is not accounted

for by the behavior of the pure enzyme The fact that part of the low pH activity is lost following ultracentrifugation suggests the involvement of a particulate fraction in this activity

Although there has been no previous report of dehydrogenase activity of glyceraldehyde-3-phosphate dehydrogenase at a low pH, the phosphatase activity of the enzyme from skeletal muscle occurs at pH values below 7 and S-acylation of the enzyme with acetyl phosphate occurs at pH 4 6 (refs 2, 3)

There are now reports in the literature of the purification of rabbit glyceraldehyde-3-phosphate dehydrogenase from skeletal muscle[4,5], brain[6] and a preliminary report on liver[7] From the information available in these papers, there is no indication of major structural differences among these purified enzymes although there are preliminary indications of a difference in the kinetic properties of the liver and muscle enzymes[1] While the purification procedure used in this paper is technically simpler than those published, the resulting enzyme does not appear to be significantly different from those obtained in the multiple-step methods The additional information in this paper leads to the conclusion that the pH dependence of the activity of the enzyme in crude extracts of the tissues is indistinguishable from that of the purified enzymes (with the exception of the heart enzyme at a low pH)

This conclusion leaves two anomalies unresolved At the physiological pH of about 7 4, the activity of glyceraldehyde-3-phosphate dehydrogenase is well below its maximal activity Obviously the low catalytic efficiency of the enzyme can be counterbalanced by increasing the intracellular concentration of the enzyme, but this is accomplished at the cost of some energy expenditure to the cell Unless there are other unknown functions which this enzyme carries out in the cell, it appears either that selective pressures have tolerated the inefficiency of this enzyme or that the local conditions within the cellular environment which may permit a more efficient turnover of the enzyme have not been reproduced experimentally

The second anomaly rests in the question of isoenzymes For the purposes of this discussion we shall limit the definition of isoenzymes to those enzymes derived from a common genetic pool which catalyse the same reaction but differ in primary structure A number of the glycolytic enzymes have now been shown to occur as isoenzymes In mammalian tissues there is now evidence for isoenzymes of hexokinase[8,9], phosphoglucomutase[10], aldolase[11], phosphoglycerate mutase[12], enolase[13,14], pyruvate kinase[15,16] and lactate dehydrogenase[17,18] Most of the other enzymes have not yet been investigated in this respect In most examples where isoenzymes have been shown to occur, the case has been made that the structural differences have been maintained because there are functional differences among the isoenzymes related to the regulation of activity within the different cell types[19] LEBHERZ AND RUTTER[20] have now shown that electrophoretically distinct isoenzymes of glyceraldehyde-3-phosphate dehydrogenase occur in turtle, perch, trout, spinach and yeast but that no such forms occur in rabbit, rat, chicken, honeybee, Euglena or *Escherichia coli* By using the isoelectric focusing technique, KOCHMAN AND RUTTER[6] have been able to demonstrate the existence of microheterogeneity in rabbit muscle and brain glyceraldehyde-3-phosphate dehydrogenase, but it is not yet clear whether these bands represent true isoenzymes Such microheterogeneity might be due instead to different states of aggregation, conformational differences or varying numbers of bound ligands within a population of identical proteins If, as is suggested by our studies, there are no func-

tional differences among the glyceraldehyde-3-phosphate dehydrogenases within a tissue or among different tissues, one must then ask why isoenzymes occur in the other species and have been lost at least in this mammal In a related question, if selective pressures have served to eliminate multiple forms of this enzyme, why have numerous other redundancies in glycolytic enzymes been retained Although the recent advances in biochemistry have focused on the important role of the regulation of enzyme activity in the adaptation of cells to changing environments, the now bewildering multiplicity of the postulated control mechanisms within the glycolytic pathway alone[21] suggests that there may be unifying mechanisms which perhaps can be deduced from a closer examination of the enzymes of this pathway

ACKNOWLEDGMENTS

This work was supported in part by grants from the U S Public Health Service (AM 11428) and National Science Foundation (GB 5925)

REFERENCES

1 G T Cori, M W Slein and C F Cori, *J Biol Chem*, 173 (1948) 605
2 J H Park, B P Meriwether, P Clodfelder and L W Cunningham, *J Biol Chem*, 236 (1961) 136
3 E Mathew, B P Meriwether and J H Park, *J Biol Chem*, 242 (1967) 5024
4 E G Krebs, in S P Colowick and N O Kaplan, *Methods in Enzymology*, Vol 1, Academic Press, New York, 1955, p 407
5 W Ferdinand, *Biochem J*, 92 (1964) 578
6 M Kochman and W J Rutter, *Biochemistry*, 7 (1968) 1671
7 C S Papadopoulos and S F Velick, *Federation Proc*, 26 (1967) 557
8 C Sharma, R Manjeshwar and S Weinhouse, *J Biol Chem*, 238 (1963) 3840
9 L Grossbard and R T Schimke, *J Biol Chem*, 241 (1966) 3546
10 J G Joshi, J Hooper, T Kuwaki, T Sakurada, J R Swanson and P Handler, *Proc Natl Acad Sci U S*, 57 (1967) 1482
11 E Pinholt, T U Rajkumar and W J Rutter, *Proc Natl Acad Sci U S*, 56 (1966) 1275
12 S Grisolia and B K Joyce, *J Biol Chem*, 234 (1959) 1335
13 H Tsuyuki and F Wold, *Science*, 146 (1964) 535
14 P J Dave, R W Kaplan and G Pfleiderer, *Biochemistry*, 345 (1966) 440
15 T Tanaka, Y Harano, F Sue and H Morimura, *J Biochem Tokyo*, 62 (1967) 71
16 W Susor and W J Rutter, *Biochem Biophys Res Commun*, 30 (1968) 14
17 C L Markert and F Moller, *Proc Natl Acad Sci U S*, 45 (1959) 753
18 N O Kaplan, M M Ciotti, M Hamolsky and R E Bieber, *Science*, 131 (1960) 392
19 N O Kaplan, *Bacteriol Rev*, 27 (1963) 155
20 H G Lebherz and W J Rutter, *Science*, 157 (1967) 1198
21 M C Scrutton and M F Utter, *Ann Rev Biochem*, 37 (1968) 249