HORMONAL CONTROL OF THE 'COMPARTMENTATION' OF THE ENZYMES OF THE PENTOSE PHOSPHATE PATHWAY ASSOCIATED WITH THE LARGE PARTICLE FRACTION OF RAT LIVER

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SUMMARY: The influence of alloxan-diabetes, insulin treatment, starvation and refeeding a high carbohydrate diet on the activity of enzymes of the pentose phosphate pathway in the large particle fraction (LPF) from rat liver has been examined. Differential extraction with 0.1M phosphate buffer and with Triton X-100 yielded two fractions, the specific activity of enzymes in the former was modified by hormonal and dietary treatments, while those in the latter remained constant. G6P dehydrogenase and 6PG dehydrogenase activity increased in parallel in the cytosol and the phosphate fraction of the LPF in contrast to transketolase and transaldolase which showed a reciprocal relationship. The possibility that cell metabolites may control binding is discussed.

INTRODUCTION

A considerable bulk of evidence is now available showing that the enzymes of the pentose phosphate pathway may occur in association with the large particle fraction (LPF) from a diversity of tissues [1-7]. In liver, electrophoretic and immunological studies have indicated the existence of different isoenzymic forms of G6P dehydrogenase which occur in variable proportions with the cytosolic, mitochondrial and microsomal fractions [5,3]. Treatment of the LPF from a range of tissues either by freezing and thawing or by Triton X-100 revealed the presence of all six enzymes of the pentose phosphate pathway with their specific activity, in some tissues, approaching that of the cytosolic fraction [7]. Preliminary studies using the fractional extraction procedure of Klingenberg [8] have shown that activity of the oxidative enzymes of the pathway could be detected in the three extracts derived from the LPF of rat liver: a readily-soluble form extracted by incubation with 0.1M phosphate buffer; a form only extractable after mechanical disruption in 0.1M phosphate buffer; a 'latent' form extracted from the residue by treatment with Triton X-100 [7].

To gain further insight into the possible functional significance of this association of the enzymes of the pentose phosphate pathway with the LPF,

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Abbreviations: G6P, glucose 6-phosphate; 6PG, 6-phosphogluconate; LPF, large particle fraction sedimenting at 1.2×10^5 *g*.min.

fractional extraction procedures have been applied to the LPF isolated from the livers of rats subjected to different dietary and hormonal treatments. The conditions selected were those already known to have an effect on the enzymes of the pentose phosphate pathway in the cytosolic fraction, i.e. alloxan-diabetes, insulin treatment, starvation and starvation followed by refeeding a high carbohydrate diet [9].

It has been found that the fraction of the LPF enzymes extracted by mechanical disruption in 0.1M phosphate buffer varied in response to the different hormonal manipulations. Treatment with insulin, or feeding high carbohydrate diet, increased the specific activity of G6P and 6PG dehydrogenases in this fraction and decreased the specific activity of transketolase and transaldolase, relative to control values. In contrast, the fraction of pentose phosphate pathway enzymes revealed by the treatment of the LPF fraction with Triton X-100 had a relatively constant specific activity in all the conditions studied.

The relationship between the activities of the cytosolic enzymes and the enzymes of the LPF extracted with phosphate buffer were also examined. Contrasting patterns of response were observed. Whereas the oxidative enzymes of the pathway (G6P and 6PG dehydrogenases) changed in parallel in the two compartments, the non-oxidative enzymes (transketolase and transaldolase) showed a reciprocal relationship, the fraction associated with the LPF decreasing as that in the cytosolic compartment increased. The possibility that the release and binding of transketolase and transaldolase might be related to the cellular concentration of intermediates, such as pentose phosphates, is discussed.

MATERIALS and METHODS

Animals. The treatment of animals was as described previously by Novello *et al.* [9]. Alloxan-diabetic rats were maintained on insulin for 5 days after injection of alloxan and killed 16 days after the last administration of insulin. The insulin-treated diabetic group received daily injections of protamine-zinc insulin (2 units) for 3 days, starting on day 22 after administration of insulin. Starvation was for 48 hr followed by refeeding a high carbohydrate diet for 3 days.

Fractional extraction procedure. The large particulate fraction from rat liver was isolated by established procedures [10] using 0.25M sucrose. The fraction sedimenting at 120,000 *g*.min was washed four times with 0.25M sucrose to remove, as far as possible, contaminating cytosolic enzymes. This washed LPF was then subjected to fractional extraction procedures essentially as described previously [7,8]. Two fractions were obtained:

(i) *Phosphate fraction*, that fraction of enzyme extracted from the LPF by

Table 1. The specific activities of enzymes of the pentose phosphate pathway in phosphate and Triton X-100 fractions from the LPF of rat liver in different hormonal and dietary conditions.

	Control	Starved	Starved and refed high carbohydrate diet	Diabetic	Diabetic + insulin
			milliunits/mg. protein		
G6P dehydrogenase					
Phosphate fraction	3.6±0.1	3.2±0.3	10.9±1.0 ↑↑↑	3.8±0.1	7.3±1.0 ↑↑
Triton X-100 fraction	10.6±1.5	9.0±1.8	14.0±2.9	9.2±1.0	10.9±0.6
6PG dehydrogenase					
Phosphate fraction	6.3±0.2	5.9±0.6	9.6±1.1 ↑↑	4.8±0.6	15.1±2.1 ↑↑↑
Triton X-100 fraction	8.8±0.8	6.9±0.3	7.2±0.7	9.0±0.3	9.1±1.2
Transketolase					
Phosphate fraction	11.3±0.9	6.1±0.4 ↓↓	8.4±0.3 ↓↓	11.4±0.1	7.4±0.4 ↓↓
Triton X-100 fraction	17.3±2.7	14.6±0.8	13.7±1.2	14.9±1.2	16.3±3.1
Transaldolase					
Phosphate fraction	13.8±1.4	7.1±0.8 ↓↓	6.7±0.7 ↓↓	11.8±1.1	7.1±0.5 ↓↓
Triton X-100 fraction	20.2±2.6	15.8±1.5	18.9±0.8	17.8±1.5	19.9±2.2
			mg. protein/LPF from 1g liver		
Protein content					
Phosphate fraction	8.2±1.0	7.9±0.7	7.4±0.5	9.9±0.3	6.6±0.7
Triton X-100 fraction	2.6±0.3	3.3±0.4	3.3±0.2	4.3±0.6	2.9±0.4

The cell fractions were prepared as described in Methods. Each value is the mean ± SEM of not less than 4 determinations. The direction and number of arrows indicates the direction of change and degree of significance of the differences relative to the control group. Fisher's P values are shown as: ↑, $P < 0.05$; ↑↑, $P < 0.01$; ↑↑↑, $P < 0.001$. Where no symbol is given the differences were not statistically significant.

Table 2. The specific activities of enzymes of the pentose phosphate pathway in the cytosol of rat liver in different hormonal and dietary conditions.

	Control	Starved	Starved and refed high carbohydrate diet	Diabetic	Diabetic + insulin
			milliunits/mg. protein		
G6P dehydrogenase	40.3±3.7	28.6±2.5 ↓	133 ±18 ↑↑	28.9±2.1 ↓	78.5±10 ↑↑
6PG dehydrogenase	45.4±1.4	35.4±1.6 ↓↓	56.3± 2.2 ↑↑	35.9±2.6 ↓	62.6± 2.1 ↑↑↑
Transketolase	17.9±0.9	18.3±1.5	19.3± 0.7	10.4±0.5 ↓↓↓	25.6± 1.1 ↑↑
Transaldolase	14.8±0.4	14.6±0.6	18.3± 0.6 ↑↑↑	11.5±0.5 ↓↓	29.8± 1.6 ↑↑↑

Each value is the mean ± SEM of six determinations. The number of arrows and their direction indicates the direction of change and degree of significance of changes relative to control values, see Table 1.

mechanical disruption in 0.1M phosphate buffer using an Ultraturrax homogeniser (2 min). This is equivalent to the combined readily soluble and soluble fractions described by Klingenberg [8].

(ii) *Triton X-100 fraction*, that fraction of enzyme revealed by treatment of the residue from the Ultraturrax extraction with Triton X-100 (1% final concentration for 1–2 hr).

Estimation of enzymes. The enzymes of the pentose phosphate pathway were estimated as previously described [11,12]. Transketolase and transaldolase were estimated by measuring the rate of formation of glyceraldehyde 3-phosphate in a system linked ultimately to the reoxidation of NADH, appropriate blank values were included to allow correction for any non-specific reoxidation of NADH by LPF [7].

RESULTS and DISCUSSION

The influence of insulin and different dietary regimen on the specific activities of the enzymes of the pentose phosphate pathway in the phosphate and Triton X-100 fractions from the LPF of rat liver is shown in Table 1. A clear distinction is apparent in the reponse of the enzymes in the two fractions. The specific activities of the enzymes occurring in the phosphate fraction varied considerably, in contrast to those of the enzymes in the Triton X-100 fraction which remained relatively constant. Treatment of alloxan-diabetic rats with insulin, or administration of a high carbohydrate diet to starved rats, increased the specific activities of G6P and 6PG dehydrogenases in the LPF-phosphate fraction, but decreased the specific activities of transketolase and transaldolase, relative to control values. The latter enzymes were also markedly depressed following 48 hours starvation.

The enzymes associated with the two fractions of the LPF thus appeared to be independently controlled. The possibility that the LPF-enzymes extractable with phosphate might be related to the cytosolic enzymes, was investigated by comparing the changes occurring in these two 'compartments' in the different hormonal and dietary conditions. The specific activity of the enzymes of the pentose phosphate pathway in the cytosolic fraction are shown in Table 2 and, comparison of these data with those in Table 1, shows that there are parallel changes in the specific activity of G6P and 6PG dehydrogenases. The direct correlation between the activities of the enzymes associated with these two 'compartments' is shown by expressing the results in logarithmic form (Fig.1) when a good linear correlation is found.

The interrelationship between the cytosol and the phosphate fraction from the LPF with respect to the distribution of transketolase and transaldolase contrasts sharply with that found for the two oxidative enzymes. In the case of the non-oxidative enzymes of the pentose phosphate pathway, there is a

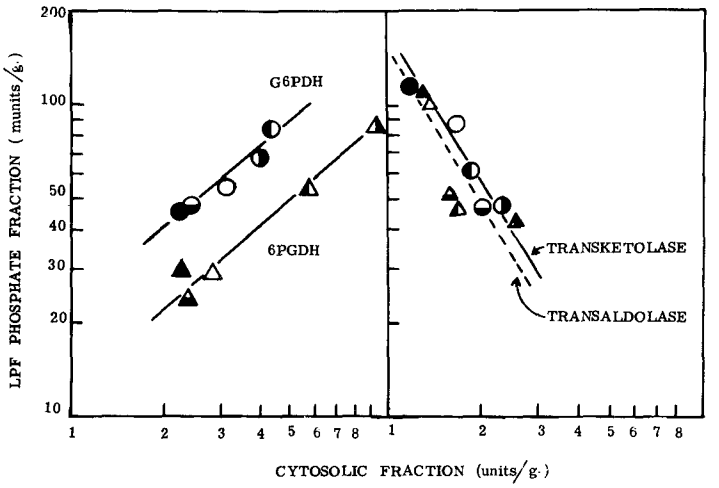

Fig.1. The relationship between cytosolic and LPF phosphate fraction enzymes of rat liver in different hormonal and dietary conditions.

The values, as milliunits in the LPF phosphate fraction or as units in the cytosolic fraction derived from 1g liver, are plotted in logarithmic form; each point is the mean of not less than 4 determinations. The different conditions are represented by: ○△, control; ●▲, alloxan-diabetic; ◐◭, alloxan-diabetic treated with insulin; ◒▲, starved 48 hr; ◑◭, starved and refed high carbohydrate diet. The circles show G6P dehydrogenase (G6PDH) and transketolase; triangles show 6PG dehydrogenase (6PGDH) and transaldolase.

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reciprocal relationship between the enzymes extracted from the LPF with phosphate and the cytosolic enzymes. This is shown in Tables 1 and 2 and in Fig.1. These results suggest that there may be a release of enzymes from the LPF in certain hormonal and dietary conditions (those associated with high levels of insulin), perhaps mediated by an accumulated metabolite. A model for such a system may be seen in the control of hexokinase binding to mitochondria, the release and binding of the hexokinase being controlled by G6P, P_i and Mg^{2+} [13].

In examining metabolite changes in relation to enzyme binding, particular attention centres upon substrates, products and effectors of that particular enzyme. In the case of transketolase, an obvious candidate is pentose phosphate. Figure 2 shows that a reciprocal relationship exists between the cell content of pentose phosphates and transketolase and transaldolase activity in the phosphate fraction, with correlation coefficients for the reciprocal of the pentose phosphate content relative to the percentage of bound transketolase and transaldolase in the LPF of 0.96 and 0.97 respectively, both values being highly significant. In this present context it should, however, be noted that if substrates can, indeed, cause release and binding of enzymes in the LPF, then the possibility of artefactual distribution profiles has to be remembered since, of

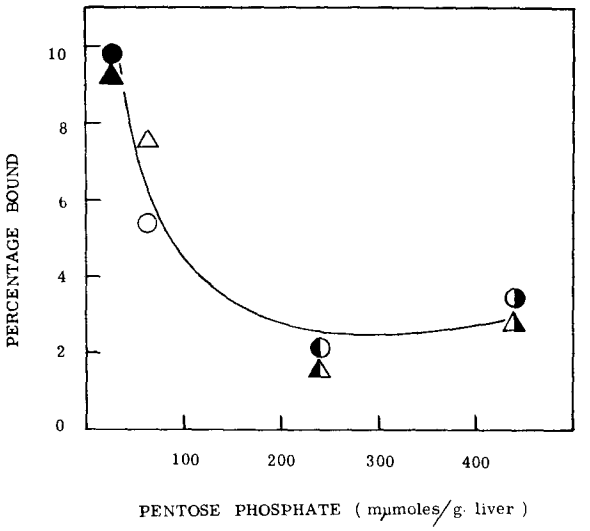

Fig.2. The relationship between binding of transketolase and transaldolase to the LPF of rat liver and the pentose phosphate content of the tissue in different hormonal and dietary conditions.

Percentage bound: the units of transketolase and transaldolase activity recovered in the phosphate fraction of the LPF as a percentage of the units found in the cytosol, all values compared on the basis of units in the fraction derived from 1g liver.
Pentose phosphates: the sum of ribose 5-phosphate, ribulose 5-phosphate, and xylulose 5-phosphate; values taken from Greenbaum *et al.* [16]. Mean values are shown as: circles for transketolase and triangles for transaldolase. The different groups are represented by: ○△, control; ●▲, alloxan-diabetic; ◐◭, alloxan-diabetic treated with insulin; ◑◭, starved and refed high carbohydrate diet.

necessity, the tissue fractionation occurs initially in the presence of substrates. The studies of Vallejo *et al.* [14] on the association of brain hexokinase with mitochondrial membranes illustrates this point with particular emphasis on the effects of the homogenisation medium, as do the results of Rendon and Waksman [15] on the effect of cations on the intramitochondrial release and binding of mitochondrial aspartate aminotransferase and malate dehydrogenase.

However, if the pentose phosphates are concerned with the release of transketolase and transaldolase from the LPF, then, for this to be a significant control mechanism it would be necessary for the mid-point of the response to approximate to the tissue content of pentose phosphates in the control animals. Thus, physiological changes of the pentose phosphate content around the control value could modify the binding of the enzymes to the LPF. This requirement appears to be met in the present study. The definitive experiment would be the

demonstration of the release of transketolase and transaldolase from isolated mitochondria by pentose phosphates *in vitro*. Preliminary experiments have shown that approximately 10% of the transketolase and transaldolase activity of the phosphate fraction from the LPF can be released by incubation of isolated mitochondria with 0.5mM ribose 5-phosphate for 30 min at 30°; G6P in the same concentration was only about half as effective despite the fact that the ribose 5-phosphate level used approximates to the cellular content of this metabolite while the G6P level used is some 4 times higher than the cell content (both values for the livers of rats fed a high carbohydrate diet [16]). It is probable that, in these initial experiments, optimal conditions for the release and stability of transketolase and transaldolase were not achieved. Nevertheless, they do suggest the possibility that the control of the association of these two enzymes with the LPF might have some resemblance to that regulating the binding of hexokinase. The existence of other factors modifying the transketolase and transaldolase of the LPF-phosphate fraction is indicated by the aberrant behaviour of the LPF isolated from the livers of starved rats. In this condition there is a low activity of transketolase and transaldolase (2.4 and 3.2% bound respectively) concurrent with a low pentose phosphate content of the liver (30 mμmoles/g) (*c.f.* alloxan-diabetic rats; see also Fig.2).

The relationship of long-chain acyl-CoA derivatives to mitochondrial function has been the subject of a number of studies [17,18] and it seems possible that the exceptional level of such derivatives in the livers of starved rats is related to the apparent low transketolase and transaldolase associated with the LPF; *in vitro* studies should clarify this point.

Neither the G6P dehydrogenase nor the 6PG dehydrogenase association with the LPF appears to be related to the steady-state concentration of their respective substrates.

The divergent behaviour of the enzymes of the oxidative and non-oxidative segments of the pentose phosphate pathway with respect to association with the LPF and the interrelationship with the cytosolic compartment, are of interest in relation to the concept advanced by Horecker [19] that the pentose phosphate pathway may operate as two parallel mechanisms for the conversion of hexose monophosphate into pentose phosphate rather than as a cycle; thus, independent control mechanisms, rather than constant proportionality, might be advantageous.

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