DEFECTIVE ALLOSTERIC REGULATION OF PHOSPHOFRUCTOKINASE IN GENETICALLY-OBESE MICE

S.S. KATYARE and J.L. HOWLAND
Committee on Biochemistry, Bowdoin College
Brunswick, Maine 04011, USA

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1. Introduction

There exists a recessive mutant of the laboratory mouse that is characterized by excessive deposition of fat, apparently resulting from increased rates of lipid synthesis [1,2]. Conversion of acetate to carbon dioxide is diminished while its conversion to lipid is significantly enhanced [3], a diversion that occurs despite normal or increased levels of mitochondrial enzymes concerned with oxide-reductions [4]. These animals exhibit a considerable variety of metabolic alterations including loss of control of gluconeogenesis [5], altered levels of blood lipids and sugars [6,7] and both hypersecretion and loss of sensitivity to insulin [8]. Although an array of metabolic changes is observed, it is clear that the mutation occurs at a single locus [1] so that it is worthwhile to look for a single metabolic defect underlying the condition. The present communication reports altered allosteric control of the important regulatory enzyme, phosphofructokinase, and suggests that such a change could, in principle, account for the obese condition of these mice.

2. Materials and methods

Mice of strain C57BL/6J-ob (obese) and normal controls were obtained from the Jackson Laboratory, Bar Harbor, Maine. Mice were killed by decapitation and a liver extract was obtained by homogenizing with a Teflon-glass homogenizer in a medium containing 20 mM Tris–HCl, pH 8.0, 5 mM $MgSO_4$, and 0.1 mM EDTA. Phosphofructokinase was partially purified by ammonium sulfate fractionation as described by Underwood and Newsholme [9].

Activity was measured essentially as described by Paetkau and Lardy [10] with the assay medium containing 33 mM imidazole, pH 7.0, 1 mM ATP, 4 mM $MgSO_4$, 2 mM fructose-6-phosphate, 50 mM KCl, 1 mM dithiothreitol, 160 μM NADH, 0.1 ml of a solution containing saturating amounts of aldolase, α-glycerolphosphate dehydrogenase, and triosephosphate isomerase, the enzymes having been dialyzed previously. The reaction mixture included either 0.1 ml of the crude homogenate or from 40–60 μl of the purified enzyme so that the optical density change was about 0.1/min. The reaction was followed in a total volume of 1.5 ml at 25°C using a Perkin–Elmer Model 356 spectrophotometer set at 340 nm. Protein was estimated by the biuret reaction; in the case of purified enzyme, protein was precipitated with 10% trichloroacetic acid prior to estimation to avoid interference from ammonium sulfate.

3. Results and discussion

Activity of phosphofructokinase in crude liver homogenates was elevated between two and four times in obese mice when compared to normal controls (table 1). When ammonium sulfate fractionation of the extract was carried out, the partially purified enzyme from the obese animals exhibited the same elevation in specific activity, comparable degrees of

Table 1
Phosphofructokinase activity in normal and obese mouse liver. Reaction conditions are as described in the methods section

		Specific activity (μmoles/min/mg)	
		Crude extract	Purified enzyme
Normal			
	a	0.0012	0.046
	b	0.0028	0.076
Obese			
	a	0.0046	0.196
	b	0.0046	0.136

purification having been obtained in each case (about 40-fold).

Since allosteric inhibition of phosphofructokinase by ATP and citrate represents an important mode of regulation of the glycolytic pathway [11] it is of interest to examine the influence of these compounds upon activity of the purified enzyme. Fig. 1 shows the characteristic activity curve for phosphofructokinase with changing concentration of ATP, where the left hand (ascending) portion reflects the role of ATP as a substrate while the right hand portion reflects its ability to serve as allosteric inhibitor. It is clear that ATP is a much less effective inhibitor of activity in the case of enzyme obtained from obese mice. A similar situation occurs with regard to citrate inhibition, where comparable concentrations produce less inhibition in the case of the obese animals (table 2). Thus, a second element of the regulation of the flow of carbon into the tricarboxylic acid cycle appears to operate with diminished sensitivity.

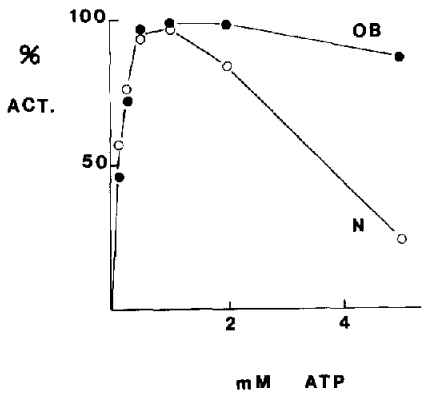

Fig. 1. The influence of ATP concentration on activity of partially purified liver phosphofructokinase. Reaction conditions were described in the methods section. N denotes enzyme isolated from normal mice; OB, enzyme from obese animals. Activity is expressed as percent of that obtained with optimal ATP which was 69 nmoles/min/mg in the case of normal mice and 190 nmoles/min/mg in that of obese.

A decline in the sensitivity with which phosphofructokinase is modulated by citrate and ATP would be expected to lead to increased synthesis of acetyl CoA and citrate. Since liver mitochondria from obese mice exhibit greater than normal oxidative capability as well as enhanced rates of oxidative phosphorylation (ref. [4] and unpublished experiments from this laboratory), there should be increased availability of ATP. High intracellular concentrations of both ATP and citrate would be likely to favor net lipid synthesis by way of the inducible enzyme, citrate lyase, [12] for which both compounds are substrates. In this connection it is worth observing that the next enzyme in the route from citrate to fatty acids in acetyl CoA carboxylase which has also been shown to be elevated in obese mice [13].

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Table 2
Inhibition of phosphofructokinase by citrate

Citrate (mM)	% Total activity	
	Normal	Obese
0	100	100
1	100	100
2	65	88
5	20	69
10	2	18

Reaction conditions were as described in the methods section with citrate concentration as shown. In this experiment, total activity was 0.054 and 0.170 μmoles/min/mg for normal and obese animals, respectively.

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