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MULTIPLE THERMAL DISCONTINUITIES IN GLUCOSE-6-PHOSPHATASE ACTIVITY

LYNN S. GRINNA

Department of Biology, University of California, Los Angeles, Calif. 90024 (U.S.A.)

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Summary

The temperature dependence of glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase EC 3.1.3.9) was studied in rat liver and kidney microsomal fractions. Arrhenius plots were non-linear and showed four distinct discontinuities in enzyme activity over the temperature range 2—41°C. The discontinuities occurred at approx. 39, 30, 20 and 12°C in the liver and were similar to this in the kidney. Changes in the energy of activation for the enzyme were noted at approx. 20°C in both tissues. The multiple discontinuities in glucose-6-phosphatase activity are viewed as a reflection of complex reorganization and/or change in physical state of the membrane components, primarily lipid.

Introduction

The dependence of membrane-bound enzymes, such as glucose-6-phosphatase, on interaction with other membrane components has been demonstrated in a variety of ways [1—5]. Membrane lipid content has been shown to be one of the major components involved in the regulation of the catalytic activity of several membrane-bound enzymes [1—5]. Further, it has recently been shown that the physical state (crystalline-liquid crystalline) of the membrane lipid also regulates the catalytic function, as well as the distribution and mobility of membrane components [6—9].

The physical state of membrane lipid has been measured by electron spin resonance (ESR) of hydrophobic probes [6,7,10] as well as by other techniques [8,11—15]. These techniques have shown that membrane lipids go through temperature-dependent phase separations. The relative sharpness of the separations depends on the complexity of the system being studied [6,10]. The more complex the lipid component mixture, such as that present in membrane systems, the broader the transition in physical state [6,10].

The activities of several membrane-bound enzymes have been found to change with temperature such that discontinuities in activity occur at specific temperatures [6,7,9]. Such discontinuities have been reported for membrane-bound enzymes of bacteria [6,8,11], cultured cells [6,7] and animal cell membranes [9,12]. These discontinuities in enzyme activity have been correlated with changes in the physical state of membrane lipid.

Recent evidence has indicated that membranes containing complex lipid mixtures generally show multiple discontinuities in lipid phase behavior and in enzyme activity when such measurements are made at approx. 1 °C intervals [6]. Using a 4 °C temperature interval, a single temperature-dependent discontinuity in enzyme activity has been reported for glucose-6-phosphatase [16]. The present studies were undertaken to investigate the thermal properties of this enzyme when measurements are taken at approx. 1 °C intervals or less.

Methods and Materials

Male Sprague-Dawley rats (6 months old), maintained on Purina Laboratory Diet, were used in all experiments. Each experiment utilized the tissues of 3—5 animals. Six separate experiments were performed on each tissue. The microsomal fractions of liver and kidney were isolated as previously described [17] and were washed one time. The fractions were used following no more than 12 h storage on ice or as frozen samples. Fresh and frozen preparations gave the same results. Assays for glucose-6-phosphatase were carried out using 0.5 mg microsomal protein, 40 mM glucose 6-phosphate, 0.1 M acetate/0.1 M succinate buffer, pH 6.1, in a final volume of 2 ml. Substrate was not limiting at any temperature. Initial velocities were measured and activity was linear with time at all temperatures. Temperature was controlled to ±0.1 °C. Above 41 °C the enzyme was inactivated. Inorganic phosphate was determined by the method of Youngburg and Youngburg [18] after centrifugation of trichloroacetic acid-precipitated samples. Protein was determined by the method of Lowry et al. [19]. All reagents were purchased from Sigma Chemical Co., St. Louis, Mo.

Results

The effects of temperature on the activity of glucose-6-phosphatase were determined in liver and kidney microsomal fractions. The Arrhenius plots describing the relationship between activity of the enzyme and temperature indicated four temperature-dependent discontinuities. In the liver these discontinuities were seen at approx. 39, 30, 20 and 12 °C (Fig. 1, Table I). In the kidney similar results were obtained and discontinuities were seen at approx. 38, 31, 21 and 11 °C (Table I). Identification of discontinuities may seem tenuous on the basis of individual experiments such as seen in Fig. 1, however, taken collectively the data of the six experiments leave no doubt that multiple discontinuities do exist and the temperatures of such discontinuities are reproducible (see insets A and B of Fig. 1). There was only slight variation as to the temperature at which these discontinuities were observed. Table I summarizes the values obtained for the four temperature discontinuities of all experiments. Although it would appear that a straight line could be fitted to the experimen-

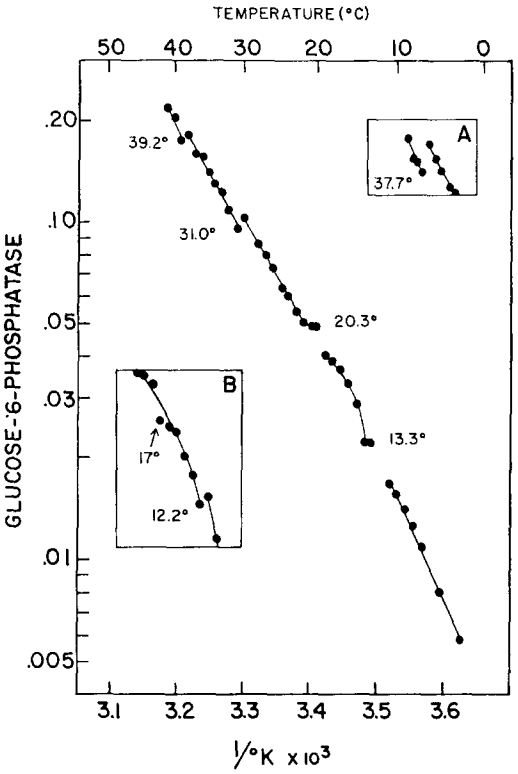

Fig. 1. Arrhenius plot of liver glucose-6-phosphatase. The Arrhenius plot is from a single representative experiment run in duplicate. Glucose-6-phosphatase activity is given in μmol inorganic phosphate released/min per mg microsomal protein and is plotted as a function of the reciprocal of the absolute temperature. Insets A and B are from other single experiments. The enzyme activity axis for the insets is the same as that for the main plot. No temperature axis is given for the insets. Points are at approx. 1 °C intervals. Indicated temperatures (in °C) apply to the upper temperature of the discontinuity. Variation between duplicate samples was negligible and smaller than the symbol size used to illustrate the experimental points.

tal points above 20°C and another straight line fitted to those below 20°C, such forced fit of straight lines has led to misinterpretation of data, as discussed by Lyons [20]. For this reason, certain portions of the Arrhenius plots are illustrated as curves rather than as straight lines (Fig. 1). A detailed theoretical explanation for such experimental observations has recently been advanced [10].

TABLE I

DISCONTINUITIES IN ARRHENIUS PLOTS

Values were determined from six separate experiments performed in duplicate. Values are given ± S.D.

	Temperature of discontinuity (°C)			
Liver	38.6 ± 0.8	30.2 ± 0.9	20.4 ± 0.7	12.3 ± 1.1
Kidney	38.3 ± 0.7	30.5 ± 0.6	21.3 ± 1.1	10.5 ± 0.6

The discontinuities were identified only if seen in all six experiments performed. In several experiments, irregularities in enzyme activity were noted in the temperature range 14—17°C. An example of this type of irregularity is given in Inset B of Fig. 1 and is indicated by an arrow. The irregularities were not seen in all experiments and are, therefore, not considered to be discontinuities. The irregularity is pointed out only as it may indicate a rearrangement of membrane components at that temperature.

Apparent energies of activation were calculated for glucose-6-phosphatase activity between all points of discontinuity from 2 to 41°C. The only discontinuity associated with a significant change in the energy of activation was that occurring at 20°C. The energy of activation values obtained were 14.1 ± 0.8 and 18.9 ± 0.4 kcal/mol in the liver and 13.3 ± 0.6 and 18.1 ± 0.9 kcal/mol in the kidney, above and below 20°C, respectively.

Discussion

Glucose-6-phosphatase of rat liver and kidney displayed four thermal discontinuities in activity on Arrhenius plots. The temperatures at which the discontinuities occurred were similar in the two tissues. The temperatures at which these discontinuities in enzyme activity occurred are in agreement with discontinuities seen in ESR spectra obtained on plasma membrane and endoplasmic reticulum of cultured mouse LM cells and on plasma membrane of chick cells [6,7]. Since it is known that glucose-6-phosphatase is modulated in its activity by membrane lipid, it is therefore suggested that the discontinuities seen in this enzyme activity are due directly or indirectly to alterations in, or rearrangement of, membrane components, primarily lipid, at the temperatures of discontinuity.

Single temperature-dependent breaks have been reported for the activity of several enzymes [12,16,21,22]. The most frequently reported temperature of such single breaks is at approx. 20°C. One such temperature-dependent break has been reported for glucose-6-phosphatase of guinea pig liver microsomes [16]. Such reports of single temperature-dependent breaks suggest that assaying enzyme activity at 3—6°C intervals reveals only the critical temperature at which change occurs in the energy of activation of the enzyme. It has been suggested that it is only when ESR spectra and/or enzyme activity measurements are taken at 1°C intervals or less that multiple discontinuities and discontinuities which do not change the slope of the Arrhenius plot become apparent [23]. In the present studies the energy of activation changed at approx. 20°C. This change in apparent energy of activation, although small, probably represents the only temperature at which conformational change in the enzyme may occur. Such conformational changes have been proposed to account for the changes seen in energy of activation [9,16,20,22].

Complex lipid mixtures, such as those of membranes, display broad boundaries of lipid phase separation and non-linear Arrhenius plots [7,8,10,13,24]. Multiple discontinuities in ESR spectra are thought to reflect multiple compartments of lipid within the membrane [6,24]. Such compartments could be due to formation of lipid clusters or to crystalline-liquid crystalline phase separations. It is unclear as to whether multiple discontinuities in

ESR spectra represent boundaries of single transitions or of separate transitions. It has recently been proposed that certain characteristic discontinuities (which are similar to those reported in this study) represent broad phase boundaries for the inner and outer monolayers of the membrane bilayer [6,7].

Multiple thermal discontinuities in enzyme activity are viewed as being a reflection of the complex and as yet poorly understood changes in the physical state or distribution of the membrane components, primarily lipid, affecting the enzyme, [6,7,10,24]. The discontinuities in glucose-6-phosphatase activity observed in this study are interpreted as reflecting such change in the interaction of the enzyme with other membrane components.

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