MINIREVIEW

Recent Advances in Hepatic Glucose 6-Phosphatase Regulation and Function

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Extensive progress has been made in the past 30 years in our understanding of "glucose 6-phosphatase" (D-glucose-6-P phosphohydrolase; EC 3.1.3.9). Comprehensive reviews of the enzyme, its functions, and its regulation were written in 1974 (1) and 1976 (2). Concepts of structure/function interrelationships were reviewed by Sukalski and Nordlie (3) in 1989. Burchell considered in detail the molecular basis for several variants of Type 1 (4–6) glycogenosis which have been characterized in the past 12 years. Our own concepts of roles of both hydrolytic and synthetic activities of hepatic glucose 6-phosphatase in the fine tuning and retuning of blood glucose in health and disease have been reviewed most recently in 1985 (7).

Significant levels of glucose 6-phosphatase have been observed in livers from a large number of mammals, birds, amphibia, reptiles, fish, and arthropods that have been examined (8–10). While most abundant in liver and kidney, true glucose 6-phosphatase in lesser amounts also has been found in the brain, small intestinal mucosa, pancreas, adrenals, brain, testes, spleen, lung (8, 11), pancreatic islets (12), and gallbladder (13).

Our purpose here was to focus upon the hepatic enzyme and to review briefly developments since 1983 regarding what is now often termed the glucose 6-phosphatase system. We will place an emphasis on the potential for regulation inherent in the several components now identified, and upon roles and regulation of biosynthetic as well as the more generally recognized hydrolytic activity of the enzyme. By choice, we will focus upon our own work and concepts, with the inclusion of some selected studies of others.

Classical Role of Glucose 6-Phosphatase

Glucose 6-phosphatase, a membrane-bound enzyme found most abundantly in liver and kidney endoplasmic reticulum, catalyzes the hydrolysis of glucose-6-P (Reaction 1) (3, 14). Thus, it serves to release free glucose in the terminal reaction common to gluconeogenic and glycogenolytic processes (1, 2, 14). Its critical role in blood glucose homeostasis is well recognized (1, 2, 14).

Multiple Activities of Glucose 6-Phosphatase

Work, much of it in our own laboratory beginning in 1961 (10, 15), has established glucose 6-phosphatase as a multifunctional enzyme. It is capable not only of catalysis of the hydrolysis of glucose-6-P (Reaction 1) and inorganic pyrophosphate (PP_i) (Reaction 4) but of glucose-6-P synthesis via potent phosphotransferase activity (e.g., Reactions 2 and 3) as well (1, 2). The V_{max} of this biosynthetic activity may equal or actually exceed that of the enzyme's hydrolytic capacity (16). We believe these novel catalytic functions for the enzyme are important for at least two reasons: (i) They may provide an alternative to insulin-dependent glucokinase for hepatic glucose phosphorylation (1, 7), and (ii) they serve as specific probes in the study of the several components and functions of what is now known to be the glucose 6-phosphatase *system* (3, 4, 17, 18).

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$$\text{Glucose-6-P} + H_2O \rightarrow \text{glucose} + \text{inorganic orthophosphate } (P_i) \quad [1]$$

$$\text{Carbamyl-P} + \text{glucose} \rightarrow \text{glucose-6-P} + NH_3 + CO_2 \quad [2]$$

$$PP_i + \text{glucose} \rightarrow \text{glucose-6-P} + P_i \quad [3]$$

$$PP_i + H_2O \rightarrow 2\ P_i \quad [4]$$

The Multicomponent Glucose 6-Phosphatase System

Studies initiated in our laboratory in the early 1960s (19–22) and continued by ourselves (reviewed in Ref. 3) and by the groups of Arion (18, 23; reviewed in Ref. 3) and Burchell (4, 5; reviewed in Ref. 3) have led to the view that glucose 6-phosphatase is not a single enzyme protein, but rather is a multicomponent enzyme *system*. At least six components have been identified to date (3, 5, 6), and more seem probable (6, 24, 25). Figure 1 depicts current concepts of structure/function relationships of the glucose 6-phosphatase system. The glucose 6-phosphatase catalytic unit is embedded within the lipid-rich membrane of the endoplasmic reticulum in such a way as to be inaccessible directly to substrates from the cytosol of the cell. An auxiliary protein, the "stabilizing protein," gives stability and Ca^{2+} sensitivity to the catalytic unit. Three membrane-bound substrate transport proteins, or "translocases," permeate the membrane and transport in a highly specific fashion glucose-6-P (translocase T_1); carbamyl-P, PP_i, or P_i (translocase T_2); and glucose (T_3). Thus, glucose-6-P hydrolysis, e.g., is accomplished by transport of glucose-6-P from the cytosol to the lumen of the endoplasmic reticulum by translocase T_1, reaction with water at the catalytic unit to yield products P_i and glucose, and finally transport of P_i (via T_2) and glucose (via T_3) to the cytosol. Hydrolysis of PP_i (Reaction 4) involves transport of PP_i from the cytosol to the lumen via T_2, hydrolysis by the catalytic unit, and transport of product P_i to the cytosol by reversed T_2 action. Synthesis of glucose-6-P via phosphotransferase action of the glucose 6-phosphatase system (Reactions 2 and 3) involves transport from the cytosol to the lumen of carbamyl-P or PP_i (via T_2) and of glucose (via T_3); reaction of carbamyl-P with glucose to produce glucose-6-P at the catalytic unit; and transport of product glucose-6-P from the lumen to the cytosol (via T_1). All of the above transpire with intact microsomes as subject of study; when the permeability barrier imposed by the microsomal membrane is disrupted, as with detergents, the catalytic unit alone is involved (3, 5, 18, 23).

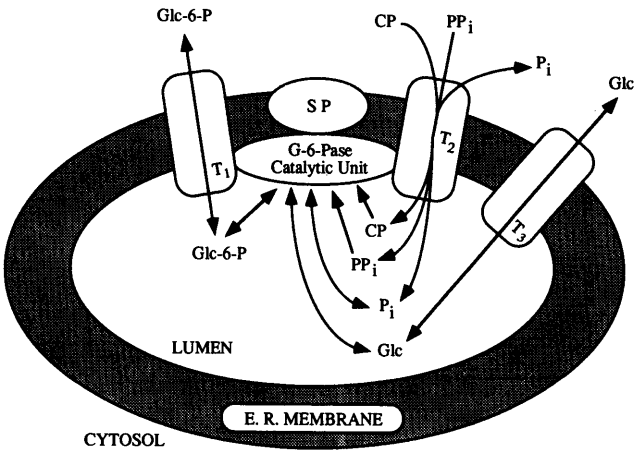

Figure 1. Structure-function relationships of the microsomal glucose 6-phosphatase system according to the substrate translocase-catalytic unit concept. A cross-section of the membrane of the endoplasmic reticulum (E.R. membrane) is indicated. SP indicates the stabilizing protein; catalytic unit is the glucose 6-phosphatase enzyme; and T_1, T_2, and T_3 are substrate/product transport proteins with the indicated specificity. Two forms of T_2 with differing specificity, $T_{2\alpha}$ and $T_{2\beta}$, have been proposed (17, 24, 25, 36). Additional details are given in the text. Modified from Foster *et al.* (49).

Purification and Characteristics of Individual Components of the Glucose 6-Phosphatase System

A catalytically inactive peptide with a molecular mass of 36.5 kDa has been purified from rat liver microsomes and identified immunochemically and on the basis of lability and labeling with radioactive substrates as the catalytic unit of glucose 6-phosphatase (26). Speth and Schulze (27) have recently reported the purification to apparent homogeneity of a rat liver peptide, molecular mass 35 kDa, which they conclude is active glucose 6-phosphatase. Recovery was only 3% of initial activity, and substrate specificity raises some question as to the peptide's enzymic identity.

One form of translocase T_2, the P_i/PP_i translocase protein, has been identified and purified with the use of antibodies raised against the rat mitochondrial phosphate/hydroxyl ion antiport protein (28). A subunit molecular mass was determined as 37 kDa. A glucose transporter has been identified in, and isolated from, rat hepatic microsomes (29, 30). This translocase, T_3, has been termed GLUT 7 because of its similarities to plasma-membrane facilitative glucose transporters (29, 30). A subunit molecular mass of 52 kDa was determined, and antibodies raised against the glucose transport-protein were used to screen a rat liver cDNA library. Sequence analysis revealed that the largest clone isolated was 2161 base pairs in length, coding for a protein of 528 amino acids (30).

Radiation inactivation analysis (31) supports a functional and chemical linkage between several components of the glucose 6-phosphatase system. A target molecular mass of about 75 kDa was observed applicable with all of glucose-6-P phosphohydrolase, mannose-6-phosphate phosphohydrolase, and carbamyl-P:glucose phosphotransferase activities. These observations were made consistently, with untreated and

disrupted microsomes derived from both normal control and diabetic rats (31).

The Need for Regulation of Glucose 6-Phosphatase

Bioregulation of glucose 6-phosphatase catalysis is strongly indicated on at least three bases. (i) Glucose 6-phosphatase, along with hepatic glucokinase, has the potential for metabolically purposeless, energy-wasting "nonsense" or "futile" cycling (Fig. 2). The combined, unconstrained sequential action of the latter plus the former would serve to hydrolyze ATP without a net biosynthetic action (32) (see Reactions 5–7). (ii) Intrinsically, glucose 6-phosphatase is

$$\text{ATP} + \text{glucose} \rightarrow \text{glucose-6-P} + \text{ADP} \quad [5]$$

$$\text{Glucose-6-P} + \text{H}_2\text{O} \rightarrow \text{glucose} + \text{P}_i \quad [6]$$

$$\text{Net: ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_i \quad [7]$$

between 15 and 60 times as active, at saturating substrate concentrations, as is glucokinase (33). Hence, any semblance of a balance between glucose phosphorylation via glucokinase and glucose-6-P hydrolysis by glucose 6-phosphatase (Fig. 2) requires constraints on the latter (even in the presence of recently described activation of the former [34]). (iii) Logically, because both glucose-6-P hydrolysis and biosynthesis are possible by actions of glucose 6-phosphatase, discriminant regulation of the phosphohydrolase (Reaction 1) and phosphotransferase activities (Reactions 2 and 3) should exist.

Multiple Opportunities for Bioregulation of the Glucose 6-Phosphatase System

The multiplicity of components of the glucose 6-phosphatase system as it is recognized today to exist in

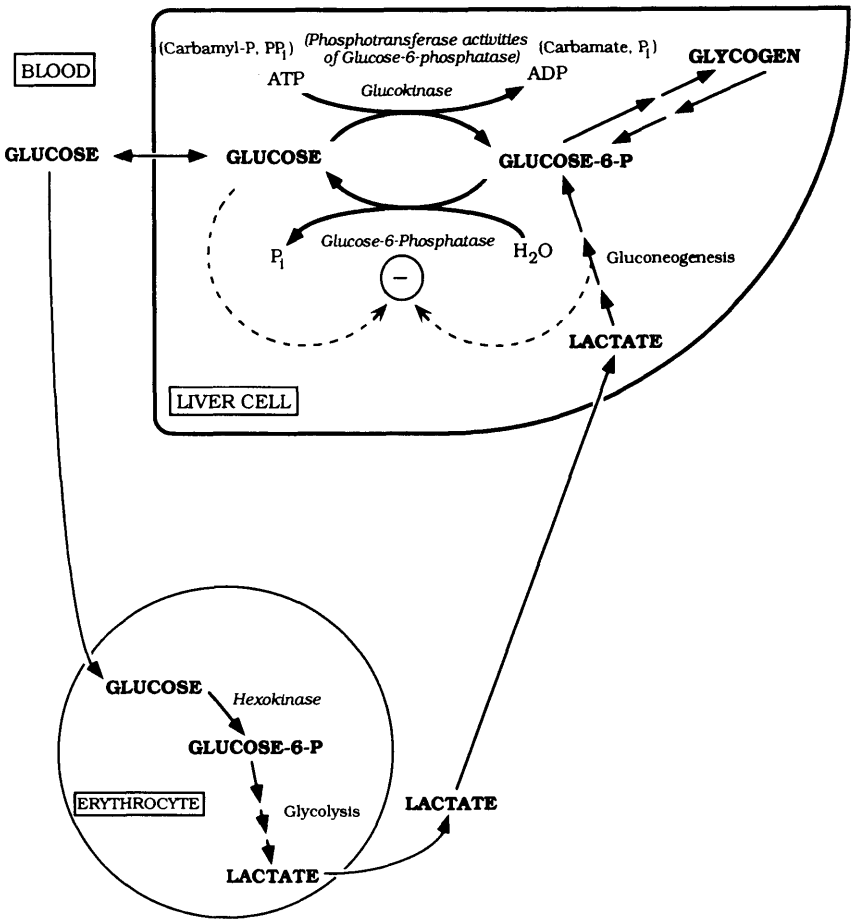

Figure 2. Interrelationships among the liver, blood, and peripheral tissues (here arbitrarily represented by the erythrocyte) with respect to glucose flow and utilization. Net rate and direction of flux of glucose between blood and the hepatocyte are determined by relative rates of glucose phosphorylation (by glucokinase) and glucose-6-P hydrolysis (by glucose 6-phosphatase). Both direct and indirect pathways of hepatic glycogenesis from glucose are represented. The former involves the direct flux within the hepatocyte from glucose to glycogen; the latter involves glycolysis in some extrahepatic tissue (erythrocyte) followed by transport of resultant lactate to the liver, where it is converted to glycogen by the glyconeogenic process (56). Inhibition of glucose-6-P phosphohydrolase by combined action of glucose plus some undefined gluconeogenic intermediate is believed to expedite the formation of glycogen by either route. Phosphotransferase activity of the glucose 6-phosphatase system is proposed by the authors to replace, supplement, or complement glucokinase in hepatic glucose phosphorylation (1, 2, 7, 33). See text for additional details. Modified from Sukalski and Nordlie (52) and Nordlie (7).

the membrane of the endoplasmic reticulum (see Fig. 1 and text, above) presents a multiplicity of possibilities for control, in a highly discriminant manner, of the several activities of the enzyme. Modifiers—classic inhibitors and activators, allosteric modulators, covalent modifications, membrane-associated conformational changes and the like—all may impact upon the enzyme system and its ultimate activity through actions at the catalytic unit, stabilizing protein, or any of the three translocase components. The credibility of the model, as well as potential for regulation of the several components of the system, has been demonstrated in recent studies including (i) characterization of genetic defects (35–37), (ii) rationalization of changes in kinetic characteristics in fasting, diabetes, and glucocorticoid administration (3, 38), and (iii) correlation of glycogenic effects of certain metabolites and other compounds with *in vivo* inhibitions of glucose-6-P phosphohydrolase activity (39–41).

Inborn Defects in the Glucose 6-Phosphatase System

To date, the congenital absence of the functional catalytic unit (Type 1a glycogenosis), translocase T_1 (Type 1b glycogenosis), translocase T_2 (Type 1c glycogenosis), stabilizing protein (Type 1aSP glycogenosis), as well as lesser aberrations in T_2 (pseudotype Ic glycogenosis) have been demonstrated (4–6, 37). In most instances, the defect indicated by immunochemical means has been correlated with altered function predicted by the model in Figure 1 (4–6, 37).

The existence of a novel variant of Type 1 glycogenosis, now termed Type Ic glycogenosis, was first demonstrated in the authors' laboratory (17). Clinical symptomology was that of the Type 1 (von Gierke's disease) patient, but glucose 6-phosphatase determined in disrupted hepatic microsomes was normal. Activities in Reactions 1–4, as well as inhibitors P_i and glucose, were used to probe the glucose 6-phosphatase system of both intact and detergent-disrupted microsomes. The absence of T_2 function, as it transports PP_i, carbamyl-P, and P_i from the cytosol to the lumen of the endoplasmic reticulum, was thus demonstrated (17). These observations have more recently been confirmed with microsomes from livers of two additional patients (36), and the absence of one T_2 transporter form has been established immunochemically. Comparable observations were made with three microsomal preparations derived from fetal livers where T_2 had not yet appeared (36). With all preparations (17, 36), P_i generated within the lumen of the endoplasmic reticulum by glucose-6-P phosphohydrolase action failed to accumulate and inhibit glucose-6-P hydrolysis as predicted. This observation indicates the existence of at least two T_2 forms, a P_i transport protein which has been termed $T_2\alpha$ and a P_i/PP_i/carbamyl-P transport protein termed $T_2\beta$ (36). The utility of the use of multiple activities as probes for the study of the glucose 6-phosphatase system, as well as the complexity of that system, is well demonstrated by these studies.

Differential Fetal Development of Glucose 6-Phosphatase Catalytic Unit and a T_2 Translocase Form

Although the ability to hydrolyze glucose-6-P is present in liver microsomes from human fetuses at 11–12 weeks of gestation, translocase $T_2\beta$ (that T_2 form transporting PP_i and carbamyl-P from the cytosol to the lumen of the endoplasmic reticulum) had not yet appeared (36, 42). This observation bears on the important question of translocase function. The physiologic significance of the differential rates of appearance of the individual components of the glucose 6-phosphatase system remains obscure. However, we would submit that translocase $T_2\beta$ may not be necessary prenatally because the mother's homeostatic mechanism serves to regulate glucose availability to the fetus. After birth, the individual is on his or her own, and synthetic activities (Reactions 2 and 3) (involving carbamyl-P and/or PP_i transport via $T_2\beta$) as well as glucose-6-P phosphohydrolase activity (Reaction 1) of the glucose 6-phosphatase system may be necessary for glucose homeostasis (see, e.g., Ref. 7; also see later sections of this Minireview).

Responses of Components of the Glucose 6-Phosphatase System to Fasting, Experimental Diabetes, and Glucocorticoid Therapy

The utility of the model in Figure 1 to rationalize complex kinetic changes in activities of glucose 6-phosphatase in response to hormonal manipulations is demonstrated by studies in which either pharmacologic doses of glucocorticoids were administered (19) or insulin levels were lowered by a 48-hr fast (38, 43) or by administration of alloxan or streptozotocin (20–22, 38). In response to glucocorticoids, there was little or no change in V_{max}, but there was a decrease in apparent K_m for glucose-6-P in the glucocorticoid-treated subjects and a diminished latency of glucose 6-phosphatase activity (23, 38, 44). (Latent activity is defined as that activity not expressed in intact microsomes, but which is brought out by disruption of microsomes with detergent [3]). These observations are interpreted in terms of the model of the glucose 6-phosphatase system as resulting from an increase in T_1 activity in response to glucocorticoid without a concomitant increase in V_{max} (38).

In contrast, in insulin deficiency (diabetes or fasting), V_{max} increased 2- to 3-fold, K_m for glucose-6-P was increased over control when determined in intact microsomes, and the latency was increased. Interpreted according to the catalytic unit/substrate translocase model (Fig. 1), these data are indicative of an increase in catalytic unit protein (23, 45), a strictly parallel

increase in catalytic unit activity (23, 45), and little or no change in translocase T_1 (23, 45). These responses provide for an enhanced rate of release of glucose from the liver through an increased V_{max} with an adaptation to retain glucose-6-P for hepatic use because of the increased apparent K_m for glucose-6-P.

Recent studies in our laboratory (44) indicate that the catalytic unit itself may exist in more than one state of activity. Responses of the catalytic unit to fasting, streptozotocin-induced diabetes, and triamcinolone injection were studied with detergent-disrupted microsomes by rapid, stopped-flow analysis (44). In this way, both pre-steady-state kinetics and steady-state kinetics could be analyzed and responses in both enzyme catalytic unit functional concentration and in kinetic parameters (K_m and V_{max}) could be measured. Catalytic unit protein newly formed in response to fasting or diabetes showed no change in K_m and an increase in V_{max} strictly proportional to the increase in functional enzyme concentration (E_o). In contrast, the enzyme newly formed in response to the glucocorticoid was less active (based on a lowered V_{max}:E_o ratio) than control. Interconversion of catalytic unit forms of greater and lesser activity are thus indicated, suggestive of alterations in activity through covalent modifications of the catalytic unit protein (44). A just-published paper strongly indicates phosphorylation/dephosphorylation as a mechanism regulating the glucose 6-phosphatase system at the level of protein synthesis (46).

Responses of the Hepatic Microsomal Glucose 6-Phosphatase System in Ehrlich Ascites-Tumor-Bearing Mice

Both untreated diabetes and the presence of a growing tumor place a marked stress upon the host for accelerated glucose production through enhanced gluconeogenesis. Therefore, we employed the steady-state kinetic approach used earlier to study responses of the glucose 6-phosphatase system to diabetes (see above) to assess possible changes in components of the system in microsomes derived from mice bearing Ehrlich ascites tumors (24, 47). In addition, immunochemical analysis was employed (47). The most striking findings in tumor-bearing mice were a 2.5-fold increase in quantity of translocase T_2 protein; an increase in the K_m value for glucose-6-P phosphohydrolase; and a decrease in the K_m, carbamyl-P values for carbamyl-P:glucose phosphotransferase, all with intact microsomes. V_{max} and apparent K_m did not change in disrupted microsomes from tumor-bearing mice compared with control mice, and the percentage of latency at V_{max} decreased for PP_i phosphohydrolase and glucose-6-P phosphohydrolase but was unchanged for the phosphotransferase. These observations support an *in vivo* tumor-related increase in translocase T_2 capacity, as it transports P_i from the microsomal lumen to the cytosol, and as it transports carbamyl-P or PP_i from the cytosol to the microsomal lumen (24, 47). The consequent increase in apparent K_m for glucose-6-P may serve to conserve glucose-6-P for hepatic use in circumstances where accelerated gluconeogenesis occurs (47).

In marked contrast, exogenously added P_i had a markedly lowered ability to inhibit glucose-6-P hydrolysis by intact microsomes derived from tumor-bearing mouse livers (24). This observation, seemingly inconsistent with an increase in T_2, is strongly supportive of the existence of at least two T_2 forms, as also indicated by our studies with microsomes from fetal livers and from Type Ic glycogenosis patients (17, 36) described above.

Sigmoidicity and Hysteresis with the Glucose 6-Phosphatase System of Intact Microsomes

Sigmoid kinetics were described for the glucose 6-phosphatase system of intact microsomes at low, near-physiologic glucose-6-P concentrations (48). The effect was more pronounced with preparations from diabetic than untreated control rats, and was abolished when the microsomal permeability barrier was destroyed. A mechanism for regulation of glucose 6-phosphatase, *in vivo*, was thus suggested. Subsequent studies, first with microsomes derived from Ehrlich ascites tumor-bearing mice (49), and then with microsomes from control and diabetic rats (50), indicated hysteretic behavior of glucose-6-P phosphohydrolase and carbamyl-P:glucose or PP_i:glucose phosphotransferase, but not inorganic PP_iase activity, with near-physiologic substrate concentrations. A quantitative analysis indicated that intramicrosomal concentrations of glucose-6-P in the region of 20–40 μM, whether attained through the addition of exogenous glucose-6-P or via intravesicular synthesis by phosphotransferase activity of glucose 6-phosphatase, may modify translocase T_1 (that for glucose-6-P transport; see Fig. 1) and thus produce the observed hysteresis (49). Hysteresis may thus provide yet another mechanism for regulation of the glucose 6-phosphatase system focused at translocase T_1.

Hysteretic behavior of another sort, observed within a much earlier time frame than the above, has been reported recently (51). These studies, carried out with a rapid, stopped-flow technique, indicate an initial "burst" of activity within the first 10 sec after initiation of activity, followed by a *decrease* rather than the increase in activity which we have seen (49). The effect was seen with both untreated and disrupted microsomes. It appears that quite different mechanisms underlie the two hysteretic phenomena. Berteloot *et al.* (51) interpret their results as inconsistent with the substrate transport model for the glucose 6-phosphatase system. Their interesting observations must be taken into account as the structure/function concepts of this complex system continue to evolve.

Metabolic Directive Effects of Inhibition of the Glucose 6-Phosphatase System at Translocase T_1

A major focus of our studies for the past several years has been upon the identification of potential metabolically important inhibitors of glucose-6-P hydrolysis and of the assessment of the impact of glucose 6-phosphatase inhibition upon metabolic processes involving glucose and glycogen in the liver. A combination of lactate plus glucose showed a pronounced synergism in regard to glycogen synthesis in isolated perfused rat liver (52), suggesting that gluconeogenic/glycolytic intermediates may inhibit glucose-6-P hydrolysis. Three specific inhibitors of glucose-6-P phosphohydrolase—fructose 1-phosphate (40), 3-mercaptopicolinate (41), and a proline metabolite (39)—have been identified and their glycogenic stimulatory effects have been demonstrated in isolated perfused livers.

Fructose 1-Phosphate as a Regulator of the Glucose 6-Phosphatase System. We tested 25 metabolites of glucose, gluconeogenic substrates, intermediates between glucose and glycogen, and related compounds as potential inhibitors of glucose-6-P hydrolysis under presumed near-physiologic conditions (40). The studies were prompted by the observations that certain gluconeogenic substrates are better precursors of hepatic glycogen than is glucose, but that glucose potentiates the flux from these compounds to glycogen, and by the observations that the "indirect pathway" (that involving glycolysis in a peripheral tissue followed by hepatic glyconeogenesis; see Fig. 2) may play a role in hepatic glycogenesis from glucose. Our thinking was that some glucose-derived metabolic intermediate might inhibit glucose-6-P hydrolysis *in vivo*, thus directing gluconeogenic flux away from glucose production and toward glycogen (Fig. 2). Of the compounds tested, only fructose 6-phosphate, ribose 5-phosphate, phosphoenolpyruvate, glyceraldehyde 3-phosphate, dihydroxyacetone-phosphate, and fructose-1-P inhibited glucose-6-P hydrolysis *in vitro*. Only the last one of these compounds showed a potential for inhibition under *in vivo* conditions, and then only after a load of fructose had been administered. Based on these observations, we (40) suggested that inhibition by fructose-1-P of glucose-6-P phosphohydrolase, along with activation of glucokinase by fructose-1-P (34), may explain in part the synergism between fructose and glucose in regard to hepatic glycogenesis (53).

3-Mercaptopicolinate. The compound 3-mercaptopicolinate (3-MP) inhibits the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (54). We (55) have used 3-MP in isolated perfused livers to block gluconeogenesis from endogenous substrates and to preclude glycogenesis from glucose via the indirect pathway (56). We have thus demonstrated net glucose uptake by perfused livers of diabetic rats from perfusate glucose concentrations at and above 4 m*M* (55). Very recent studies show that 3-MP has effects on enzymes other than phosphoenolpyruvate carboxykinase (41). It inhibits glucose-6-P phosphohydrolase (Reaction 1) but not carbamyl-P:glucose phosphotransferase (Reaction 2) of the glucose 6-phosphatase system (41, 57). This inhibition correlates with 3-MP's effects in our isolated perfused liver system in stimulating net glucose uptake, enhancing glycogenesis from glucose and from the gluconeogenic substrates fructose and dihydroxyacetone, and increasing steady-state hepatic glucose-6-P concentration (41). All of these effects are explainable through inhibition by 3-MP of the hydrolysis of glucose-6-P whether formed from glucose phosphorylation or via glyconeogenesis from substrates proximal to carboxykinase (41). Previous (41) and current (57) studies show that the inhibition is time dependent and irreversible, and appears to involve translocase T_1, specifically, of the glucose 6-phosphatase system. With the use of 3-MP to block glucose-6-P hydrolysis, we (57) have demonstrated recently glucose-concentration-dependent glycogenesis in perfused livers of 48-hr fasted rats, with a K_m of 30 m*M*.

A Proline-Derived Metabolite. The mechanism underlying the stimulation by amino acids of glycogenesis from glucose and gluconeogenic substrates has been unclear for some years (58). Recently, evidence has been presented suggesting a common mechanism through a simple increase in osmolarity (58). The sole exception was the amino acid L-proline (58). Therefore, we compared L-proline and L-glutamine as substrates for glucose and glycogen formation in isolated perfused livers (39). Perfusion with both resulted in glucose production. However, proline was glyconeogenic but glutamine was not. Hepatic glucose-6-P levels rose with proline but not glutamine. These observations suggested that a proline-derived metabolite was inhibiting glucose 6-phosphatase and thus diverting glucose-6-P away from glucose formation and toward glycogen (Fig. 2). This thesis has been confirmed in studies indicating that the presence of proline (with 3-MP present to block carboxykinase) enhances glycogenesis from dihydroxyacetone at the expense of glucose formation, enhances net glucose uptake and glycogenesis from glucose with 30 m*M* glucose present, and inhibits glucose production from endogenous hepatic glycogen. The metabolite has not been identified; carbamyl-P, metabolites between α-ketoglutarate and glucose-6-P (which are common to proline and glutamine), and Δ^1-pyrrolidine-5-carboxylate have been eliminated as possibilities (39, 59). These studies (39) establish the credibility of glucose-6-P phosphohydrolase as a focus for metabolic regulation, *in situ* (Fig. 2).

Multiple Effects of Arachidonate on the Glucose 6-Phosphatase System

The polyunsaturated fatty acid arachidonate [20:4;5,8,11,14] potently inhibited glucose-6-P phos-

phohydrolase activity of both intact and disrupted rat liver microsomes. Inhibitions were seen with 5–200 μM arachidonate (60). Dixon plots were linear with disrupted microsomes but curvilinear (concave upward) with intact microsomes. Inhibition of carbamyl-P:glucose phosphotransferase was also observed. K_i values of about 2.5–17 μM were determined, depending on conditions and activity. These observations suggest two sites of action of the polyunsaturated fatty acid on the glucose 6-phosphatase system: the catalytic unit and translocase T_1. Arachidonate is one of the most potent inhibitors of glucose 6-phosphatase yet identified. These observations suggest that arachidonate derived from the action of phospholipases upon phosphatidylinositol phosphates or other phospholipids could serve as a messenger, perhaps integrating arachidonate with Ca^{2+} and glucose 6-phosphatase in the regulation of carbohydrate metabolism (60).

Effects of Chloride Ion and General Ionic Strength Effects on the Glucose 6-Phosphatase System

Chloride ion has been shown to inhibit, competitively against phosphate substrates, both hydrolytic (61, 62) and synthetic (62) activities of glucose 6-phosphatase. The K_i was about 70 mM with disrupted microsomes and 90 mM with intact microsomes. These values, taken with physiologic Cl^- concentrations, indicate the potential for significant inhibition by cellular Cl^- (33).

An activation of both hydrolase and phosphotransferase activities of glucose 6-phosphatase progressive with increasing concentration has been observed with sodium and potassium salts of the buffers Hepes, cacodylate, and acetate. The effect was seen with both disrupted and intact microsomes, but was greater with the latter. Maximally, activation was about 3.5-fold. A correspondence of activation patterns with the several buffers indicates a general ionic strength effect, which may be important within the normal cellular range.

Recent Evidence Supporting Phosphotransferase Activity of the Glucose 6-Phosphatase System in Hepatic Glucose Phosphorylation

Further support for the credibility of the model of glucose 6-phosphatase system structure/function is provided by recent evidence indicative of the function of phosphotransferase activity of the system, *in vivo*. We have long advocated physiologic functions for carbamyl-P:glucose phosphotransferase (Reaction 2) and perhaps PP_i:glucose phosphotransferase (Reaction 3) activities of the glucose 6-phosphatase system (e.g., see Refs. 1, 2, 7, and 33). Activity of the former at V_{max} is, e.g., maximally more than 1.5 times that of glucose-6-P phosphohydrolase activity (Reaction 1) (16).

Fine Tuning of Blood Glucose Concentrations. Our tuning/retuning hypothesis involving both hydrolytic and synthetic functions of the glucose 6-phosphatase in glucose homeostasis has been described by Nordlie (7). Very briefly, the concept rests upon a controlled but adjustable balance between hepatic glucose phosphorylation and glucose-6-P hydrolysis as critical determinants of the direction and net rate of flux of glucose between the blood and the liver cell (Fig. 2, liver cell). Tradition has it that glucose phosphorylation is catalyzed by hepatic glucokinase and glucose-6-P hydrolysis by glucose 6-phosphatase. Recognizing that the former enzyme's activity is very low or absent in many species (9) and that it is critically dependent upon insulin as an inducer, we propose that biosynthetic function of glucose 6-phosphatase (Reactions 2 or 3) may act supplementally to (or in place of) glucokinase for hepatic glucose phosphorylation as circumstances may dictate (1, 2, 7). A gradual replacement of glucokinase with increasing amounts of carbamyl-P:glucose phosphotransferase activity of glucose 6-phosphatase (insulin is a *repressor* here) as diabetes becomes increasingly pronounced would serve to raise progressively and continuously ambient blood glucose levels, directly proportional to the severity of the insulin insufficiency. This is because the K_m, glucose for phosphotransferase activity of glucose 6-phosphatase (~40 mM [16]) is several-fold greater than that for glucokinase (6–12 mM [34]). A progressive increase in the ratio of the high K_m enzyme/lower K_m enzyme (i.e., carbamyl-P:glucose phosphotransferase/glucokinase) as diabetes develops progressively "tunes" the liver to maintain increasingly higher ambient blood glucose levels. The interested reader is directed to Refs. 1, 2, and 7 for quantitative kinetic details.

Hepatic Glucose Phosphorylation by a High K_m Enzyme Other than Glucokinase. Evidence supportive of the involvement of a high K_m enzyme other than glucokinase—possibly synthetic activity of glucose 6-phosphatase—in hepatic glucose phosphorylation was summarized most recently in Ref. 7.

A series of studies with isolated, perfused rat livers, isolated hepatocytes, and individual enzymes studied kinetically, begun in 1977 by Alvares and Nordlie (63) and continuing to this date, supports the phosphorylation of glucose in mammalian livers by a high K_m enzyme system acting complementary to, or in place of, insulin-dependent glucokinase. We believe that carbamyl-P:glucose phosphotransferase activity of the glucose 6-phosphatase system (Reaction 2) is the most likely candidate for this role (7, 33). Alternatively, PP_i produced in the uridinebisphosphate glucose synthase reaction, in the urea cycle, and in many other biosynthetic processes may be involved (Reaction 3) (7, 33). Some evidence in support of this proposal is summarized very briefly in the following paragraphs.

Perfusion studies with livers of 48-hr fasted rats, in which the hepatic glucose-6-P level was assessed as a

function of perfusate glucose concentration, support a metabolic "push," i.e., by glucose phosphorylation, as a major determinant of glucose-concentration-dependent metabolic flux from glucose to glycogen (64). Our very recent studies, in which 3-MP was used as a tool to block endogenous gluconeogenesis, to inhibit the indirect pathway, and to prevent glucose-6-P rehydrolysis, indicate a K_m, glucose of 30 m*M* for glycogenesis from glucose in perfused livers of 48-hr fasted rats, consistent with phosphotransferase activity of glucose 6-phosphatase as a major contributor to hepatic glucose phosphorylation (57). Earlier studies by Nordlie *et al.* (55) demonstrated net glucose uptake by perfused livers of glucokinase-devoid diabetic rats with glucose levels as low as 4 m*M* when 3-mercaptopicolinate was included to inhibit gluconeogenesis from endogenous substrates. (We now know that 3-MP also inhibits the hydrolysis of glucose-6-P, but not its synthesis, by activities of glucose 6-phosphatase [57]).

Kinetic analysis (33) of our own data (63) and those of others (65) indicated a K_m, glucose of 60–90 m*M* (33), and studies of Singh and Nordlie (66) showed a progressive increase in K_m, glucose from 33 m*M* to 48 m*M* for phosphorylation of [2-^{3}H]D-glucose by hepatocytes derived from rats fasted progressively from 0 to 72 hr. All of these K_m, glucose values are considerably larger than can be explained by glucokinase, for which a $K_{0.5}$ of about 6–12 m*M* applies (34).

Studies with filipin-permeabilized hepatocytes (67) and isolated liver nuclei and nuclear membranes (68) indicate that latency of phosphotransferase activities of the glucose 6-phosphatase system is *not* an impediment to physiologic function, *in situ*, nor is physiologic pH (67). Furthermore, normal cellular levels of carbamyl-P and glucose have been shown to be high enough to maintain physiologically credible levels of carbamyl-P:glucose phosphotransferase activity (33).

A competition between substrates carbamyl-P, PP_i, and glucose-6-P at the catalytic unit has been demonstrated *not* to be a problem with the glucose 6-phosphatase system where the apparent K_m values are an order of magnitude or more greater than normal physiologic concentrations of substrates (69). It has been demonstrated by kinetic analysis that under these conditions, the several activities of the enzyme behave almost completely as though they were catalyzed by several enzymes, each distinct for an individual activity (69). Thus, e.g., glucose-6-P hydrolysis and carbamyl-P:glucose phosphotransferase activities may proceed individually and nearly totally noninteractively, catalyzed by the glucose 6-phosphatase system in a single hepatocyte. Very recent evidence for multiple forms of T_2, i.e., $T_2\alpha$ and $T_2\beta$, with differing specificities (24, 25, 36) eliminate a competition by P_i against carbamyl-P or PP_i transport (18) as an impediment to glucose-6-P synthesis via phosphotransferase activities of the glucose 6-phosphatase system with intact cellular preparations.

Net concentration-dependent uptake of glucose by perfused diabetic livers in the presence of 3-MP (55) mentioned above has been complemented more recently by kinetic studies with perfused livers of 48-hr fasted rats with 0.5 m*M* 3-MP present. The K_m for glucose as substrate for glycogenesis of 30 m*M* supports a high K_m enzyme in addition to glucokinase in hepatic glucose phosphorylation (57), consistent with the progressive increase in K_m for glucose phosphorylation observed with the duration of an extended fast (66). With progressive fasting, glucokinase decreases and glucose 6-phosphatase increases.

N-Acetylglucosamine, an inhibitor of glucokinase, was employed in both liver perfusion (52) and isolated hepatocyte (70) studies to demonstrate hepatic glucose phosphorylation by a high K_m system other than glucokinase. Liver and hepatocyte preparations from 48-hr fasted rats showed net glucose uptake and phosphorylation of 2-^{3}H-labeled glucose which could not be explained on the basis of residual glucokinase (52, 70), or, in the former case, by the indirect pathway of hepatic glycogenesis from glucose (52). Likewise, net glucose uptake with 15 or 30 m*M* glucose by livers from fasted or fed rats was not explainable exclusively by glucokinase activity and/or the indirect pathway (52).

Recent and Ongoing Studies Indicating Hepatic Glucose Phosphorylation by Carbamyl-P:Glucose Phosphotransferase Activity. The potential for hepatic glucose phosphorylation by carbamyl-P:glucose phosphotransferase activity of the glucose 6-phosphatase system was first demonstrated in studies by Lueck and Nordlie in 1972 (71). Those studies were prompted by the observations of Natale and Tremblay (72, 73) that carbamyl-P generated via carbamyl-P synthase I in the mitochondrion may become available for extramitochondrial pyrimidine biosynthesis. Lueck and Nordlie (71) demonstrated that a reconstituted system consisting of hepatic mitochondria (which contain carbamyl-P synthase I) and microsomes (which contain the multifunctional glucose 6-phosphatase/phosphotransferase system) synthesized glucose-6-P when provided with glucose and the substrates and activator for carbamyl-P synthase I. The system was absolutely dependent upon *N*-acetylglutamate (an activator of carbamyl-P synthase I) and ATP (the phosphoryl donor for carbamyl-P formation). Ornithine, which competes for carbamyl-P via ornithine transcarbamylase, inhibited the synthesis. ATP was ineffective without *N*-acetylglutamate, and glucose-6-P formation did not occur in the absence of mitochondria.

Recognizing that the studies described to this point support, in principle, the formation of glucose-6-P by phosphotransferase activity of glucose 6-phosphatase, but do not unequivocally establish that this takes place,

in vivo, or identify specifically the phosphoryl donor(s), we carried out further experiments in which isolated perfused liver studies were combined with enzyme measurements.

Evidence exists for the channeling of carbamyl-P derived from mitochondrial carbamyl-P synthase I into the urea cycle (74), and for channeling of carbamyl-P from cytosolic carbamyl-P synthase II into pyrimidine biosynthesis (75). Nonetheless, studies currently ongoing in our laboratory provide strong experimental support for the involvement, specifically, of carbamyl-P:glucose phosphotransferase activity in glucose phosphorylation in the liver of the 48-hr fasted rat under near-physiologic conditions. The earlier work of Lueck and Nordlie (71) referred to above established the feasibility of glucose-6-P formation via coupled action of carbamyl-P synthase I and carbamyl-P:glucose phosphotransferase activity of glucose 6-phosphatase. We have now shown that 10 m*M* proline, which in the presence of glucose produced an increase in carbamyl-P, also increased glycogenesis from glucose but did not accelerate ureagenesis (76, 77). Ornithine, a substrate for ornithine transcarbamylase, lowered hepatic glucose uptake and glycogenesis from glucose while increasing ureagenesis (76). Glutamine, which not only provides nitrogen for carbamyl-P synthesis but also serves as precursor for the urea cycle intermediate L-aspartate (78), accelerated ureagenesis and decreased glycogenesis, relative to proline's effects. Norvaline, an inhibitor of ornithine transcarbamylase, promoted net glucose uptake and glycogenesis from glucose and lower ureagenesis (A. B. Bode, R. C. Nordlie, unpublished observations). Ethoxyzolamide, an inhibitor of carbonic anhydrase (79) which has been shown to inhibit ureagenesis as a consequence of lowered bicarbonate (a substrate for carbamyl-P synthase I [78]), inhibited *both* urea production and glycogenesis from glucose in our perfused liver preparations including either 8 or 30 m*M* glucose (A. B. Bode, R. C. Nordlie, unpublished observations).

Thus, when there is an increased demand by the urea cycle for carbamyl-P, glycogenesis from glucose is decreased; when the urea cycle is inhibited and carbamyl-P consequently becomes more available, glucose uptake and glycogenesis from glucose are increased; and when carbamyl-P formation is inhibited, *both* the urea cycle and glycogenesis from glucose are depressed. All of this strongly supports interrelationships between the urea cycle and glycogenesis from glucose focused upon the relative availability of carbamyl-P to the two processes. The possibility of channeling of some mitochondrial-derived carbamyl-P to glucose phosphorylation via the glucose 6-phosphatase system is strengthened, we believe, by the report by Katz *et al.* (80) of a continuum between the outer mitochondrial membrane and the endoplasmic reticulum where the glucose 6-phosphatase system is located.

The preceding studies all were and are being performed with perfused livers derived from 48-hr fasted rats, where glucokinase has dropped to about one quarter the normal level. These same strategies will subsequently be employed to assess hepatic glucose phosphorylation mechanisms in the liver of the diabetic rat. There, elevated glucose, increased flux of amino acids to the liver, elevated carbamyl-P synthase, severely depressed glucokinase, and increased glucose 6-phosphatase phosphotransferase all should favor carbamyl-P:glucose phosphotransferase over insulin-dependent glucokinase as the preferred mechanism of hepatic glucose phosphorylation. Such phosphorylation persists in the diabetic liver even though net glucose production via accelerated gluconeogenesis dominates (55).

Conclusion and Future Directions

Studies leading to the present concepts of glucose 6-phosphatase structure/function began 30 years ago with the report by Nordlie and Arion (15) of the enzyme's multifunctional nature. Subsequent studies, reviewed above and elsewhere (1–7), showed that the glucose 6-phosphatase system is much more complex—structurally, catalytically, and physiologically—than originally thought. With these newly elucidated complexities comes the need for discriminant regulation of each of these components, activities, and functions. Because of these complexities of the system, its membrane-bound nature, and its consequent instability, glucose 6-phosphatase has been the most refractory of the gluconeogenic enzymes to study at the molecular level both in regard to its individual proteins and regulation of their biosynthesis at the transcriptional and translational levels. The next few years will be exciting ones as this most complex of gluconeogenic enzymes receives further, intensive study by research groups worldwide.

Areas which we believe merit immediate intensive study include (i) isolation and characterization, chemically and functionally, of all of the individual components of the system; (ii) characterization of possible multiple forms of transport components, as we have done with T_2 (24, 25, 36); (iii) optimistically, the reassembly of these components into a functional unit within synthetic biomembranes; (iv) characterization of the impact of components of biomembranes upon function of the various components; (v) cloning of all of the individual proteins of the system; (vi) characterization of mechanisms regulating the biosynthesis of the individual proteins at the transcriptional and translational levels; (vii) demonstration of possible auxiliary functions of translocases other than exclusively with the glucose 6-phosphatase system; (viii) characterization of possible physical associations of some components of

the system; (ix) characterization of the glucose 6-phosphatase system from nonhepatic sources; (x) characterization of physiologic roles of the glucose 6-phosphatase system, and possibly its individual components, in nonhepatic tissues; (xi) elucidation of the physiologic significance of differential rates of appearance of individual components of the system during development; (xii) further determination of the physiologic significance of the multiple activities (hydrolytic and biosynthetic) of the glucose 6-phosphatase system; and (xiii) identification of additional factors—metabolite inhibitions, covalent modifications through protein phosphorylation/dephosphorylation and the like—which may regulate both hydrolytic and biosynthetic functions in discriminant, short-term fashion.

Future studies of this least understood and most complex of gluconeogenic enzymes seem to us poised at the edge of a golden era. We have been a part of the events leading to that positioning, and hope to be a part of future exciting developments as well.

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