# HUMAN LIVER CARBAMYL PHOSPHATE-GLUCOSE PHOSPHOTRANSFERASE ACTIVITY: CATALYTIC PROPERTIES AND PHYSIOLOGICAL PHOSPHORYLATIVE POTENTIAL

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Received 25 August 1971

## 1. Introduction

Studies over the past 7 years [1–3] indicate that D-glucose-6-P phosphohydrolase (EC 3.1.3.9; eq. 1) is a multifunctional enzyme capable of catalyzing potent phosphotransferase as well as hydrolase reactions (see eqs. 1–3, for example). $PP_i$-glucose phosphotransferase activity of the enzyme (eq. 2) of rat liver has been particularly intensively studied. Nordlie [3, 4] has concluded that, although of possible potential physiological importance, significant phosphorylation of glucose by this activity within the liver cell under normal conditions may be questioned on 3 counts: (a) cellular unavailability of $PP_i$ (but see [5]); (b) the acidic pH-activity profile and low pH optimum [1]; and (c) the high $K_m$ value for glucose of 80 to 90 mM [1, 4, 6].

$$\text{Glucose-6-P} + H_2O \rightarrow \text{glucose} + P_i \quad (1)$$
$$PP_i + \text{glucose} \rightarrow \text{glucose-6-P} + P_i \quad (2)$$
$$\text{Carbamyl-P} + \text{glucose} \rightarrow \text{glucose-6-P} + \text{carbamate} \quad (3)$$

Carbamyl-P:glucose phosphotransferase activity (eq. 3), first noted by us in rat liver [8] has now been studied in some detail in human liver autopsy samples which recently have become available to us. Catalytic properties of this activity are described in this paper, and a comparison is made of the relative capacities of human liver carbamyl-P:glucose phosphotransferase and glucokinase for glucose phosphorylation at pH 7. It is concluded that criticisms previously directed at a physiologically important synthetic role for phosphotransferase activities of liver glucose 6-phosphatase appear obviated on the basis of the described pH-activity profiles and $K_m$ (glucose) values (28–50 mM) of carbamyl-P:glucose phosphotransferase, and that the latter activity may well be of metabolic significance in the human liver under physiological conditions.

## 2. Materials and methods

Sources of substrates and other reagents, as well as analytical methods, were as employed in previous studies [1, 4, 6–8]. Glucose-6-P phosphohydrolase and $PP_i$-glucose phosphotransferase activities were assayed as described by Nordlie and Arion [7], and carbamyl-P:glucose phosphotransferase was measured by the method of Nordlie and Arion [7] as modified by Lueck and Nordlie [8]. All incubations were carried out for 10 min at 30 ± 0.1°. Activity was a linear function of incubation time under the conditions employed. Additional details are given in the text and in the legends to the figures. Liver specimens were obtained from human males (43 and 52 yr of age) at autopsy, and were cooled and maintained at −20° until used. Such tissues were thawed at 0°, homogenized in 0.25 M sucrose solution in a Waring blendor for 1 min, then processed further in a Potter-Elvehjem homogenizer at 600 rpm for 2 min, diluted to 10 ml per g liver and centrifuged at 15,000 *g* for 10 min. This supernatant material was further centrifuged at 88,000 *g* for 50 min; the resulting supernatant fraction was discarded, and the sedimented pellet (containing the enzymic acitivity) was resuspended in 0.25 M sucrose, stored frozen in small aliquots at −20°, and melted at 0° as needed for the experiments described

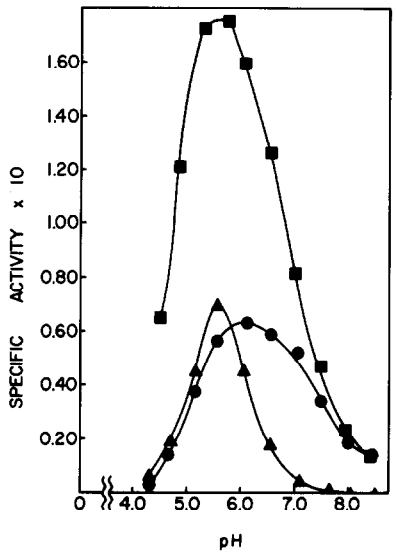


Fig. 1. Effects of assay pH on carbamyl-P:glucose phosphotransferase (■), $PP_i$-glucose phosphotransferase (▲), and glucose-6-P phosphohydrolase (●). Assay mixtures contained, in 1.5 ml, 40 mM buffer, 10 mM phosphate substrate, and 180 mM D-glucose (phosphotransferase). Buffers were as follows: Na acetate, pH ≦ 5.0; Na acetate + Na cacodylate, pH 5.5; Na cacodylate, pH 6.0 and 6.5; Na cacodylate + HEPES, pH 7.0; HEPES, pH 7.5; HEPES + Tris-Cl, pH 8.0; and Tris-Cl, pH > 8.0. Specific activity = μmoles glucose-6-P formed (transferase) or hydrolyzed (hydrolase)/min/mg protein. Assay mixtures were prepared in duplicate; activity was measured in one series and the pH determined with a Beckman expanded scale meter in the second series.

in figs. 1 and 2, A–C. Such enzyme preparations, or the liver homogenates in the experiment described in fig. 2D, were supplemented with sodium deoxycholate (to 0.1%, w/v) prior to assay. Pentex (Miles Laboratories, Inc., Kankakee, Ill. USA), "fatty-acid poor" bovine albumin which had been further subjected to ethanol extraction, was included in all assay mixtures (10 mg/1.5 ml reaction mixture). These conditions were found optimal for maximal activity in supplementary studies. Ionic strength was maintained constant at 0.10 by the addition of supplementary NaCl to assay mixtures.

## 3. Results

Activity-pH profiles for carbamyl-P:glucose phosphotransferase, $PP_i$-glucose phosphotransferase, and glucose-6-P phosphohydrolase, determined with constant levels of phosphate substrates (10 mM) and glucose (180 mM; phosphotransferase only), are compared in fig. 1. Maximal activity was noted at pH 5.5 with both phosphotransferases and at pH 6 with the hydrolase. Carbamyl-P:glucose phosphotransferase levels exceeded those of the other activities at all pH values. In contrast with $PP_i$-glucose phosphotransferase, synthetic activity with carbamyl-P as substrate remained significantly high at and above pH 7.

Fig. 2, A–C, depicts the results of two-substrate kinetic studies carried out as described by Florini and Vestling [9] and as previously performed at pH 6 with rat liver $PP_i$-glucose phosphotransferase activity by Nordlie and coworkers [6, 10]. Unlike the latter activity, the carbamyl-P:glucose phosphotransferase system of human liver at physiological pH shows a marked dependence of apparent $K_m$ values on concentration of the second substrate, as indicated by varying $x$-axis intercepts of experimental double-reciprocal plots [11] in fig. 2, A and B. Apparent $K_m$ values for glucose, determined as negative reciprocals of $x$-axis intercepts of extrapolations of experimental plots in fig. 2A, are listed on this figure along with levels of carbamyl-P to which they correspond. Such experimental values ranged from 28 mM with 0.5 mM carbamyl-P to 51 mM with 10 mM carbamyl-P present; theoretical lower and upper limits, calculated as $-1/x$-coordinate of the common point of convergence of experimental lines in fig. 2A [12] and from the secondary plot (fig. 2C) [9] for carbamyl-P concentrations approaching 0 and infinity, respectively, were 26 mM and 70 mM. Apparent $K_m$ values for carbamyl-P likewise exhibited a dependence on glucose concentrations (see fig. 2B), and ranged experimentally from 4.7 mM (20 mM glucose) to 7.8 mM (200 mM glucose) and theoretically from 3.5 mM (glucose concentration → 0; see fig. 2B) to 9.2 mM (glucose concentration → infinity; see fig. 2C).

The capacity of human liver to catalyze glucose-6-P synthesis via carbamyl-P:glucose phosphotransferase at pH 7 in the presence of various concentrations of glucose is described in fig. 2D. Activity values, expressed in terms of μmoles of glucose-6-P formed/min/g wet liver, are based on assays carried out with liver homogenates. Carbamyl-P concentration (except where otherwise noted) was maintained at 0.5 mM or

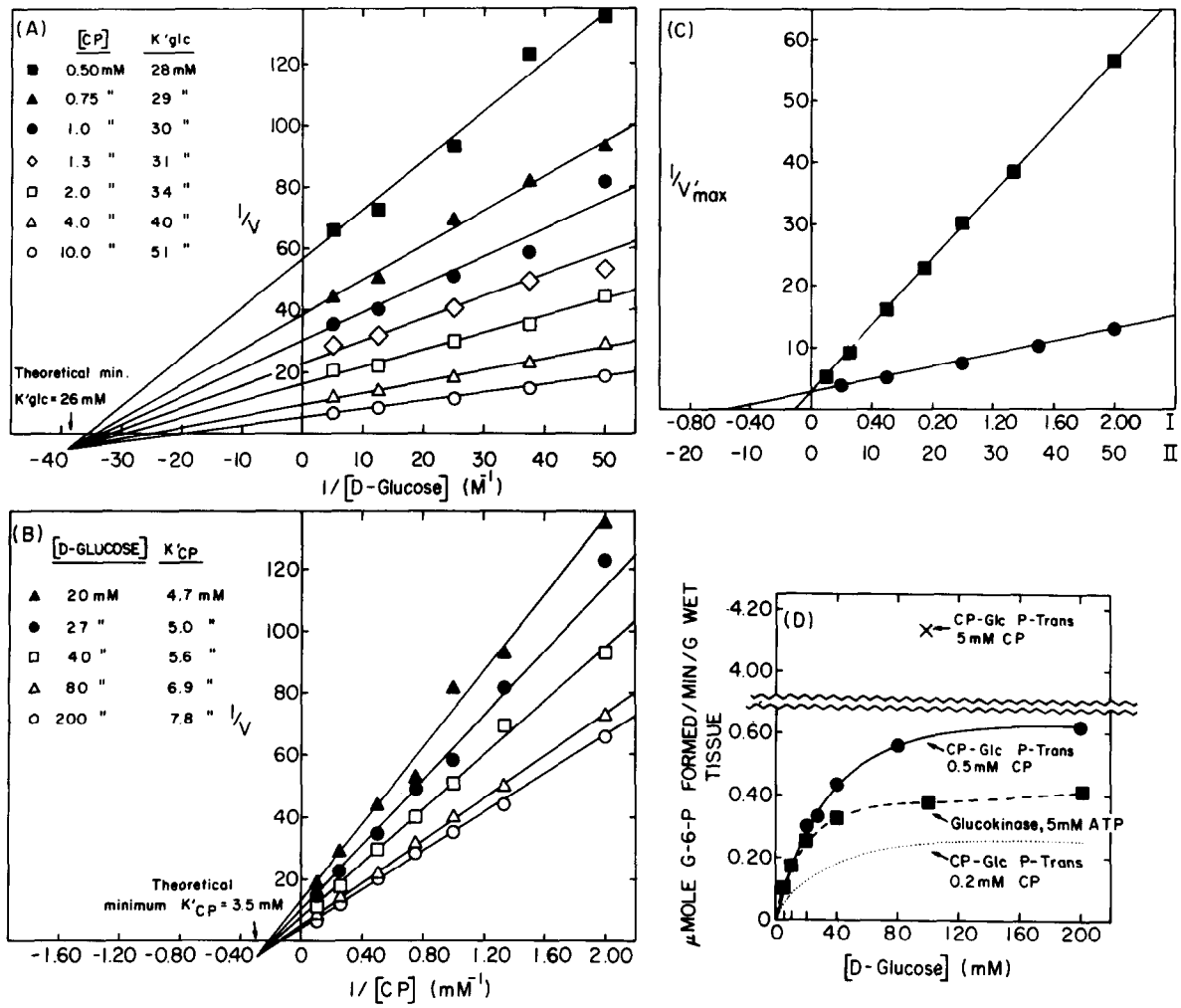


Fig. 2. Effects of variations in substrate concentrations on carbamyl-P:glucose phosphotransferase activity at pH 7. All phosphotransferase assay mixtures contained, in 1.5 ml, 20 mM cacodylate + 20 mM HEPES buffer; in A–C the 88,000 *g* pellet (0.5 mg protein/1.5 ml reaction mixture) served as enzyme source, while in D 2 mg liver homogenate protein/1.5 ml assay mixture was employed. (A) Carbamyl-P:glucose phosphotransferase studied as a function of varied glucose concentrations (20 to 200 mM) at several constant levels of carbamyl-P [CP]. Velocity, $v$, is expressed as μmoles glucose-6-P formed/min/mg protein. Data are plotted in conventional double-reciprocal form. Apparent Michaelis constant values for glucose ($K'_{glc}$), determined as negative reciprocals of $x$-axis intercepts of extrapolations of experimental plots obtained with the various, designated concentrations of carbamyl-P present, are indicated on the figure. (B) Carbamyl-P:glucose phosphotransferase activity studied as a function of varied carbamyl-P concentrations (0.5 to 10 mM) at several constant levels of D-glucose. Apparent Michaelis constant values for carbamyl-P ($K'_{CP}$) obtained at various glucose concentrations are indicated on the figure. Other details are as in fig. 2A. (C) Secondary plots [9] of $y$-axis intercepts from fig. 2, A and B, against reciprocals of millimolar concentrations of carbamyl-P (I) and molar concentrations of D-glucose (II). The intersection of the plots with the $y$-axis evaluates $1/V_{max}$; the $x$-axis intercepts are equal to $-1/K_{CP}$ (■) and $-1/K_{glc}$ (●). $V_{max}$, the maximal reaction velocity with infinite levels of both substrates, is 0.36 μmoles/min/mg protein, which corresponds with a value of 14.4 μmoles/min/g wet liver. $K_{CP}$ and $K_{glc}$, the theoretical maximum Michaelis constant values for carbamyl-P and glucose with infinite concentrations of second substrate present are, respectively, 9.2 mM and 71 mM. (D) Comparison of the relative abilities of human liver carbamyl-P:glucose phosphotransferase (CP-Glc P-Trans) and glucokinase to phosphorylate glucose at pH 7. Assay conditions for the former activity were as in fig. 2A except that carbamyl-P concentration was either 0.2 mM, 0.5 mM, or 5 mM as indicated, and homogenates were used as enzyme source. Glucokinase values were calculated as indicated in the text. Carbamyl-P:glucose phosphotransferse values represent averages from assays of liver preparations from 2 males, ages 43 and 52 yr (5 mM glucose = 90 mg/100 ml).

0.2 mM, values which approximate that for this compound in rat liver (see footnote*). Glucokinase values, based on activity levels (5 mM ATP and 100 mM glucose) reported for human liver by Brown et al. [14], adjusted to pH 7 (see [15]) and 30°, and calculated for 5 mM ATP and varied concentrations of glucose on the basis of a $K_m$ value of 15 mM for the hexose [16], also are included for comparative purposes. With 5 mM carbamyl-P, carbamyl-P:glucose phosphotransferase activity 7 times that with 0.5 mM carbamyl-P was observed (see X in fig. 2D).

## 4. Discussion

Previous contraindications for a physiologically significant role for the phosphotransferase activities of glucose 6-phosphatase in hepatic glucose phosphorylation appear obviated by the observations, described above, relating to catalytic properties of carbamyl-P: glucose phosphotransferase activity of human liver. This activity: (a) involves a metabolically established metabolite as phosphoryl group donor, (b) is quite active even at and above pH 7 (fig. 1), and (c) exhibits appareant $K_m$ values for glucose of less than 30 mM at low, possibly physiological (see footnote*), concentrations of carbamyl-P (see fig. 2A). The latter values compare quite favorably with $K_m$ values of 15 mM [16] to 20 mM [15] for glucokinase (see fig. 2D).

Maximally, at pH 7, carbamyl-P:glucose phosphotransferase is the most active glucose phosphorylating system yet characterized for human liver (note the $V_{max}$ value, fig. 2C, of 14.4 μmoles glucose-6-P formed/min/g wet liver with saturating levels of both substrates). As indicated by data in fig. 2D, levels of this activity in liver of mature (43–52 yr old) human males compare favorably with those of glucokinase even with physiological levels of substrates. Collipp et al. [17] previously have reported that glucose 6-phosphatase levels in young children may be 5 to 10 times those of adults; this suggests that in a significant segment of the population carbamyl-P: glucose phosphotransferase activity levels may be considerably higher than those reported here.

* In preliminary studies involving the freeze-clamp technique, Raijman and Jones [13] have observed carbamyl-P levels of 0.1–0.2 mM in livers of normal, fed rats.

Although these observations by themselves do not unequivocally establish the biological significance of this activity, they do indicate its intrinsic capacity to catalyze highly significant amounts of glucose phosphorylation in human liver under physiological conditions. And because relatively high levels of hepatic glucose 6-phosphatase appear considerably more widely present in nature than is liver glucokinase (see [18]), it is suggested that carbamyl-P:glucose phosphotransferase activity of the former enzyme may well prove to be the more generally metabolically important "high $K_m$" hepatic glucose phosphorylating system involved in the control of blood sugar levels.

## Acknowledgements

This work was supported by the Hill Family Foundation, U.S. Public Health Service grant AM 07141, National Science Foundation grant GB-14752, and the American Diabetes Association.

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