Glucose 6-Phosphate Produced by Glucokinase, but Not Hexokinase I, Promotes the Activation of Hepatic Glycogen Synthase*

(Received for publication, May 22, 1996, and in revised form, July 8, 1996)

Joan Seoane‡, Anna M. Gómez-Foix‡, Robert M. O’Doherty§, Cristina Gómez-Ara‡, Christopher B. Newgard‡, and Joan J. Guinovart‡§

From the §Departament de Bioquímica i Biologia Molecular, Facultat de Química, Universitat de Barcelona, E08028 Barcelona, Spain and §Departments of Biochemistry and Internal Medicine, Gifford Laboratories for Diabetes Research, University of Texas Southwestern Medical Center, Dallas, Texas 75235

In a previous study (O’Doherty, R. M., Lehman, D. L., Seoane, J., Gómez-Foix, A. M., Guinovart, J. J., and Newgard, C. B. (1996) J. Biol. Chem. 271, 20524–20530), we demonstrated that adenovirus-mediated overexpression of glucokinase but not hexokinase I has a potent enhancing effect on glycogen synthesis in primary hepatocytes. In an effort to understand the underlying mechanism of this differential effect of the two hexokinase isoforms, we have investigated changes in key intracellular metabolites and the activation state of glycogen synthase in cells treated with recombinant adenoviruses expressing the liver isoform of glucokinase (AdCMV-GKL) or hexokinase I (AdCMV-HKI).

Glucose 6-phosphate (Glu-6-P) levels are elevated from approximately 1.5 nmol/mg protein to 8–10 nmol/mg protein in both AdCMV-GKL- and AdCMV-HKI-treated hepatocytes as glucose is raised from 1 to 5 mM, levels four times higher than those in untreated cells. In AdCMV-GKL-treated cells, Glu-6-P continues to accumulate at glucose levels greater than 5 mM, reaching a maximum of 120 nmol/mg protein in cells incubated at 25 mM glucose, a value 10 and 50 times greater than the maximal levels achieved in AdCMV-HKI-treated and untreated cells, respectively. In parallel with the changes observed in Glu-6-P levels, increases in UDP-Glc in AdCMV-HKI and AdCMV-GKL-treated cells were most pronounced at low (1–5 mM) and high (25 mM) glucose levels, respectively. Despite the significant increases in Glu-6-P and UDP-Glc achieved in AdCMV-HKI-treated cells, only AdCMV-GKL-treated cells exhibited increases in glycogen synthase activity ratio and translocation of the enzyme from a soluble to a particulate form relative to untreated control cells. We conclude that Glu-6-P produced by overexpressed glucokinase is glycogenic because it effectively promotes activation of glycogen synthase. Glu-6-P produced by overexpressed hexokinase, in contrast, appears to be unable to exert the same regulatory effects, probably due to the different subcellular distribution of the two glucose-phosphorylating enzymes.

In mammals in the postabsorptive state, glycogen synthesis is potently activated in the liver in response to increased circulating glucose levels. The mechanism by which this occurs is not a settled matter. It was originally suggested that unmetabolized glucose can indirectly activate glycogen synthase (GS),† the key enzyme in the control of glycogen synthesis, by relieving the inhibitory effect of phosphorylase a on the protein phosphatases (phosphatases 1 and 2A) responsible for dephosphorylation of, and hence activation of, GS (1). Several lines of evidence, however, argue that free glucose cannot be solely responsible for the activation of glycogen synthesis. First, activation of glycogen synthase and accumulation of phosphorylase a can occur simultaneously in liver cells (2, 3). Second, glucose phosphorylation is required to activate glycogen synthase but not to inactivate phosphorylase a (4–7). Finally, increases in the glycogen synthase activation state are proportional to the intracellular glucose 6-phosphate (Glu-6-P) level (7, 8). These observations suggest that glucose phosphorylation is a key step in the activation of glycogen synthesis.

There are several mechanisms by which Glu-6-P can regulate activation of glycogen formation. Increases in intracellular Glu-6-P lead to the allostERIC activation of GS, this effect being reversible when Glu-6-P returns to basal levels (9). Additionally, Glu-6-P promotes the covalent activation of glycogen synthase, possibly by inducing a conformational change that favors the dephosphorylation of the enzyme by phosphatases 1 and 2A (10). Finally, increases in Glu-6-P levels trigger translocation of GS between a supernatant fraction and a pellet fraction, a process that appears to play an important role in the regulation of glycogen synthesis (11, 12).

Gluose is phosphorylated to Glu-6-P in mammalian cells by members of the hexokinase gene family. Hepatocytes contain primarily glucokinase (GK or HKIV), and small amounts of hexokinase I (HKI) (13). GK differs from HKI, HKII, and HKIII in that it is approximately half as large, is not allosterically inhibited by Glu-6-P, and has a much higher $S_{0.5}$ for glucose (8 mM versus 50–100 $\mu$M) (14). In a recent study, we showed that overexpression of GK in hepatocytes by adenovirus-mediated gene transfer causes profound enhancement of glycogen synthesis, with the major effect occurring at glucose concentrations in excess of 5 mM, whereas overexpression of HKI has no effect at any concentration of the sugar (15). The current study was undertaken to elucidate the mechanism of this surprising differential effect of the two glucose-phosphorylating enzymes, with particular emphasis on their impact on Glu-6-P levels and the activation state of glycogen synthase.

MATERIALS AND METHODS

Preparation of Recombinant Adenoviruses—Recombinant adenoviruses containing the cDNAs encoding rat hexokinase I (AdCMV-HKI) or...
Activation of Glycogen Synthase by Glucokinase Overexpression

23757

RESULTS

GK and HKI Overexpression in Rat Hepatocytes—Demonstration of the efficacy of the AdCMV-HKI and AdCMV-GKL recombinant adenoviruses for overexpression of glucose-phosphorylating enzymes has been documented previously (15). In the current series of studies, glucose-phosphorylating capacity in untreated hepatocytes was 13.5 ± 0.8 μmol/min/g protein when assayed by the spectrophotometric method at 100 μM glucose and 2.3 ± 0.2 μmol/min/g protein with 20 μM glucose when assayed by the radiometric assay. The reasons for the difference in sensitivity of the two assays has been detailed in a previous article (15). When assayed by the radiometric method, the glucose phosphorylation capacity was found to be increased by 9- and 8-fold at 20 mM glucose and by 10- and 23-fold in 3 mM glucose in extracts from AdCMV-GKL- and AdCMV-HKI-treated hepatocytes, respectively, relative to extracts from untreated cells assayed at the same glucose concentrations. These values are comparable to those previously reported (15).

Glu-6-P and UDP-Glc Accumulation in AdCMV-GKL- and AdCMV-HKI-treated Hepatocytes—Our previous article demonstrated that overexpression of GK but not HKI increases glycogen synthesis in hepatocytes (15). To address potential mechanisms underlying this observation, Glu-6-P and UDP-Glc levels were measured in untreated, AdCMV-GKL-treated, and AdCMV-HKI-treated hepatocytes. Untreated and transduced cells preincubated for 42 h at 1 mM glucose and then transferred to media containing variable glucose concentrations in the range of 1–25 mM for 2 h had Glu-6-P levels that increased in a glucose concentration-dependent manner (Fig. 1). In both AdCMV-HKI- and AdCMV-GKL-treated cells Glu-6-P was significantly increased over untreated control cells at all glucose levels (Fig. 1A). In AdCMV-HKI-treated cells, however, the greatest increases in Glu-6-P occurred between 1 and 5 mM glucose, with smaller increases at higher glucose levels (Fig. 1B). In AdCMV-GKL-treated cells, there was a sigmoidal increase in Glu-6-P accumulation with an inflection at glucose levels greater than 5 mM, reaching a value of 120 nmol/mg protein (approximately equivalent to a 30 mM intracellular concentration) in cells incubated in 25 mM glucose (Fig. 1A). The level of Glu-6-P in AdCMV-GKL-treated cells incubated in 25 mM glucose was 10- and 50 times greater than the corresponding levels in AdCMV-HKI and untreated cells, respectively. In parallel with the changes observed for Glu-6-P, the increases in UDP-Glc in AdCMV-HKI- and AdCMV-GKL-
treated cells were most pronounced at low (1–5 mM) and high (25 mM) glucose levels, respectively (Fig. 2). At 1 mM glucose, UDP-Glc levels in AdCMV-HKI-treated cells were more than double those in AdCMV-GKL-treated or untreated cells, whereas at 25 mM glucose, AdCMV-GKL-treated cells had UDP-Glc levels that were 3-fold greater than in AdCMV-HKI-treated cells and 7-fold greater than in untreated control cells.

Glycogen Synthase Activity in AdCMV-GKL- and AdCMV-HKI-treated Hepatocytes—A possible mechanism that can explain the ability of GK but not HK overexpression to activate glycogen synthesis (15) is that the Glu-6-P derived from GK but not HK increases the proportion of glycogen synthase that is in the active state. To investigate this hypothesis, we measured changes in the glycogen synthase activation state in response to changes in media glucose and intracellular Glu-6-P levels. Total glycogen synthase activity (measured in the presence of 6.6 mM Glu-6-P) was not statistically different in untreated cells, AdCMV-HKI-treated cells, and AdCMV-GKL-treated cells (2.06 ± 0.05, 2.30 ± 0.09, and 2.12 ± 0.11 milliunits/10^6 cells, respectively; mean ± S.E. for five determinations). A dramatic difference was noted, however, in the glycogen synthase activity ratio (activity measured in the absence of Glu-6-P divided by activity measured in the presence of 6.6 mM Glu-6-P), which increased sharply as a function of glucose concentration in the supernatant fraction from AdCMV-GKL-treated cells to a ratio of 0.56 ± 0.04 at 25 mM glucose, a value three times greater than that achieved in extracts from untreated or AdCMV-HKI-treated cells (Fig. 3A). Similar results were obtained in the 10,000 × g pellet fraction from these cells (Fig. 3B). Fig. 4, A and B, demonstrates that the increase in the GS activation state in both the supernatant and pellet fractions from AdCMV-GKL-treated hepatocytes is correlated with Glu-6-P levels. No increase in the GS activity ratio was observed in AdCMV-HKI-treated cells relative to untreated cells (Fig. 3), despite the fact that Glu-6-P levels are increased to a similar extent in AdCMV-GKL and AdCMV-HKI-treated hepatocytes (Fig. 1B) at glucose concentrations (5 mM, for example) that induce GS activation in AdCMV-GKL-treated cells. These data support the hypothesis that Glu-6-P produced from GK has a different regulatory impact on glycogen synthase activation than Glu-6-P produced by hexokinase I.

In addition to its role in promoting the activation of glycogen synthase, Glu-6-P can also induce the translocation of the enzyme from a cytosolic pool to a 10,000 × g pellet pool (12). Total glycogen synthase activity (measured in the presence of 6.6 mM Glu-6-P) decreased in the supernatant fraction from 1.5 ± 0.1 milliunits/10^6 cells in untreated cells to 0.73 ± 0.07 milliunits/10^6 cells in AdCMV-GKL-treated cells incubated at 25 mM glucose. Concomitantly, total GS activity increased in the pellets from 0.53 ± 0.08 to 1.44 ± 0.08 milliunits/10^6 cells, respectively. The combination of translocation and activation led to an increase in active GS in the pelletable fraction in AdCMV-GKL-treated but not AdCMV-HKI-treated cells. As shown in Fig. 5, incubation of AdCMV-GKL-treated but not AdCMV-HKI-treated hepatocytes with increasing concentrations of glucose caused a large increase in active glycogen synthase (measured in the absence of Glu-6-P) in the 10,000 × g pellet fraction. In cells incubated at 5 mM glucose, active glycogen synthase in the pellet from glucokinase-overexpressing cells was twice that in untreated or hexokinase-overexpressing cells, becoming five times greater in cells incubated at 25 mM glucose. The corresponding increases in the supernatant fraction were less marked, being only 39% and 2-fold at 5 and 25 mM glucose, respectively. These data provide further support for a differential regulatory impact of Glu-6-P produced by...
hexokinase versus glucokinase in liver cells.

**Relationship between Glu-6-P and Lactate Accumulation**—The foregoing results indicate that Glu-6-P produced from glucokinase has a greater glycogenic potential than Glu-6-P produced from hexokinase. To investigate the relationship between the source of Glu-6-P and the glycolytic rate, intracellular Glu-6-P levels and lactate levels were measured in untreated and AdCMV-GKL- and AdCMV-HKI-treated cells cultured at glucose concentrations in the range of 1–5 mM (Fig. 1B). As shown in Fig. 6, a tight linear relationship exists between the intracellular Glu-6-P level and the media lactate concentration for all three groups of cells, indicating that Glu-6-P produced by glucokinase has no advantage relative to Glu-6-P produced by hexokinase for entry into the glycolytic pathway.

**DISCUSSION**

The purpose of this study was to further evaluate the role of glucose phosphorylation in the control of glycogen synthase and to elucidate the mechanism underlying the differential metabolic impact of glucokinase and hexokinase I overexpression in hepatocytes. Previous studies have shown that glucose must be phosphorylated to promote the activation of hepatic glycogen synthase (4, 6, 7). The results of the current study clearly support this hypothesis but also introduce the new concept that the potency of Glu-6-P for activation of glycogen synthase and glycogenesis is determined by the hexokinase isoform that is responsible for its production.

We have used the recombinant adenovirus system to overexpress glucokinase or hexokinase I, thereby raising Glu-6-P to levels much higher than found in untreated hepatocytes expressing only endogenous hexokinases. The accumulation of Glu-6-P in AdCMV-HKI- and AdCMV-GKL-treated cells reflects the known kinetic properties of hexokinase I and glucokinase. The increase in Glu-6-P levels in cells overexpressing glucokinase is described by a sigmoidal curve, with an inflection point at 5 mM glucose, in accord with the enzyme’s high \( K_{0.5} \) for glucose and its cooperative behavior (13, 14). In cells overexpressing hexokinase I, Glu-6-P accumulation is enhanced to the same degree as in AdCMV-GKL-treated cells up to glucose concentrations of 5 mM, but at higher concentrations of the sugar, much more Glu-6-P accumulates in the hexokinase-overexpressing cells. Since glucose-phosphorylating activity in extracts of AdCMV-GKL- and AdCMV-HKI-treated cells was equivalent when measured at 20 mM glucose, the modest further accumulation of Glu-6-P at high glucose concentrations in hexokinase-overexpressing cells is likely explained by the inhibition of this enzyme by its reaction product (14).

At low glucose concentrations, Glu-6-P does accumulate in...
AdCMV-HKI-treated cells to levels that are 5–9 times higher than in untreated control cells. Importantly, this Glu-6-P does not have the same regulatory impact as the identical amount of Glu-6-P produced by overexpression of glucokinase. This is best illustrated by comparison of events in glucokinase- versus hexokinase-overexpressing cells incubated at 5 mM glucose. Glu-6-P levels are elevated from approximately 1.5 nmol/mg protein (approximately 0.3 mM intracellular concentration) to 8–10 nmol/mg protein (2.0–2.5 mM) in both AdCMV-GKL- and AdCMV-HKI-treated cells (Fig. 1B), but only the glucokinase-overexpressing cells exhibit increases in the glycogen synthase activity ratio (Fig. 3), translocation of glycogen synthase (Fig. 5), and, as shown in our previous study (15), activation of glycogen synthase at this concentration of the sugar. Glu-6-P is thought to bind to glycogen synthase and to cause a conformational change that activates the enzyme and renders it a better substrate for protein phosphatases (10). The increase in the activation state of glycogen synthase in AdCMV-GKL-treated cells suggests that accumulation of glucokinase-derived Glu-6-P enhances dephosphorylation of the enzyme more effectively than Glu-6-P derived from overexpressed hexokinase I.

One potential explanation for the lack of metabolic impact of Glu-6-P on glycogen synthesis in hexokinase I-overexpressing cells could have been a failure to divert this pool of the intermediate to UDP-Glc. Our data show, however, that this is not the case, since UDP-Glc levels are actually more elevated in hexokinase-overexpressing cells than in glucokinase-overexpressing cells incubated at 1 or 5 mM glucose (Fig. 2). Thus, UDP-Glc formation is clearly not limiting at 5 mM glucose, a concentration sufficient to activate glycogen accumulation in glucokinase-overexpressing cells but not hexokinase-overexpressing cells. These data show that the increase in UDP-Glc achieved at 5 mM glucose is not sufficient to activate glycogen synthesis through a “push” mechanism, as has been proposed (26), and that other regulatory events, such as Glu-6-P-mediated activation of glycogen synthase, must accompany accumulation of the proximate precursor.

Recent studies have demonstrated that hepatic glucokinase translocates from a bound to a free state in response to high glucose concentrations or to micromolar concentrations of fructose or sorbitol, which are precursors of fructose 1-phosphate (27). Translocation of glucokinase by these substrates correlates with stimulation of glycogen synthesis (28). In contrast to glucokinase, the low $K_m$ hexokinases do not translocate in response to either elevated glucose concentrations or the presence of fructose or sorbitol (29). The failure to activate glycogen synthase with increasing Glu-6-P in hexokinase-overexpressing cells may be explained by compartmentation of this pool of Glu-6-P at a site that is not accessible to glycogen synthase. Translocation of glucokinase to a “glycogenic site” may also help explain the results of Cahill et al. (30), who showed nearly 40 years ago in rat liver slices that glycolysis becomes saturated at approximately 20 mM glucose, whereas rates of glyco-
genesis continue to increase at much higher levels of the hexose (30).

In sum, we have demonstrated that the distinct effects of glucokinase and hexokinase I overexpression on glycogen synthesis in hepatocytes can be explained by the differential impact of Glu-6-P produced by the two enzymes on activation and translocation of glycogen synthase. These studies confirm the importance of Glu-6-P in regulation of glycogenesis and provide important new evidence for a second level of control conferred by the hexokinase isoform that is responsible for glucose phosphorylation. Whether the differential potency of glucokinase and hexokinase in regulation of glycogen synthase is related to differences in their subcellular localization will be an important topic for future study.

Acknowledgments—We thank Anna Adrover for skilled technical assistance and Dr. Loranne Agius (University of Newcastle) for helpful comments and suggestions.

REFERENCES

1. Stalmans, W., DeWulf, H., Hue, L., and Hers, H. G. (1974) Eur. J. Biochem. 41, 123–124
2. Ciudad, C. J., Massague, J., and Guinovart, J. V. (1979) FEBS Lett. 99, 321–324
3. Ciudad, C. J., Carabaza, A., Busch, F., Gómez-Font, A. M., and Guinovart, J. V. (1988) Arch. Biochem. Biophys. 264, 30–39
4. Carabaza, A., Ciudad, C. J., Baque, S., and Guinovart, J. V. (1992) FEBS Lett. 296, 211–214
5. Newgard, C. B., Hwang, P. K., and Fletterick, R. J. (1989) CRC Crit. Rev. Biochem. Mol. Biol. 24, 69–99
6. Massillon, D., Bollen, M., De Wulf, H., Overloop, K., Vanstapel, F., Van Hecke, P., and Stalmans, W. (1995) J. Biol. Chem. 270, 19351–19356
7. Ciudad, C. J., Carabaza, A., and Guinovart, J. V. (1986) Biochem. Biophys. Res. Commun. 141, 1195–1200
8. Fernandez-Novell, J. M., Roca, A., Bellido, D., Vilaro, S., and Guinovart, J. V. (1996) Eur. J. Biochem. 238, 570–574
9. Roach, P. J. (1980) in Enzymes (Boyer, P. D., and Krebs, E. G., eds) 3rd Ed., Vol. 17, pp. 499–539, Academic Press, New York
10. Villar-Palasi, C. (1991) Biochem. Biophys. Acta 1244, 203–208
11. Fernandez-Novell, J. M., Arino, J., Vilaro, S., and Guinovart, J. V. (1992) Biochem. J. 281, 443–448
12. Fernandez-Novell, J. M., Arino, J., Vilaro, S., Bellido, D., and Guinovart, J. V. (1992) Biochem. J. 286, 497–501
13. Weinhouse, S. (1970) Curr. Top. Cell Regul. 11, 1–50
14. Wilson, J. E. (1984) in Regulation of Carbohydrate Metabolism (Beitner, R., ed) pp. 45–85, CRC Press, Boca Raton, FL
15. O’Doherty, R. M., Lehman, D. L., Sosna, J., Gómez-Font, A. M., Guinovart, J. V., and Newgard, C. B. (1996) J. Biol. Chem. 271, 20524–20530
16. Becker, T. C., BeltranRodrigo, H., Noel, R., Johnson, J. H., and Newgard, C. B. (1994) J. Biol. Chem. 269, 21234–21238
17. Becker, T. C., Noel, R. J., Johnson, J. H., Lynch, H. M., Hirose, H., Tokuyama, Y., Bell, G. I., and Newgard, C. B. (1996) J. Biol. Chem. 271, 390–394
18. Massague, J., and Guinovart, J. V. (1977) FEBS Lett. 82, 317–320
19. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
20. Kawajima, M., Newgard, C. B., Foster, D. W., and McGarry, J. D. (1986) J. Biol. Chem. 261, 8849–8853
21. Thomas, J. A., Schledner, K. R., and Larner, J. (1968) Anal. Biochem. 25, 486–489
22. Chan, T. M., and Exton, J. H. (1976) Anal. Biochem. 71, 96–105
23. Lang, G., and Michal, G. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed) Vol. 3, pp. 1238–1242, Academic Press, New York
24. Keppler, D., and Beck, K. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed) Vol. 3, pp. 524–530, Academic Press, New York
25. Gutmann, I., and Wahlefeld, A. W. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed) Vol. 3, pp. 1464–1468, Academic Press, New York
26. Van de Werve, G., and Jeannenaud, B. (1984) Am. J. Physiol. 247, E271–E275
27. Agius, L., and Peak, M. (1993) Biochem. J. 296, 785–796
28. Agius, L. (1994) Biochem. J. 296, 30–39
29. Agius, L. (1994) Biochem J. 303, 841–846
30. Cahill, G. F., Jr., Hastings, A. B., Ashmore, J., and Zottu, S. (1958) J. Biol. Chem. 230, 125–135
Glucose 6-Phosphate Produced by Glucokinase, but Not Hexokinase I, Promotes the Activation of Hepatic Glycogen Synthase

Joan Seoane, Anna M. Gómez-Foix, Robert M. O'Doherty, Cristina Gómez-Ara, Christopher B. Newgard and Joan J. Guinovart

J. Biol. Chem. 1996, 271:23756-23760.
doi: 10.1074/jbc.271.39.23756

Access the most updated version of this article at http://www.jbc.org/content/271/39/23756

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 29 references, 12 of which can be accessed free at http://www.jbc.org/content/271/39/23756.full.html#ref-list-1