Differing Roles of Akt and Serum- and Glucocorticoid-regulated Kinase in Glucose Metabolism, DNA Synthesis, and Oncogenic Activity*

Received for publication, February 3, 2003, and in revised form, April 30, 2003
Published, JBC Papers in Press, May 6, 2003, DOI 10.1074/jbc.M301127200

Hideyuki Sakoda‡, Yukiko Gotoh§, Hideki Katagiri¶, Mineo Kurokawa, Hiraku Oono‡, Yukiko Onishi‡, Motonobu Anai‡, Takehide Oghara, Midori Fujisiro, Yasushi Fukushima, Miho Abe, Nobuhiro Shojima, Masatoshi Kikuchi, Yoshitomo Oka§, Hisamaru Hirai, and Tomoichiro Asano**

From the ‡Institute for Adult Diseases, Asahi Life Foundation, 1-9-14 Nishishinjuku, Shinjuku-ku, Tokyo 116, Japan, the ¶Department of Internal Medicine, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan, the §Department of Molecular Biology, Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan, and the **Division of Molecular Metabolism and Diabetes, Department of Internal Medicine, Tohoku University Graduate School of Medicine, 2-1 Seiryou, Aoba-ku, Sendai 980-8575, Japan

Serum- and glucocorticoid-regulated kinase (SGK) is a serine kinase that has a catalytic domain homologous to that of Akt, but lacks the pleckstrin homology domain present in Akt. Akt reportedly plays a key role in various cellular actions, including glucose transport, glycogen synthesis, DNA synthesis, anti-apoptotic activity, and cell proliferation. In this study, we attempted to reveal the different roles of SGK and Akt by overexpressing active mutants of Akt and SGK. We found that adenovirus-mediated overexpression of myristoylated (myr-) forms of Akt resulted in high glucose transport activity in 3T3-L1 adipocytes, phosphorylated glycogen synthase kinase-3 (GSK3) and enhanced glycogen synthase activity in hepatocytes, and the promotion of DNA synthesis in interleukin-3-dependent 32D cells. In addition, stable transfection of myr-Akt in NIH3T3 cells induced an oncogenic transformation in soft agar assays. The active mutant of SGK (D-SGK, substitution of Ser422 as Asp) and myr-SGK were shown to phosphorylate GSK3 and to enhance glycogen synthase activity in hepatocytes in a manner very similar to that observed for myr-Akt. However, despite the comparable degree of GSK3 phosphorylation between myr-Akt and D-SGK or myr-SGK, D-SGK and myr-SGK failed to enhance glucose transport activity in 3T3-L1 cells, DNA synthesis in 32D cells, and oncogenic transformation in NIH3T3 cells. Therefore, the different roles of SGK and Akt cannot be attributed to ability or inability to translocate to the membrane thorough the pleckstrin homology domain, but rather must be attributable to differences in the relatively narrow substrate specificities of these kinases. In addition, our observations strongly suggest that phosphorylation of GSK3 is either not involved in or not sufficient for GLUT4 translocation, DNA synthesis, or oncogenic transformation. Thus, the identification of substrates selectively phosphorylated by Akt, but by not SGK, may provide clues to clarifying the pathway leading from Akt activation to these cellular activities.

PI 3-kinase has been implicated in the regulation of numerous cellular processes (1–3). The lipid product of PI 3-kinase reportedly activates several AGC kinases, including Akt, atypical protein kinases C, p70 S6 kinase, and SGK (3, 45). Among these AGC kinases, Akt was found to mediate various insulin- and growth factor-induced cellular responses such as stimulation of GLUT4 translocation to the plasma membrane (10, 11, 46, 47), inhibition of GSK3 (22), and promotion of cell survival by inhibiting apoptosis (4–8). In addition, as shown by the v-Akt data, constitutively activated Akt has transforming activity (9). These cellular activities have been shown to be induced by overexpression of constitutively activated Akt or its membrane-targeted mutant. For example, glucose uptake is reportedly increased in both constitutively activated Akt-overexpressing 3T3-L1 adipocytes and L6 myotubes (10, 11), whereas Akt2-deficient mice show impaired glucose tolerance due to decreased insulin-induced glucose uptake in skeletal muscle and increased hepatic glucose production (12).

On the other hand, SGK, the expression level of which is increased by glucocorticoid and serum stimulation in cultured cells (13, 48), is the one member of the AGC kinase family with a highly conserved kinase domain compared with that of Akt (54% identical amino acids) (14, 15). Indeed, the substrate specificity of the kinase domain of SGK has been reported to be similar to that of Akt (16). Furthermore, it was also reported that the PI 3-kinase pathway activates SGK through PDK1/2, i.e. in the same manner as for Akt (17, 49). However, the most apparent difference in the structures of SGK and Akt is that SGK lacks the PH domain that Akt has. Thus, SGK is considered to not be able to translocate to the membrane like Akt in response to growth factor stimulation. In fact, it was reported that insulin stimulation induces the translocation of Akt to the membrane fraction, but that this does not occur with mutant Akt lacking the PH domain or with SGK (18).

Although recent studies revealed a possible role of SGK in aldosterone-induced apical translocation of the epithelial sodium channel in distal nephrons (19–21), it seems that our understanding of the roles of SGK remains limited. In this

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

** To whom correspondence should be addressed. Tel.: 81-3-3815-5411 (ext. 33133); Fax: 81-3-5803-1874; E-mail: asano-tky@umin.ac.jp.

† The abbreviations used are: PI, phosphatidylinositol; SGK, serum- and glucocorticoid-regulated kinase; GLUT4, glucose transporter-4; GSK3, glycogen synthase kinase-3; PDK, phosphoinositide-dependent kinase; PH, pleckstrin homology; myr-, myristoylated; DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; IL-3, interleukin-3; BrdUrd, bromodeoxyuridine; WT, wild-type.
study, we investigated whether SGK induces the cellular functions known to be induced by activated Akt. Akt activation reportedly plays a key role in glucose metabolism, including increased glycogen synthase and GLUT4 translocation to the plasma membrane, as well as in inhibition of apoptosis and promotion of cell growth. The induction of these cellular functions by Akt has been well demonstrated, and they are also induced by overexpressing myr-Akt (10, 11, 22). Thus, we constructed the active mutant of SGK and myr-SGK and investigated whether these SGK mutants can exert the same actions as myr-Akt.

This is the first report clearly demonstrating the different roles of Akt and SGK. In addition, interestingly, these differences are attributable not to the presence or absence of the PH domain responsible for membrane targeting, but very possibly to differences in the substrate specificities of the kinase domains of SGK and Akt.

EXPERIMENTAL PROCEDURES

Antibodies—Anti-Myc tag (clone 9E10), anti-phospho-Ser**2** AKT1, and anti-SGK antibodies were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). Anti-Akt and anti-phospho-Ser**4** AKT1 antibodies were purchased from Cell Signaling Technology (Beverly, MA).

Cell Culture—Hepatocytes were isolated from fasted rats by collagenase perfusion as described previously (23) and plated in collagen-coated 25-cm**2** flasks at a density of 1.0 × 10**5** cells/cm**2** in DMEM supplemented with 10% FCS, 0.5 mg/ml insulin, 1 μM dexamethasone, and 10 mg/ml epidermal growth factor. After a 6-h attachment period, hepatocytes were transfected using adenoviral gene transfer. 3T3-L1 fibroblasts were maintained in DMEM supplemented with 10% donor calf serum (Invitrogen) under a 10% CO**2** atmosphere at 37 °C. 2 days after the fibroblasts reached confluence, differentiation was induced by incubating the cells for 48 h in DMEM supplemented with 10% fetal bovine serum, 0.5 mM 3-isobutyl-1-methylxanthine, and 4 mg/ml dexamethasone. Thereafter, the cells were maintained in DMEM containing 10% FCS and 0.25 ng/ml IL-3. After incubation, the cells were IL-3-starved for an additional 24 h, and then DNA synthesis assays were performed. In 3T3-L1 adipocytes and 32D cells, all recombinant adenoviruses were used at a concentration of 3.0 × 10**9** plaque-forming units/ml. Under these conditions, none of the cells infected with LacZ exhibited significant differences in glycogen synthesis or glucose transport activity compared with non-infected cells, and 95–100% of the cells were infected as judged by blue coloration after 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) incubation 36–48 h after infection.

Immunoprecipitation and Immunoblotting—The cells were collected and boiled in Laemmli sample buffer containing 100 mM/liter dithiothreitol. SDS-PAGE and immunoblotting were performed as described previously (27) using each antibody as a probe.

Glycogen Synthase Assay—After 3 h of serum starvation, cells in 25-cm**2** flasks were stimulated with or without 100 ng/ml insulin for 30 min. The cells were homogenized in homogenizing buffer containing 50 mM Tris, 10 mM EDTA, and 100 mM NaF. 50 μg of liver samples were then assayed in glycogen synthase buffer containing 8.9 mM UDP-[**6**-**3**H]glucose (1 Ci/mmol) in the absence or presence of 10 mM Glu-6-P for 20 min at 30 °C. UDP-[**6**-**3**H]glucose incorporation into glycogen was measured by liquid scintillation counting.

Glucose Transport Assay—3T3-L1 adipocytes in 24-well culture dishes were serum-starved for 3 h in DMEM containing 0.2% bovine serum albumin. They were next incubated for 45 min in glucose-free Krebs-Ringer phosphate buffer (137 mM NaCl, 4.7 mM KCl, 10 mM sodium phoshate (pH 7.4), 0.5 mM MgCl**2**, and 1 mM CaCl**2**) and then incubated with or without 100 nm insulin for 15 min. Basal and stimulated uptakes of 2-deoxy-[**6**-**3**H]glucose were then measured as described previously (28).

DNA Synthesis Assay—32D cells were maintained in RPMI 1640 medium supplemented with 10% FCS and 0.25 ng/ml IL-3. After incubation in IL-3-free RPMI 1640 medium for 24 h, the cells were incubated with BrdUrd labeling solution for 6 h. BrdUrd incorporation was measured by liquid scintillation counting.

Construction of SGK Mutants and myr-Akt—3A-SGK, a dominant-negative form of SGK, was constructed by substituting lysine 127, threonine 256, and serine 422 with alanines. n-SGK was constructed by substituting serine 422 with aspartic acid. myr-SGK contains an src myristoylation signal sequence at the N terminus. MAA-Akt was constructed by substituting lysine 179, threonine 308, and serine 473 with alanines. myr-Akt, which contains an src myristoylation signal sequence, was described previously (25). All the constructs were designed to contain a Myc tag at the C terminus.

FIG. 1. Schematic presentation of recombinant adenoviruses of Akt and SGK. 3A-SGK was constructed by substituting lysine 127, threonine 256, and serine 422 with alanines. D-SGK was constructed by substituting serine 422 with aspartic acid. myr-SGK and myr-Akt contain an src myristoylation signal sequence (myr) at the N terminus. MAA-Akt was constructed by substituting lysine 179, threonine 308, and serine 473 with alanines. All constructs were designed to contain a Myc tag at the C terminus. a.a., amino acids.
Different Cellular Actions Mediated by Akt and SGK

Effect of SGK or Akt Overexpression on Glucose Transport—WT-Akt, MAA-Akt, myr-Akt, WT-SGK, 3A-SGK, and myr-SGK were overexpressed in 3T3-L1 adipocytes. Immunoblotting with anti-Myc tag antibody revealed the expression levels of Akt and SGK and their mutants to be comparable (Fig. 2A, first panel). In addition, the expression levels of WT-Akt, MAA-Akt, and myr-Akt were approximately five times those of endogenously expressed Akt judging from the immunoblotting results using anti-Akt antibody (Fig. 2A, second panel), and basal phosphorylation was observed in myr-Akt (third panel). On the other hand, endogenously expressed SGK was not detectable in 3T3-L1 adipocytes, whereas overexpressed WT-SGK, 3A-SGK, and myr-SGK were detected by immunoblotting (Fig. 2A, fourth panel). Increased phosphorylation of GSK3β was observed in 3T3-L1 adipocytes overexpressing myr-Akt, n-SGK, and myr-SGK, whereas overexpression of wild-type and dominant-negative mutants of Akt and SGK did not increase GSK3

phosphorylation (Fig. 2A, fifth panel). Finally, interestingly, although 2-deoxy-D-[3H]glucose uptake into 3T3-L1 adipocytes was increased 8-fold by overexpression of myr-Akt compared with that of LacZ, n-SGK or myr-SGK overexpression had essentially no effect on 2-deoxy-D-[3H]glucose uptake (Fig. 2B).

Effect of SGK or Akt Overexpression on Phosphorylation of GSK3 and Glycogen Synthesis—To confirm that phosphoryl-
ated GSK3 elevates glycogen synthesis irrespective of whether Akt or SGK phosphorylates GSK3, active mutants of Akt (myr-Akt) and SGK (p-SGK and myr-SGK) were overexpressed in primary cultured rat hepatocytes. The expression levels of myr-Akt, D-SGK, and myr-SGK were comparable, as shown by immunoblotting using anti-Myc tag antibody (Fig. 3A). Similar to the observations in 3T3-L1 adipocytes, overexpression of these active mutants of Akt and SGK induced an apparent increase in phosphorylation of GSK3, as did insulin stimulation (Fig. 3B). As shown in Fig. 3C, glycogen synthase activity was shown to be significantly increased (~1.5-fold) either by 30 min of stimulation with insulin or by overexpression of myr-Akt, p-SGK, or myr-SGK compared with the control. Thus, it is clear that phosphorylation of GSK3 results in the elevation of glycogen synthesis irrespective of phosphorylation by either Akt or SGK.

**Effect of SGK or Akt Overexpression on DNA Synthesis**—WT-Akt, MAA-Akt, myr-Akt, WT-SGK, 3A-SGK, and myr-SGK were overexpressed in 32D cells by adenoviral transfer so that their expression levels were similar (Fig. 4A), and the effects on DNA synthesis were examined by measuring BrdUrd incorporation. Because 32D is an IL-3-dependent cell line, removal of IL-3 from the medium reportedly induces apoptosis, which can be shown to be the result of suppressed DNA synthesis. Overexpression of myr-Akt, D-SGK, and myr-SGK induced GSK3 phosphorylation to a similar degree, whereas that of WT-Akt, MAA-Akt, WT-SGK, and 3A-SGK did not (Fig. 4B). However, interestingly, overexpression of only myr-Akt significantly enhanced the protection from apoptosis achieved by lack of IL-3 (Fig. 4C). On the other hand, overexpression of p-SGK or myr-SGK had no inhibitory effect on apoptosis, despite phosphorylation of GSK3.

**myr-Akt Overexpression Alone Can Transform NIH3T3 Cells**—Finally, we analyzed the transforming ability of Akt and SGK by evaluating the capacity for anchorage-independent growth. NIH3T3 cells stably transfected with either Akt or SGK constructs were seeded in DMEM containing 0.3% agar and 20% FCS, and colony formation was estimated as a representative of anchorage-independent growth ability. Three independent experiments were performed using three different clones of each derivative, and a representative one is shown in Fig. 5 (A and B). Stable expression of Akt and SGK constructs was confirmed by immunoblotting with anti-Myc tag, anti-Akt, and anti-SGK antibodies (Fig. 5B). Similar to observations in cells transiently overexpressing Akt or SGK due to adenoviral transfer, stable overexpression of myr-Akt, D-SGK, and myr-SGK significantly increased the phosphorylation level of GSK3, whereas that of other constructs did not. These cells were subjected to soft agar assay to evaluate their transforming activity. As shown in Fig. 5B (middle panel), myr-Akt-expressing cells formed many macroscopic colonies within 14 days after being seeded. In contrast, although p-SGK- and myr-SGK-expressing cells showed phosphorylation of GSK3, they apparently could not make colonies (Fig. 5B, upper and lower panels). The colonies (counted from the three independent clones for each of the Akt and SGK constructs) are shown as bar graphs in Fig. 5C.

**DISCUSSION**

Numerous serine/threonine kinases exist in the cell, and they play specific roles by inducing individual cellular functions. As to the molecular mechanisms underlying the various roles of each kinase protein, the substrate specificity of the kinase domain and subcellular distribution are considered to be major contributors. SGK and Akt are considered to be very similar kinase proteins, both of which belong to the AGC family. They are activated via phosphorylation by PDK1 and PDK2, located downstream from PI 3-kinase. However, the presence and absence of a PH domain in Akt and SGK, respectively, should result in different subcellular distributions of these two kinases. In the case of Akt, Akt translocates to the membrane fraction via binding of the PH domain with PI-3,4,5-P_3 produced by PI 3-kinase, and phosphorylation of
Thr306 and Ser473 of Akt by PDK1 and PDK2 takes place, thereby activating Akt kinase (30–32). myr-Akt is located at the plasma membrane without stimulation and is constitutively activated (10, 33). Then, Akt phosphorylates several substrates that transmit signals (6).

In this study, to investigate the different roles of Akt and SGK, active as well as membrane-targeted active mutants of these kinases were overexpressed. Previous studies have established the functions induced by and the roles of activated Akt, and all or most of these cellular actions, for which Akt is reportedly responsible, are indeed induced by overexpression of myr-Akt (33–37). Thus, although this study was carried out employing an overexpression system, the cellular actions described herein are considered to be physiological functions induced by Akt. For example, it is well established that Akt plays a major role in glucose metabolism, including increased glycolysis and GLUT4 translocation to the plasma membrane, functions that can be induced by overexpressing myr-Akt (10, 11). Indeed, translocation of Akt to the plasma membrane is suggested to be important for glucose transport.

In addition, the oncogenic activity of Akt is also well known since v-Akt has been identified as an oncogenic protein, and this promotion of cell growth activity is similarly observed upon overexpression of myr-Akt (38, 39).

We obtained two important conclusions from this comparative study of SGK and Akt mutants. The first is that SGK can mediate GSK3 phosphorylation and the resultant glycogen synthesis, but not other cellular functions induced by Akt such as increased glucose transport, inhibition of apoptosis, and oncogenic transformation. Thus, it is possible that increased expression of SGK inhibits the phosphorylation of Akt and the resultant cellular functions such as increased glucose transport, inhibition of apoptosis, and cell proliferation because these two kinases are similarly phosphorylated by PDK1 and PDK2. Conversely, an increase in SGK may contribute to increased glycolysis by increasing the phosphorylation of GSK3. In addition, a previous report showed that overexpression of the myristoylated and PH domain-deleted form of Akt induces an increase in glucose transport activity in 3T3-L1 adipocytes (10). Therefore, these different roles of the two kinases are apparently not attributable to membrane-targeting ability or an unidentified mechanism of signal transduction from the PH domain, because myristoylated and PH domain-deleted Akt, but not myr-SGK, enhanced glucose transport, inhibition of apoptosis, and cell proliferation.

GSK3 is well established as a regulator of glycogen metabolism (22, 40–42). Many proteins in this family are related to protein synthesis. Wnt signaling and transcription factors have been reported to be substrates of GSK3, and Somervaille et al. (43) described GSK3 and Bax as being involved in the suppression of apoptosis induced by growth factor withdrawal. However, our results suggest that GSK3 is very likely to be independent of Akt-induced cellular actions such as increased glucose transport, inhibition of apoptosis, and cell proliferation.

As discussed above, the different cellular functions induced by Akt and SGK cannot be attributed to the difference in membrane targeting through the PH domain. Although it has been reported that substrate specificities for synthetic peptides differ minimally between Akt and SGK based on an in vitro experiment using glutathione S-transferase fusion proteins and synthetic peptides (16), we speculate that considerable differences in terms of in vivo substrate specificity exist between these kinases, which account for the apparent differences in the roles of Akt and SGK. In other words, there may be some unidentified proteins phosphorylated by Akt, but not by SGK, that play key roles in glucose transport and anti-apoptotic effects, whereas GSK3 plays only minor roles in these functions.

In conclusion, we have demonstrated for the first time an important difference between Akt and SGK and that this difference is not due to membrane localization, but rather possibly to the difference in their kinase activities. The identification of substrates selectively phosphorylated by Akt, but not by SGK, may provide clues to clarifying the pathway leading from Akt activation to the aforementioned cellular activities.
41. Cohen, P., and Frame, S. (2001) Nat. Rev. Mol. Cell. Biol. 2, 769–776
42. Brady, M. J., Kartha, P. M., Aysola, A. A., and Saltiel, A. R. (1999) J. Biol. Chem. 274, 27497–27504
43. Somervaille, T. C., Linch, D. C., and Khwaja, A. (2001) Blood. 98, 1374–1381
44. Glowacki, J., Mizuno, S., and Greenberger, J. S. (1998) Cell Transplant. 7, 319–326
45. Czech, M. P., and Corvera, S. (1999) J. Biol. Chem. 274, 1865–1868
46. Wang, Q., Somwar, R., Bilan, P. J., Liu, Z., Jin, J., Woodgett, J. R., and Klip, A. (1999) Mol. Cell. Biol. 19, 4008–4018
47. Foster, L. J., Li, D., Randhawa, V. K., and Klip, A. (2001) J. Biol. Chem. 276, 44212–44221
48. Mizuno, H., and Nishida, E. (2001) Genes Cells 6, 261–268
49. Virbasius, J. V., Song, X., Pomerleau, D. P., Zhan, Y., Zhou, G. W., and Czech, M. P. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 12908–12913
Differing Roles of Akt and Serum- and Glucocorticoid-regulated Kinase in Glucose Metabolism, DNA Synthesis, and Oncogenic Activity
Hideyuki Sakoda, Yukiko Gotoh, Hideki Katagiri, Mineo Kurokawa, Hiraku Ono, Yukiko Onishi, Motonobu Anai, Takehide Ogihara, Midori Fujishiro, Yasushi Fukushima, Miho Abe, Nobuhiro Shojima, Masatoshi Kikuchi, Yoshitomo Oka, Hisamaru Hirai and Tomoichiro Asano

J. Biol. Chem. 2003, 278:25802-25807.
doi: 10.1074/jbc.M301127200 originally published online May 6, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M301127200

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 49 references, 30 of which can be accessed free at http://www.jbc.org/content/278/28/25802.full.html#ref-list-1