Inhibition of Glucose and Dehydroascorbic Acid Uptakes by Resveratrol in Human Transformed Myelocytic Cells

Jae B. Park[†]

Phytonutrients Laboratory, Building 307, Room 313, ARS, USDA, Beltsville, Maryland 20705

Received August 21, 2000

Resveratrol (*trans*-3,5,4′-trihydroxystilbene) is a natural phytochemical found in grapes and wine. Numerous biological effects of resveratrol have been reported in the last 10 years. In this paper, the competitive inhibition of intracellular uptake of glucose and dehydroascorbic acid in U937 and HL-60 cells by resveratrol is reported.

Resveratrol (*trans*-3,5,4′-trihydroxystilbene) has been reported to have numerous biological effects, including antiinflammatory, antiplatelet, and anticarcinogenic effects in humans.[1−6] These effects may be due to its antioxidant property and/or to its chemical structure.[4,7,8] For instance, resveratrol is presumed to inhibit ribonucleotide reductase and cyclooxygenase-2 due to its antioxidant property[7,8] and to play the role of an antagonist for estrogen receptors due to its chemical structure.[9]

Resveratrol is abundant in grapes and wine. It is usually consumed by humans in conjunction with other dietary components and may therefore interact with other constituents of the diet. However, resveratrol has largely been studied as a single variable.[4−9] A lack of studies of possible interactions diminishes the ability to evaluate the true effects of resveratrol in humans.[7−9] Because flavonoids inhibit glucose uptake in U937 and HL-60 cells,[10] and because there is a structural similarity (in the A and B rings) between flavonoids and resveratrol, the effect of resveratrol as an inhibitor of glucose uptake in these cells was investigated. The effect of resveratrol on dehydroascorbic acid uptake was also studied, since dehydroascorbic acid (an oxidized form of vitamin C) is transported into these cells via glucose transporters (Glut 1 and Glut 3).[11−14]

To demonstrate that resveratrol inhibits glucose uptake, two myelocytic cell lines (U937 and HL-60) known for their capability of transporting glucose were utilized in these studies. Glucose uptake in both U937 and HL-60 cell lines was inhibited approximately 50% by resveratrol at concentrations of 73 and 75 μM, respectively (Figure 1A). To define the pattern of the inhibition and to determine K_i values of resveratrol, kinetic studies were performed using U937 cells. Glucose uptake was competitively inhibited by resveratrol; the K_i value calculated using the Michaelis−Menton plot was 89 μM (Figure 1b). Similarly, in HL-60 cells, the K_i value was 85 μM. Glucose uptake occurs via both sodium-independent and -dependent glucose transporters: glucose transporters 1−5 are sodium-independent transporters, and SGLT (sodium/glucose cotransporter) is a sodium-dependent transporter.[15−17] Therefore, glucose uptake was also investigated in the presence and absence of sodium (see Experimental Section). In U937 and HL-60 cells, most glucose uptake is sodium-independent and is inhibited by resveratrol (data not shown). The data in this study indicate that most of the glucose uptake in U937 and HL-60 cells is independent of sodium and that resveratrol inhibited this uptake. Although in most replications, resveratrol usually inhibited glucose uptake in U937 and HL-60 cells, incomplete inhibition (approximately 20%) (Figure 1a) was occasionally observed and could not be eliminated even at high concentrations of resveratrol (>500 μM). A plausible reason for the incomplete inhibition might be the existence of a small quantity of sodium-dependent glucose transporters in U937 and HL-60 cells. In the absence of sodium, the incomplete inhibition was reduced to less than 10% at resveratrol concentrations > 100 μM. This also supports the data that resveratrol inhibits sodium-independent glucose transport.

Since dehydroascorbic acid uptake occurs via two sodium-independent glucose transporters (Glut1 and Glut 3),[11−14] and resveratrol inhibited sodium-independent uptake of glucose in U937 and HL-60 cells, the effect of resveratrol on dehydroascorbic acid uptake was investigated in these cells. Dehydroascorbic acid uptake was inhibited by resveratrol, and the inhibition was concentration-dependent (Figure 2a). Resveratrol inhibited 50% of dehydroascorbic acid uptake at concentrations of 38 and 32 μM in U937 and HL-60 cells, respectively (Figure 2a). In U937, the inhibition by resveratrol was competitive, and K_i values calculated using the Michaelis−Menton plot were approximately 35 μM (Figure 2b). To confirm that this competitive inhibition of dehydroascorbic acid uptake was sodium-independent, the experiments were repeated in the presence and absence of sodium. As expected, the inhibition of dehydroascorbic acid uptake was independent of sodium in the buffer (data not shown). Since resveratrol has antioxidant properties, another mechanism by which resveratrol could inhibit dehydroascorbic acid uptake in U937 and HL-60 cells would be by reducing dehydroascorbic acid to ascorbic acid. However, this possibility was ruled out when HPLC analysis showed no ascorbic acid produced from dehydroascorbic acid, even when high concentrations of resveratrol (>500 μM) were used. The antioxidant properties of resveratrol are therefore not involved in the inhibition of dehydroascorbic acid uptake in U937 and HL-60 cells.

Since dehydroascorbic acid uptake occurs via sodium-independent glucose transporters (Glut 1 and Glut 3)[12−14] and resveratrol inhibition of the uptake of dehydroascorbic acid and glucose was independent of sodium, it is likely that resveratrol inhibits both dehydroascorbic acid and glucose uptakes by blocking sodium-independent glucose transporters. This likelihood was investigated by measuring the inhibitory effects of resveratrol on glucose and dehydroascorbic acid uptakes in Chinese hamster ovary

[†] Tel: 301-504-8365. Fax: 301-504-9456. E-mail: Park@307.bhnrc.usda.gov.

10.1021/np000411t
Published on Web 02/15/2001

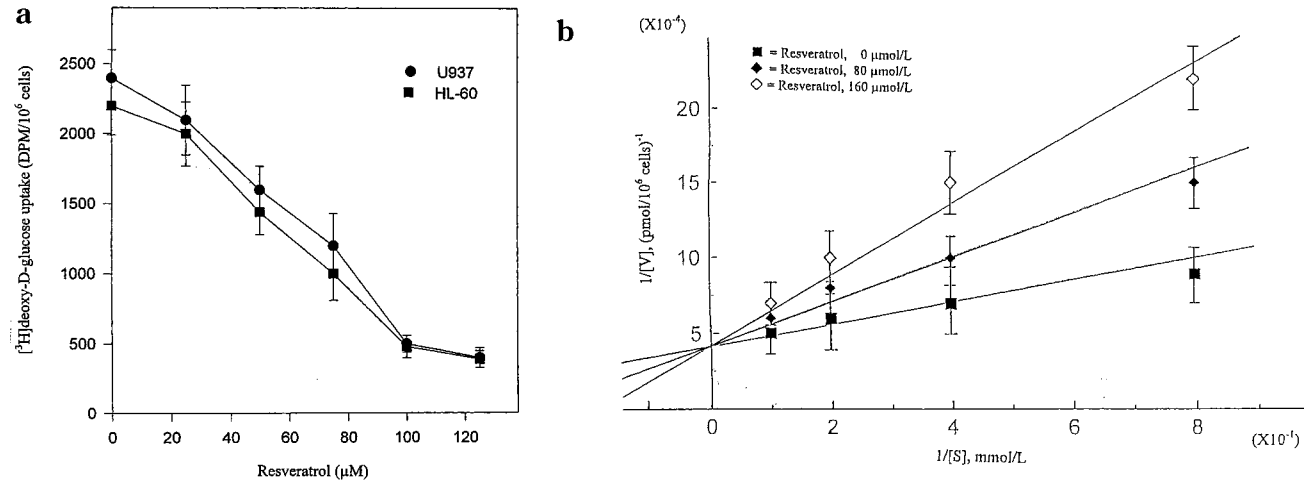

Figure 1. Inhibition of glucose uptake by resveratrol in U937 and HL-60 cells. (a) In U937 and HL-60 cells, glucose uptake was inhibited by resveratrol. The solid squares and circles represent HL-60 and U937 cells, respectively. (b) In U937 cells, the value of K_i was determined using the Michaelis−Menton plot. Transport of deoxyglucose at 1.25, 2.5, 5, and 10 mM was measured in the presence of 0, 80, and 160 μM of resveratrol. Data points in all figures represent the mean ± SD, $n = 4$.

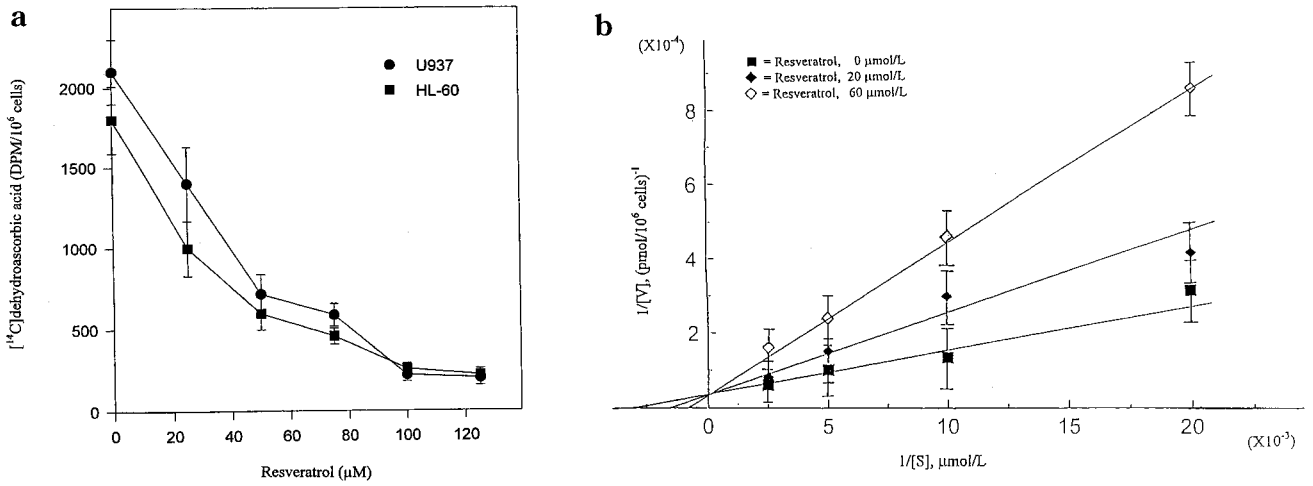

Figure 2. Inhibition of dehydroascorbic acid uptake by resveratrol in U937 and HL-60 cells. (a) In U937 and HL-60 cells, dehydroascorbic acid uptake was inhibited by resveratrol. The solid squares and circles represent HL-60 and U937, respectively. (b) In U937 cells, the value of K_i was determined using the Michaelis−Menton plot. Transport of dehydroascorbic acid at 50, 100, 200, and 1000 μM was measured in the presence of 0, 20, and 60 μM of resveratrol. Values are means ± SD, $n = 5$.

(CHO) cells overexpressing sodium-independent glucose transporters, Glut 1 and Glut 3.[12,14]

Glut1 and Glut 3 are sodium-independent glucose transporters that transport both glucose and dehydroascorbic acid into cells. Glucose uptake and dehydroascorbic acid uptake were individually measured in wild-type CHO cells (control) versus the CHO cells transiently transfected with Glut 1. The cells transfected with Glut 1 exhibited a 3−8-fold increase in [^{3}H]deoxyglucose uptake and a 4−6-fold increase in [^{14}C]dehydroascorbic acid uptake as compared to the control (CHO) cells (Figure 3a,b). The increased uptakes of glucose and dehydroascorbic acid were gradually abolished by increasing the concentration of resveratrol. Uptakes of glucose and dehydroascorbic acid were also measured individually in wild-type CHO cells (control) versus the CHO cells transiently transfected with Glut 3. The cells transfected with Glut 3 exhibited a 4−7-fold increase in [^{3}H]deoxyglucose uptake and a 5−6-fold increase in [^{14}C]dehydroascorbic acid uptake compared to the control (CHO) cells (Figure 3a,b). Thus, CHO cells transfected with Glut1 and Glut3, sodium-independent glucose transporters, show increased uptake of both glucose and dehydroascorbic acid, as compared to control CHO cells. This increased uptake of both compounds is inhibited by resveratrol, presumably as a result of blocking the Glut1 and Glut3 transporters in the transfected cells. Since resveratrol also inhibits the uptake of both glucose and dehydroascorbic acid in U937 and HL-60 cells, it can be speculated that the uptake is blocked by a similar mechanism in these cells as well.

Since resveratrol inhibits both glucose and dehydroascorbic acid uptakes in U937 and HL-60 cells, it raises the question of whether these cells accumulate resveratrol, and, if so, whether glucose and dehydroascorbic acid inhibit the accumulation. The two cell lines were determined to accumulate resveratrol in a similar manner (data shown only for U937 cells). At resveratrol concentrations of 20 to 1000 μM, U937 cells accumulated resveratrol, as shown in Figure 4. The accumulation was sodium-independent, and saturation occurred above 500 μM. To determine whether glucose and dehydroascorbic acid inhibit the accumulation of resveratrol in the cells, the accumulation was measured at various concentrations of glucose and/or dehydroascorbic acid. Neither inhibited the accumulation of resveratrol (data not shown). These data demonstrate clearly that resveratrol inhibits sodium-independent uptake of both glucose and dehydroascorbic acid, but glucose and/or de-

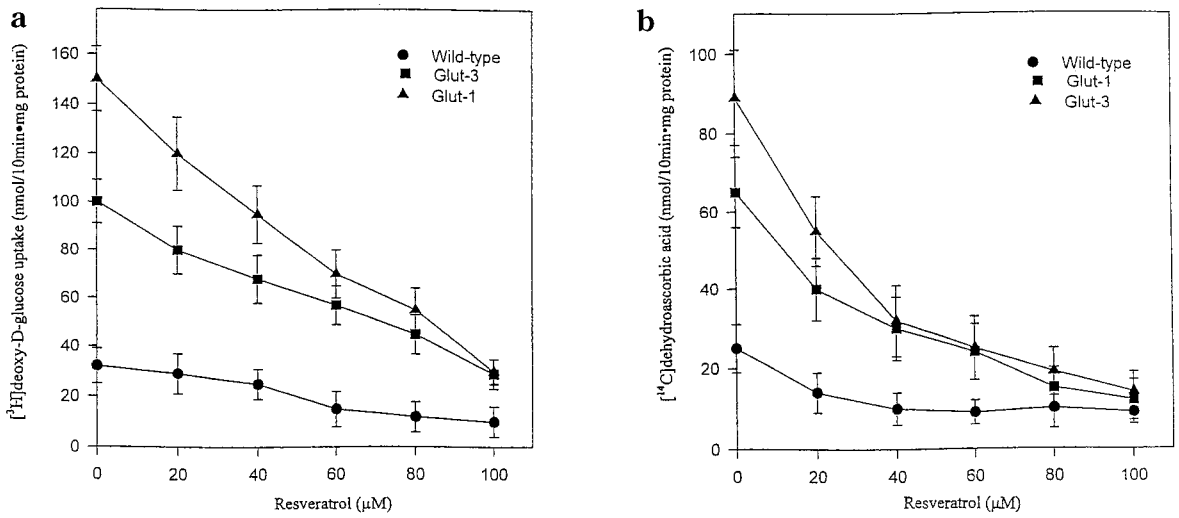

Figure 3. Inhibition of glucose and dehydroascorbic acid uptakes in CHO cells overexpressing glucose transporters. (a) Wild-type CHO and CHO cells overexpressing Glut 1 or Glut 3 were incubated in six-well plates at room temperature for 10 min in Krebs medium containing the indicated concentrations of resveratrol and 1 μmol/L [^{3}H]deoxyglucose. Glucose uptake was measured as described in the Experimental Section. (b) Inhibition of dehydroascorbic acid uptake by resveratrol was performed using [^{14}C]dehydroascorbic acid in wild-type CHO and CHO cells overexpressing Glut 1 or Glut 3, as described in ref 14. All values are means ± SD, $n = 6$.

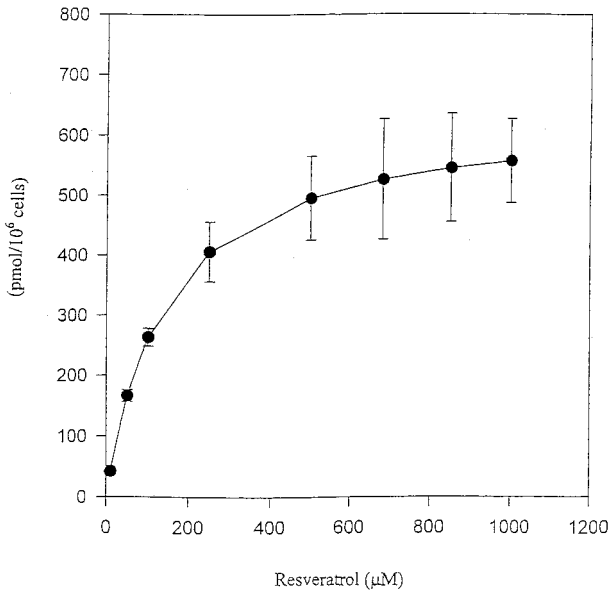

Figure 4. Intracellular accumulation of resveratrol in U937. U937 cells accumulated resveratrol over the concentrations from 20 to 1000 μM. The concentrations of resveratrol were determined using the HPLC method as described in the Experimental Section. All values are means ± SD, $n = 5$.

hydroascorbic acid do not inhibit the accumulation of resveratrol in these cell lines.

Inhibitors for sodium-dependent and -independent glucose uptake have been sought to characterize its transporters. This is the first report of resveratrol as a potential inhibitor of sodium-independent uptake of glucose and dehydroascorbic acid. To date, only a few pharmacokinetic studies in a limited range of concentrations have been performed on resveratrol, and resveratrol as a potential inhibitor for glucose and dehydroascorbic acid uptake has not been investigated.[18−21] Therefore, in the future, the concentrations of resveratrol in human intestine and in plasma must be extensively determined to validate its purported biological functions.

Experimental Section

Materials. Resveratrol (*trans*-3,5,4′-trihydroxystilbene) was purchased from Sigma (St. Louis, MO). U937 and HL-60 cells were purchased from ATCC (Rockville, MD).

Cell Culture Conditions. U937 and HL-60 cells were cultured in RPMI medium supplemented with 10% fetal bovine serum. Cell viability was determined microscopically by trypan blue exclusion, and the number of cells was counted by hemacytometer.[22] For the uptake experiments the cells were cultured to a cell density of 2×10^6.

Glucose Uptake Assay. A total of $(1-2) \times 10^6$ cells were resuspended in 1 mL of HEPES/phosphate buffer containing 147 mM NaCl, 5 mM KCl, 1.9 mM KH_2PO_4, 1.1 mM Na_2HPO_4, 1.5 mM glucose, 0.3 mM $MgSO_4 \cdot 7H_2O$, 1 mM $MgCl_2 \cdot 6H_2O$, 0.3 mM $CaCl_2 \cdot 2H_2O$, and 10 mM HEPES, pH 7.4. The reaction was initiated by adding 1.0 μCi 2-[1,2-^{3}H(N)]deoxy-D-glucose (specific activity 25 mCi/mmol). After 10 min, the cells were centrifuged and washed twice in cold PBS (pH 7.4) to terminate the reaction.[10] Uptake activity as whole cells was measured using scintillation spectrometry. For inhibition studies, various amounts of resveratrol were added to the reaction mixtures, and uptake activity was measured as described above. Since resveratrol is sensitive to light, all incubations with resveratrol were performed in the dark. To test the sodium dependence of glucose uptake, the sodium-free buffer was prepared by replacing NaCl and Na_2HPO_4 in HEPES buffer with choline chloride and K_2HPO_4.

Dehydroascorbic Acid Preparation and Uptake Assay. [^{14}C]Dehydroascorbic acid was prepared from [^{14}C]ascorbic acid (NEN Life Science Products Inc., 6.6 mCi/mmol).[23] For the uptake assay of dehydroascorbic acid, $(1-2) \times 10^6$ cells were resuspended in 1 mL of the same HEPES/phosphate buffer. The reaction was initiated by adding [^{14}C]dehydroascorbic acid. After 10 min, the reaction was terminated by centrifugation, and the cell pallet was washed twice in cold PBS (pH 7.4).[11] Uptake activity as whole cells was measured using scintillation spectrometry, or the activity was calculated as ascorbic acid reduced from dehydroascorbic acid. Ascorbic acid concentration was measured by high-performance liquid chromatography (HPLC) with coulometric electrochemical detection.[23,24]

Inhibition by Resveratrol of Glucose and Dehydroascorbic Acid Uptake in CHO Cells Overexpressing Glut1 or Glut 3. Chinese hamster ovary cells (CHO) were transfected with rat glucose transporter 1 or human glucose transporter 3, using Transfectam reagent (Promega, Madison, WI).[14,25] The cells were maintained in Ham's F-12 with 10% fetal calf serum and 1000 mg/mL penicillin/streptomycin. For experiments measuring the inhibition of glucose and dehydroascorbic acid uptake in CHO cells, confluent cells in a six-well dish (well size, 35 mm) were washed two times with Krebs buffer (30 mM HEPES, 130 mM NaCl, 4 mM KH_2PO_4, 1 mM $MgSO_4$, 1 mM $CaCl_2$, pH 7.4) and incubated at 23 °C for 2

min with Krebs buffer containing different concentrations of resveratrol. The reaction was initiated by adding 1.0 μCi [1,2-^{3}H(N)]deoxy-D-glucose or 1.0 μCi [^{14}C]dehydroascorbic acid. After 10 min, the reaction was terminated by washing the cells twice in cold PBS (pH 7.4),[10] and the cells were solubilized in 0.1 N NaOH, 1% CHAPS (Calbiochem., La Jolla, CA). Dehydroascorbic acid and glucose uptakes were assayed as described above. The efficiency of transfection was monitored by cotransfection with pGL2 promoter vector, with measurement of luciferase according to the manufacturer's protocol (Promega, Madison, WI).

HPLC Measurement of Resveratrol. A total of (1−2) × 10^6 cells were resuspended in 1 mL of the same HEPES/phosphate buffer used for the uptake experiments. Several concentrations of resveratrol (′from 20 to 1000 μM) were added to the cell suspension and incubated for 10 min. After incubation, the cells were washed with cold PBS (pH 7.4) to terminate the reaction. The cells were extracted twice with 80% methanol, and resveratrol content in the supernatant was determined. Resveratrol was measured by HPLC as follows: Nova-Pak C18 (Waters, 2.1 × 150 mm) was used as the stationary phase, and an isocratic buffer of 50 mM NaH_2PO_4 containing 45% methanol (pH 4.3) was used as the mobile phase. Resveratrol standards were injected by an autosampler (Alliance 2690, Waters) and were measured by an electrochemical detector with four electrode channels (CoulArray) and calculated with the manufacturer's software (v.1.0). For optimal measurement of resveratrol, the four channels were set at 50, 250, 450, and 600 mV.

Kinetics. Analyses of the inhibition of dehydroascorbic acid and glucose uptakes by resveratrol were performed as previously described.[26] The values of K_i were determined using the Michaelis−Menton plot. Data points in all figures represent the mean of three samples ± SD

References and Notes

(1) Jang M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W.; Fong, H. H.; Farnsworth, N. R.; Kinghorn, A. D.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. *Science* **1997**, *5297*, 218−20.
(2) Kimura Y.; Okuda, H.; Kubo, M. *J. Ethnopharmacol.* **1995**, *2*, 131−9.
(3) Orsini F.; Pelizzoni, F.; Verotta, L.; Aburjai, T.; Rogers, C. B. *J. Nat. Prod.* **1997**, *11*, 1082−7.
(4) Clement M. V.; Hirpara, J. L.; Chawdhury, S. H.; Pervaiz, S. *Blood* **1998**, *3*, 996−1002.
(5) Belguendouz L.; Fremont, L.; Gozzelino, M T. *Biochem. Pharmacol.* **1998**, *6*, 811−6.
(6) Pace-Asciak C. R.; Hahn, S.; Diamandis, E. P.; Soleas, G.; Goldberg, D. M. *Clin. Chim. Acta* **1995**, *2*, 207−19.
(7) Fontecave, M.; Lepoivre, M.; Elleingand, E.; Gerez, C.; Guittet, O. *FEBS Lett.* **1998**, *3*, 277−9.
(8) Fontecave M.; Jang, J.; Pezzuto, M.; Dannenberg, A. J. *J. Biol. Chem.* **1998**, *34*, 21875−82.
(9) Gehm, B. D.; McAndrews, J. M.; Chien, P. Y.; Jameson, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *25*, 14138−43.
(10) Park, J. B. *BBRC.* **1999**, *260*, 568−574.
(11) Welch, R. W.; Wang, Y.; Crossman, A.; Park, J. B.; Kirk, K. L.; Levine, M. *J. Biol. Chem.* **1995**, *270*, 12584−12592.
(12) Park, J. B.; Levine, M. *J. Nutr.* **2000**, *130*, 1297−1302.
(13) Vera, J. C.; Reyes, A. M.; Carcamo, J. G.; Velasqez, F. V.; Rivas, C. I.; Zang, R. H.; Strbel, P.; Iribarren, R.; Scher, H. I.; Sleb, J. C.; Golde, D. W. *J. Biol. Chem.* **1996**, *271*, 8719−8724.
(14) Rumsey, S. C.; Kwon, O.; Xu, G. W.; Burant, C. F.; Simpson, I.; Levine, M. *J. Biol. Chem.* **1997**, *272*, 18982−18989.
(15) Mueckler, M.; Caruso, C.; Baldwin, S. A.; Panico, M.; Blench, I.; Morris, H. R.; Allard, W. J.; Lienhard, G. E.; Lodish, H. F. *Science* **1985**, *229*, 941−945.
(16) Nishimura, H.; Pallardo, F. V.; Seidner, G. A.; Vannucci, S.; Simpson, I. A.; Birnbaum, M. J. *J. Biol. Chem.* **1993**, *268*, 8514−8520.
(17) Deezay, O.; Baghdiguian, S.; Fantini, J. *J. Biol. Chem.* **1995**, *270*, 12536−12541.
(18) Scheen, A. J.; Lefebvre, P. J. *Drugs* **1998**, *55*, 225−236.
(19) Bertelli, A. A.; Giovannini, L.; Stradi, R.; Bertelli, A.; Tillement, J. P. *Int. J. Tissue React.* **1996**, *18*, 67−71.
(20) Wilson, T.; Knight, T. J.; Beitz, D. C.; Lewis, D. S.; Engen, R. L. *Life Sci.* **1996**, *59*, PL15−21.
(21) Zhu, Z.; Klironomos, G.; Vachereau, A.; Neirinck, L.; Goodman, D. W. *J. Chromatogr. B. Biomed. Sci. Appl.* **1999**, *19*, 389−392.
(22) Grankvist, K.; Lernmark, A.; Taljedal, I. B. *Biochem. J.* **1977**, *162*, 19−24.
(23) Park, J. B.; Levine, M. *Biochem. J.* **1996**, *315*, 931−938.
(24) Levine, M.; Dhariwal, K.; Welch, R.; Wang, Y.; Park, J. B. *Am. J. Clin. Nutr.* **1995**, *62* (Suppl), 1347S−1356S.
(25) Chen, C.; Okayama, H. *Mol. Cell. Biol.* **1987**, *7*, 2745−2752.
(26) Neale, K.D.; Richards, T. G. *Elementary Kinetics of Membrane Carrier Transport*; John Wiley & Sons: New York, 1972; p 102

NP000411T