Phosphorylation of Human Glutamine:Fructose-6-phosphate Amidotransferase by cAMP-dependent Protein Kinase at Serine 205 Blocks the Enzyme Activity*

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Glutamine:fructose-6-phosphate amidotransferase (GFAT) is the rate-limiting enzyme in glucosamine synthesis. Prior studies from our laboratory indicated that activation of adenylate cyclase was associated with depletion of O-GlcNac modification. This finding and evidence that human GFAT (hGFAT) might be regulated by cAMP-dependent protein kinase (PKA) led us to investigate the role of PKA in hGFAT function. We confirmed that adenylate cyclase activation by forskolin results in diminished O-GlcNac modification of several cellular proteins which can be overcome by exposure of the cells to glucosamine but not glucose, suggesting the PKA activation results in depletion of UDP-GlcNac for O-glycosylation. To determine if GFAT is indeed regulated by PKA, we expressed the active form of the enzyme using a vaccinia virus expression system and showed that the activity of the enzyme was to decrease to undetectable levels by PKA phosphorylation. We mapped the PKA phosphorylation sites with the aid of matrix-assisted laser desorption ionization mass spectroscopy and showed that the protein was stoichiometrically phosphorylated at serine 205 and also phosphorylated, to a lesser extent at serine 235. Mutagenesis studies indicated that the phosphorylation of serine 205 by PKA was necessary for the observed inhibition of enzyme activity while serine 235 phosphorylation played no observable role. The activity of GFAT is down-regulated by cAMP, thus placing regulation on the hexosamine pathway that is in concert with the energy requirements of the organism. During starvation, hormones acting through adenylate cyclase could direct the flux of glucose metabolism into energy production rather than into synthetic pathways that require hexosamines.

For single cell organisms, the concentration of extracellular nutrients depends on the environment while multicellular organisms normally maintain the concentration of these nutrients at relatively constant level. In vertebrates, the extracellular glucose concentration is tightly maintained despite changes in the availability of dietary carbohydrates. This homeostasis is accomplished by appropriate hormone signaling that directs glucose into energy yielding pathways during starvation versus synthetic and storage pathways in the fed state. Insulin is the major hormone that coordinates the utilization of glucose for synthetic and storage functions in the fed state while a variety of other hormones are secreted in response to stress and starvation to coordinate the utilization of glucose for energy production. A key intermediary metabolite of glucose is fructose 6-phosphate (Fru-6-P). Fru-6-P metabolism is tightly regulated allosterically and by hormones to be in concert with the nutritional status of the intact organism. Fru-6-P can be metabolized through glycolysis to create ATP and/or it can be metabolized to glucosamine for use in glycoprotein synthesis by the enzyme GFAT (1). Furthermore, in liver and kidney, Fru-6-P can be utilized for gluconeogenesis. Thus, this substrate plays a pivotal role in the flux of glucose into energy yielding pathways or into synthetic and storage pathways. In hormone responsive tissues, starvation or stress is signaled by glucagon and epinephrine, and in both cases, these hormones are coupled to the accumulation of cyclic AMP (cAMP). The impact of cAMP accumulation in the liver on Fru-6-P metabolism is to direct the flux of Fru-6-P into gluconeogenesis (2) while in the heart, cAMP accumulation through epinephrine stimulation results in the flux of Fru-6-P into glycolysis (3). Thus, the net effect of starvation or stress is for the liver to release glucose to provide for the energy requirements of muscle and heart. From this consideration, it would be reasonable to predict that part of this concerted control on Fru-6-P utilization during starvation or stress would be the cessation of Fru-6-P flux into glucosamine synthesis and hence into glycoprotein synthesis.

Previously, we reported that glucose starvation combined adenylate cyclase activation by forskolin treatment of NRK fibroblasts resulted in the depletion of the transcription factor Sp1 by a process that involves proteasomes (4, 5). Under normal circumstances, Sp1 (4, 6, 7) and several other transcription factors (8–11) and nuclear proteins (12) are modified by the covalent O-linkage of the monosaccharide N-acetylg glucosamine (O-GlcNAc) to serine or threonine residues in the protein backbone. However, under these conditions of adenylate cyclase activation and glucose starvation, Sp1 and several other proteins were observed to undergo nearly complete removal of the O-GlcNAc modification (4). Conversely, exposure of cells to glucose or glucosamine resulted in an increase in the modifi-

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‡ The abbreviations used are: Fru-6-P, fructose 6-phosphate; GFAT, glutamine:fructose-6-phosphate amidotransferase; O-GlcNAc, O-linked N-acetylg glucosamine; GST, glutathione S-transferase; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight; NRK, normal rat kidney; PAGE, polyacrylamide gel electrophoresis; PKA, protein kinase A; RP-HPLC, reverse phase-high performance liquid chromatography; PCR, polymerase chain reaction; BrdUrd, 5-bromodeoxyuridine.
cation of proteins by O-GlCNAC (4, 13). We postulated that the O-GlCNAC state of Sp1 or other proteins controlled the degradation of this transcription factor by the proteasome (4, 5).

Since Sp1 is critically important for the transcription of TATA-less housekeeping genes (14), this loss of Sp1 would result in the down-regulation of those genes that encode the bulk of cellular proteins under conditions of nutrient deprivation or stress (cAMP). A role for the O-GlCNAC modification in the control of protein synthesis at the translational level has also been suggested by the studies of elongation factor 2 (15–17). Together, these studies suggest that the O-GlCNAC state of certain intracellular proteins may coordinate the level of macromolecular synthesis in the cell in a manner that reflects the nutritional status.

The studies described in this article resulted from our attempt to understand how the O-GlCNAC status of Sp1 and other proteins could be modified by exposure of cells to the adenylyl cyclase activator, forskolin. Either this treatment resulted in a decreased rate of modification or an increased rate of O-GlCNAC removal from these proteins. Since we had shown that O-GlCNAC modification is a substrate driven in certain cell types (13, 18, 19), we focused our attention on the enzyme that controls the synthesis of glucosamine, GFAT. Analysis of the predicted amino acid sequence of GFAT indicated the presence of two potential cAMP-dependent protein kinase (PKA) phosphorylation sites (20). While recently evidence has suggested that PKA phosphorylation of liver-derived rat GFAT activates this enzyme (20), we showed that PKA phosphorylation of recombinant hGFAT shut down enzymatic activity, thereby reducing the availability of substrate for O-GlCNAC modification.

This notion would fit with the idea that stress and starvation should cause the flux of glucose carbons into energy yielding pathways rather than synthetic pathways. Our results are compatible with GFAT playing a regulatory role in glycoprotein synthesis that is in concert with the metabolic signals that regulate Pru-6-P utilization.

**EXPERIMENTAL PROCEDURES**

**Materials**—Protein kinase A catalytic subunit, 5-bromo-2′-deoxyuridine (BrdUrd), l-glutamine, glutamic acid, 3-acytylpyridine adenosine dinucleotide, myophenolic acid, thoramin, and trifluoroacetic acid were purchased from Sigma. γ-[32P]ATP was purchased from NEN Life Science Products Inc. Glutamate dehydrogenase, sequencing grade trypsin, fructose 6-phosphate, and ATP were purchased from Roche Molecular Biochemicals. Glutathione-Sepharose 4B was purchased from Amersham Pharmacia Biotech. RP-HPLC column was purchased from Vydac.

**Cell Culture**—BSC40 cells and NRK cells were grown in Dulbecco's modified Eagle's medium with 10% fetal calf serum (Life Technologies, Inc., Grand Island, NY), 100 μg of penicillin/ml, and 50 μg of gentamicin/ml at 37 °C in a humidified incubator with 7.5% CO2.

**Western Blot Detection of Intracellular O-GlCNAC-modified Proteins**—Cultured NRK cells at 70% confluency in 10-cm culture dishes were incubated in glucose-free Dulbecco's modified Eagle's medium with 10% fetal calf serum for 24 h. The next morning, the cells were subjected to SDS-PAGE followed by transfer onto a nitrocellulose membrane. The O-GlCNAC signal was detected by a monoclonal anti-Rac2 antibody (4, 13, 18, 21) using the Enhanced Chemiluminescence System (Amersham Pharmacia Biotech).

**Cloning and Expression of Recombinant Human GST-GFAT Construct**—Parental human GFAT (hGFAT) cDNA was kindly provided by Dr. G. McKnight in the Bluescript plasmid (22). For cloning into the ptM3 expression construct, the 5,152-base pair region of hGFAT cDNA was amplified by PCR using oligonucleotide primers with the following sequences: 5′-CATGAAATCTGTTGATATTGGCT-3′ and 5′-CATGGACCCTGGTCACATTTT-3′. The PCR product was ligated into the pT7T3 plasmid between EcoRI and BamHI. The cloned PCR product was sequenced to confirm fidelity of the PCR amplification and isozyme which is used in this work. The GFAT coding sequence was excised from the GFAT cDNA with PstI and SalI and ligated downstream of the 900-base pair fragment of GFAT that had already been cloned into GST-pTM3. This yielded a construct that encodes the full-length GFAT as a fusion protein with GST. The procedures for generation of recombinant GST-hGFAT vaccinia virus were described (4, 5, 23) using both mycoplasmolic and BrdUrd selections. The vaccinia virus system was a kind gift from Dr. B. Moss.

**Enzyme Assay of hGFAT**—hGFAT was expressed as a fusion protein with GST in BSC40 cells using the viral expression system. 24 h after infection, the cells were lysed in high-salt lysis buffer containing 50 mM Tris-Cl (pH 7.5), 0.5 mM NaCl, 1 mM phenylmethanesulfonyl fluoride, 1 mM dithiothreitol, 1 mM EDTA, 20% glycerol by three cycles of freeze-thaw. The supernatant was recovered by centrifugation and pelleted by incubation with a 50% slurry of glutathione-Sepharose beads at 4 °C for 30 min. The beads (100 μl) were washed with cold enzyme assay buffer containing 20 mM Tris-Cl (pH 7.5), 2.5 mM CaCl2, 50 mM NaCl, 10 mM MgCl2, 1 mM dithiothreitol, and 10% glycerol. The fusion protein was eluted from the bead with 10 mM reduced glutathione and the concentration of the protein was determined by both standard protein assay (Bio-Rad Dc) and SDS-PAGE. The enzyme activity was determined by a spectrophotometric method as described (22, 24). Essentially, in a standard 1-ml assay, the purified GST-GFAT fusion protein (1 μg) bound to the glutathione-Sepharose beads was incubated with 10 μM glutamine, 10 μM fructose 6-phosphate, 0.5 mM 3-acytylpyridine adenosine dinucleotide, and 20 units of glutamate dehydrogenase in enzyme assay buffer at 37 °C for 1 h. The supernatant was collected after incubation and absorbance at 365 nm was determined. A blank calibration control consisted of the entire reaction mixture with GST alone bound to the beads.

**Phosphorylation of hGFAT**—The purified GST-hGFAT fusion protein (1 μg) was phosphorylated in vitro while bound on glutathione-Sepharose beads by the catalytic subunit of cAMP-dependent PKA in phosphorylation reaction buffer containing 50 mM Tris-Cl (pH 7.5), γ-[32P]ATP (10 μCi), 100 μM ATP, 10 mM MgCl2, 5 mM dithiothreitol at 30 °C for 20 min. After the phosphorylation reaction, the beads were washed with enzyme assay buffer. An aliquot of beads was removed and the bound protein was separated by SDS-PAGE. The gel was fixed and stained with Coomassie Blue and the level of phosphorylation was determined by autoradiography. The remainder of the beads was assayed for GFAT enzymatic activity as described above. An unphosphorylated GST-hGFAT control was treated identically in the phosphorylation buffer without PKA but in the presence of ATP.

**Mutagenesis Study of hGFAT**—The two potential PKA phosphorylation sites (Ser205, Ser235) were mutated to alanine by PCR, one site at a time or in combination of the two sites. The enzyme activity and phosphorylation assays were performed as described above.

**Cloning of the Escherichia coli GFAT Isoform**—DH5α cells (5 μg DNA was used as a template for PCR amplification of the E. coli GFAT. Two oligonucleotide primers, designed based on the sequence of the E. coli GFAT gene (Genebank accession numbers: AE000450 and U000996) (5'-CATCTCAAGATTGAGAAATGTGCGG and 5'-CATAGGCTTCTACCAACCGTAAACTTGTGC) were used to amplify the E. coli GFAT gene (1.8 kilobase). The PCR product was purified and cloned into Bluescript between XbaI and SstI and the insert sequence was confirmed by restriction digestion and automated sequencing. For expression of the E. coli enzyme, the gene was cloned into the GST-pTM3 construct between SpeI and SstI and expressed as a GST fusion protein following generation of recombinant E. coli GFAT vaccinia virus. The assay of E. coli GFAT enzymatic activity was performed exactly as described for the hGFAT.

**RP-HPLC Separation of Radiolabeled hGFAT Trypatic Digests**—Purified wild type GST-hGFAT fusion protein (20 μg) bound to glutathione-Sepharose beads was phosphorylated using γ-[32P]ATP (ATP concentration of 0.1 μM, specific activity: 3000 Ci/mmol) and 100 units of PKA catalytic subunit at 30 °C for 15 min. An equal aliquot of PKA and 10 μg cold ATP was added to the labeling reaction and incubation was
only 100 mM Tris-Cl (pH 8.0). The bound and labeled GST-hGFAT was digested with sequencing grade trypsin in the 100 mM Tris-Cl (pH 8.0) buffer at room temperature for 20 min. The protein solution was concentrated by a size exclusion filter (Microcon), after which the GST-hGFAT fusion protein was digested with sequencing grade trypsin in the 100 mM Tris-Cl (pH 8.0) buffer at an enzyme/protein ratio of 1:10 at room temperature overnight. The GST-hGFAT tryptic peptides were separated by RP-HPLC on a Microsorb-MV C18 column (Rainin, Woburn, MA). Elution of the column was with a linear increasing concentration (5–75%) of acetonitrile in water containing 0.1% trifluoroacetic acid and a flow rate of 0.2 ml/min. The radioactivity in an equal aliquot from each collected fraction was determined using a scintillation counter.

MALDI-TOF Mass Spectrometry to Identify Phosphorylated GFAT Peptides—The HPLC fractions containing the 32P-labeled peptides were vacuum dried, solubilized in 5% trifluoroacetic acid in water and the mass of the peptides in the fraction was determined by MALDI-TOF as described previously (5, 7, 25). The instrument was calibrated with Neurotensin and Substance P. An unphosphorylated GST-GFAT tryptic digest and trypsin blank were also run as controls.

RESULTS
Forskolin Stimulation of NRK Cells Results in Reduced O-GlcNAc Modification of Intracellular Proteins—Previously, we showed that glucose starvation and forskolin stimulation of NRK cells results in reduced modification of Sp1 and other intracellular proteins by O-GlcNAc (4). To determine whether this response results from depletion of substrate for the enzyme O-GlcNAc transferase, the cells were starved of glucose, then pretreated with or without forskolin prior to exposure to glucose or glucosamine (Fig. 1). The level of protein modification on Western blots can be blocked by preadsorption of the anti-STAT3 antibody to control for protein loading. Only small fluctuations in the STAT3 signal were evident. This result agrees with our earlier observations (4) and is compatible with the notion that forskolin treatment of the cells blocks the activity of GFAT, thereby depriving O-GlcNAc transferase of substrate for protein modification and a reduction of modification on the O-GlcNAc proteins. This substrate restriction can be overcome in forskolin-treated cells by the provision of glucosamine in the extracellular medium.

PKA Treatment Induces a Loss of hGFAT Activity in Vitro—To directly test the effect of PKA phosphorylation on hGFAT, we expressed and purified recombinant hGFAT and exposed the enzyme to the catalytic subunit of PKA. To express hGFAT, we developed a recombinant vaccinia virus that encodes hGFAT as a fusion protein with GST. The GST-hGFAT was expressed in BSC40 cells infected with the recombinant vaccinia virus and the fusion protein was purified to near homogeneity on a glutathione affinity column (see below). To determine if it was necessary to cleave hGFAT from the GST tag, we compared the activity of GST-hGFAT bound to the glutathione affinity beads with the activity of hGFAT cleaved from the GST with thrombin. The GFAT activity of the fusion protein bound to the beads was the same as the activity of the enzyme cleaved from the GST (and the beads) with thrombin (data not shown). Therefore, the phosphorylation studies on GFAT could be performed directly on the purified and bound GST-hGFAT. This approach gave certain advantages. Because the phosphorylation step was performed on the GST-hGFAT bound to the affinity column, the subsequent removal of the catalytic subunit of PKA and a buffer exchange for the optimal measurement of GFAT activity was simplified. Treatment of 10 pmol (1 μg) of GST-hGFAT fusion protein with various doses of PKA in this manner resulted in a dose-dependent loss of GFAT activity from the fusion protein (Fig. 2A) and a corresponding dose-dependent increase in the phosphorylation of GST-GFAT (Fig. 2B). The half-maximal effect of PKA on GFAT activity occurred at a dose of approximately 50 units (1 unit of PKA transfers 1 pmol of phosphate/min) and at 150 units, no residual GFAT activity could be measured. Correspondingly, incorporation of [32P]phosphate into GFAT was dose-dependent with half-maximal phosphorylation occurring at a dose of approximately 50 units of PKA (Fig. 2B). A similar inhibition of GFAT activity was observed when the enzyme activity was measured following cleavage of the GFAT by thrombin to remove GST (data not shown). The phosphorylation and activity studies were performed on equal quantities of GST-GFAT protein (Fig. 2C). Since the GFAT activity assay was performed...
after the removal of ATP and PKA by washing the glutathione affinity beads, the possibilities of an allosteric effect of ATP or a phosphorylation effect on the read out enzyme for the GFAT activity assay, glutamate dehydrogenase, was made much less likely.

To further control for potential interference of the PKA phosphorylation reagents with the GFAT enzyme assay, we performed similar studies on recombinant GFAT cloned from *E. coli*. The *E. coli* GFAT shows roughly 35% sequence homology with the mammalian homolog and is not feedback inhibited by UDP-GlcNAc (25). *E. coli* GFAT also contains a potential PKA phosphorylation motif (serine 342), but this serine residue is in a region of the *E. coli* GFAT that is not conserved in mammalian GFAT. The *E. coli* GFAT cDNA was expressed as a fusion protein with GST using the same viral expression system. Functional studies showed that this cloned *E. coli* GFAT fusion protein was enzymatically active. Exposure of 10 pmol of *E. coli* GFAT to the same dose range of PKA resulted in no significant effect on the activity of the bacterial enzyme (Fig. 2A). This result suggested that the sensitivity of hGFAT to PKA treatment results from specific structural determinants in the human enzyme and not from an effect of PKA on the GFAT enzyme assay system.

**RP-HPLC Separation of Phosphorylated hGFAT Tryptic Peptides**—Examination of the sequence of hGFAT reveals two potential PKA phosphorylation sites (20) at serine 205 and serine 235. To determine the actual sites of phosphorylation, we conducted a phosphopeptide mapping study of hGFAT. To this end, purified GST-GFAT fusion protein was labeled sequentially with [γ-32P]ATP then with cold ATP catalyzed in each case by 100 units of PKA to ensure stoichiometric phosphorylation of the protein. The protein was then cleaved with sequence grade trypsin and the resulting peptides were separated by HPLC. Fig. 3A shows the UV absorbance at 214 nm of the eluted peptides while the lower panel shows the profile of radiolabeled peptide. Two major peaks of radioactivity with retention times of 5–10 and 25–29 min and one lesser peak of radioactivity with a retention time of 20–21 min were eluted.
Based on the number of basic amino acids in the hGFAT sequence, 80 tryptic peptides can be generated from hGFAT. The predicted tryptic peptides containing the PKA phosphorylation motifs with serine 205 and serine 235 have a net mass of 991.86 Da (panel A) and 815.70 Da (panel B), respectively. The peptides identified with these masses are indicated with an asterisk. The insets indicate the sequence of the individual tryptic peptides of hGFAT that contain the potential PKA serine phosphorylation sites and the calculated unmodified molecular weight of these peptides. Phosphorylation contributes 80 Da to the mass. The other mass spectrometric peaks represent tryptic GST peptides (1009.85 Da, 1031.85 Da), GFAT peptide (763.61 Da), and other unidentified peptides.

The S205A Mutant hGFAT Abolished the Sensitivity to PKA Treatment—To determine the functional significance of these PKA phosphorylation sites in hGFAT, the sites were mutated.
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FIG. 5. GST-GFAT fusion proteins. Vaccinia expressed GST-GFAT fusion proteins, either wild-type or with mutations, were purified by glutathione affinity chromatography, then analyzed by SDS-PAGE and stained with Coomassie Blue. lane 1, GST-E. coli GFAT; lane 2, wild-type GST-hGFAT; lane 3, GST-hGFAT with a serine 205 to alanine mutation; lane 4, GST-hGFAT with a serine 235 to alanine mutation; lane 5, GST-hGFAT with alanine mutations at both serine 205 and serine 235. The molecular weight standards are shown on the left side of the figure.

Three mutant forms of hGFAT were generated in which the two serines (205 and 235) in the PKA recognition sites were mutated to alanine, one at time or in combination. These GST-hGFAT mutants were expressed using the same viral expression vectors, and purified to near homogeneity by glutathione affinity chromatography (Fig. 5). Functional studies showed that the three mutant forms of hGFAT protein exhibited the same specific enzyme activity as the wild-type form. However, in vitro PKA treatment of both single serine 205 and double serine 205 + serine 235 mutants resulted in no significant effect on the GFAT activity (Fig. 6A). However, similar to wild type GFAT, the single serine 235 mutant exhibited the loss of enzyme activity following PKA treatment (Fig. 6A). Consistent with the functional study, the double serine 205 + serine 235 mutant showed no phosphate incorporation when treated with PKA (Fig. 6B), indicating that the mutagenesis eliminated all potential phosphorylation sites in the GST-hGFAT protein. This mutagenesis study indicates that the phosphorylation of serine 205 by PKA is necessary for the observed inhibition of GFAT activity. Since inhibition of GFAT activity by PKA phosphorylation does not occur in the serine 205 mutant, then other potential phosphorylation sites do not play a role in this control of enzyme activity. In particular, while serine 235 may also be phosphorylated by PKA, this phosphorylation has no significant effect on the activity of the enzyme when measured in vitro.

DISCUSSION

In our studies of the transcription factor, Sp1, we showed that activation of PKA by forskolin resulted in a marked decrease in the glycosylation of Sp1 and other proteins (4). Since the O-GlcNAc modification may involve the same serine or threonine residues that can be phosphorylated, the notion has been raised that glycosylation and phosphorylation may occur as reciprocal events (8). Indeed, in some systems, this reciprocal relationship seems to hold because the modification sites map to the same residue in the proteins (28, 29). In addition to the Sp1 example cited here, a recent study on cerebellar neurons also demonstrated that an activation of PKA was coupled to a reduction of the O-GlcNAc level in cytoskeletal proteins (30). However, when cells were exposed to a selective inhibitor of the O-GlcNAc-β-N-acetylglucosaminidase (O-GlcNAcase) only a slightly reduced phosphorylation level of Sp1 was observed (31) despite the accumulation of O-GlcNAc on the transcription factor. Furthermore, the generalized loss of the O-GlcNAc signal in many proteins in response to PKA activation, while not ruling out the idea that there may be sites that can switch between phosphorylation and glycosylation, and the observation that the level of O-GlcNAc modification of many intracellular proteins can be affected by substrate availability prompted us to explore another hypothesis, that PKA activation may limit UDP-GlcNAc availability for protein modification through an action on GFAT, the rate-limiting step in glucosamine synthesis. GFAT has been noted to contain potential PKA phosphorylation sites (20), raising the possibility that phosphorylation of the protein could alter the enzyme activity. To test this idea, we expressed and purified hGFAT using a vaccinia virus expression system that allowed the recovery of active enzyme. This mammalian expression system also allowed us to place mutations into the protein. Our studies showed that hGFAT was indeed phosphorylated by the catalytic subunit of PKA. Mapping studies indicated that the RRGS<sup>205</sup> motif in GFAT was stoichiometrically phosphorylated while it appeared that the KKGS<sup>235</sup> motif was less efficiently phosphorylated. Failure to identify the unphosphorylated form of the KKGS<sup>235</sup> peptide made it impossible to accurately assess the stoichiometry of phosphorylation of this site. This phosphorylation was associated with a complete loss of GFAT enzymatic activity and phosphorylation of the RRGS<sup>205</sup> but not the KKGS<sup>235</sup> motif was necessary for this loss of activity. That a mutation in the RRGS<sup>205</sup> motif completely blocked the inhibitory effect of PKA suggests that this phosphorylation site is the only site in the hGFAT molecule that mediates the inhibitory effect of PKA on GFAT activity. These findings with regard to hGFAT suggest that the observed deglycosylation of intracellular proteins in response to PKA activation by forskolin results largely from inhibition of glucosamine synthesis and substrate restriction for the O-GlcNAc transferase enzyme.

Our finding that GFAT activity is inhibited by PKA-mediated phosphorylation differs from the report of Zhou et al. (20) who showed that the activity of rat GFAT is stimulated by phosphorylation. There are several possible explanations for...
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