Phosphorylation of Mouse Glutamine-Fructose-6-phosphate Amidotransferase 2 (GFAT2) by cAMP-dependent Protein Kinase Increases the Enzyme Activity*

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Yong Hu‡, Lacinda Riesland‡, Andrew J. Paterson§, and Jeffrey E. Kudlow‡§

From the Departments of ‡Cell Biology and §Medicine, Division of Endocrinology, Diabetes, and Metabolism, University of Alabama at Birmingham, Birmingham, Alabama 35294

A protein encoded by a new gene with approximately 75% homology to glutamine-fructose-6-phosphate amidotransferase (GFAT) was termed GFAT2 on the basis of this similarity. The mouse GFAT2 cDNA was cloned, and the enzyme was expressed with either an N-terminal glutathione S-transferase or His tag. The purified protein expressed in mammalian cells had GFAT activity. The \( K_m \) values for the two substrates of reaction, fructose 6-phosphate and glutamine, were determined to be 0.8 \( \text{mM} \) for fructose 6-phosphate and 1.2 \( \text{mM} \) for glutamine, which are within the ranges determined for GFAT1. The protein sequence around the serine 202 of GFAT2 was conserved to the serine 205 of GFAT1, whereas the serine at 235 in GFAT1 was not present in GFAT2. Previously we showed that phosphorylation of serine 205 in GFAT1 by the catalytic subunit of cAMP-dependent protein kinase (PKA) inhibits its activity. Like GFAT1, GFAT2 was phosphorylated by PKA, but GFAT2 activity increased approximately 2.2-fold by this modification. When serine 202 of GFAT2 was mutated to an alanine, the enzyme not only became resistant to phosphorylation, but also the increase in activity in response to PKA also was blocked. These results indicated that the phosphorylation of serine 202 was necessary and sufficient for these alterations by PKA. GFAT2 was modestly inhibited (15%) by UDP-GlcNAc but not through detectable O-glycosylation. GFAT2 is, therefore, an isoenzyme of GFAT1, but its regulation by cAMP is the opposite, allowing differential regulation of the hexosamine pathway in specialized tissues.

The hexosamine biosynthetic pathway, a universally conserved pathway in all life forms, metabolizes fructose 6-phosphate in conjunction with glutamine and acetate to UDP-N-acetylhexosamine as its main end product. These UDP-N-acetylhexosamines are used mainly for the synthesis of exported proteins that are modified by complex carbohydrate side chains prior to their exit from the interior of the cell. In addition, UDP-N-acetylhexosamines also serve as essential substrates for the synthesis of the glycosyl side chain of glycolipids, and the ratio of UDP-N-acetylglucosamine (UDP-GlcNAc) to UDP-N-acetylgalactosamine usually is 3:1 (1, 2). UDP-GlcNAc is an active precursor of chitin that is polymerized by bacterial, fungal, and insect cells to form the major component of the wall and insect cuticle.

In higher eukaryotes, part of the UDP-GlcNAc is used by the enzyme O-GlcNAc transferase (OGT) to modify nucleocytoplasmic proteins by the addition of the monosaccharide GlcNAc to the hydroxyl groups on serine or threonine residues of proteins. This O-GlcNAc modification can be removed from proteins by the enzyme O-GlcNase. Important observations have been linked to this modification of proteins by OGT. Overexpression of OGT causes insulin resistance in adipocytes (3, 4), whereas inhibition of the counteracting enzyme O-GlcNase is associated with pancreatic \( \beta \) cell and brain hippocampal neuron apoptosis (5, 6) and a hormone secretory defect (7). Although the modification has been found in 80 or more proteins, many of which are transcription factors, only recently has there been concrete evidence for a direct role of the O-GlcNAc modification in the control of protein functions (8). The enzyme OGT has been localized to gene-targeted co-repression complexes (9), and the modification of the transcription factor Sp1 has been shown to repress the transcriptional activation capacity of OGT (10). The 26 S proteasome is also regulated reversibly by this modification: OGT inhibits the function of proteasomes, whereas O-GlcNase activates the organelle (11). Because the proteasome controls the abundance of many regulatory proteins, including proapoptotic and developmental factors, and gene co-repression similarly determines cellular outcome, the hexosamine pathway, with its ability to sample the metabolism of sugars (glucose backbone of GlcNAc), amino acids (nitrogen in GlcNAc), and lipids (acetate in GlcNAc), could provide a metabolic input into vital cell processes. Indeed, an absence of the OGT gene on the X chromosome is lethal (12).

The rate-limiting step in glucosamine synthesis is catalyzed by the enzyme glutamine-fructose-6-phosphate amidotransferase (GFAT). The flux of glucose into the hexosamine pathway can therefore be controlled by the activity of the GFAT enzyme. GFAT activity has been detected in almost every organism and tissue. The enzyme is composed of two domains: a glutaminase domain, which catalyzes the hydrolysis of glutamine to glutamate and ammonia, and a synthase domain, which catalyzes amination and isomerization of fructose 6-phosphate to glucosamine 6-phosphate (13). The Escherichia coli GFAT has been crystallized, and the structure of the enzyme has been elucidated (14). Because the activity of GFAT controls the entry of glucose
into the hexosamine pathway, the control of GFAT activity might have implications for these and other regulatory processes. Previously, our laboratory expressed and purified recombinant GST-GFAT in a mammalian expression system and showed that the phosphorylation of serine 205 of the GFAT protein by cAMP-dependent protein kinase (PKA) inhibited the activity of the enzyme. Such inhibition of GFAT would confer the preferential metabolism of glucose to ATP rather than glucosamine in pancreatic β cells, perhaps causing more ATP-stimulated insulin secretion (15) for a given glucose challenge. Because incretins such as Glp1, which are made in the gastrointestinal tract in response to food, increase cAMP in the β cell (16), part of their mechanism of action might result from this shift in the metabolism of glucose.

When GFAT was purified from liver and subjected to phosphorylation by PKA, however, another investigative group (17) found that the enzyme activity was increased by approximately 1.7-fold. At approximately the same time of that study, a gene encoding a protein with approximately 75% homology to GFAT was cloned. The gene product was named GFAT2 on the basis of this homology but not on the basis of activity. Northern blot analysis revealed different tissue distribution between GFAT1 and GFAT2. GFAT1 was more highly expressed in the pancreas, placenta, and testis than GFAT2, whereas GFAT2 was expressed throughout the central nervous system, especially in the spinal cord. The GFAT2 locus was mapped to human chromosome 5q and mouse chromosome 11 (18).

To determine whether GFAT2 indeed has GFAT enzyme activity and the effect of PKA phosphorylation on the enzyme activity, GFAT2 was cloned, expressed, and purified. Our studies showed that GFAT2 has GFAT enzyme activity. It is phosphorylated at a site similar to GFAT1, but instead of inhibiting its activity on phosphorylation, the modification accentuated the enzyme activity. These isozymes of GFAT would allow different tissues under hormonal stimulation to metabolize glucose differently.

**EXPERIMENTAL PROCEDURES**

**Materials**—Protein kinase A catalytic subunit, protein phosphatase catalytic subunit, fructose 6-phosphate, 5-bromo-2′-deoxyuridine, 1-glutamine, glutamic acid, 3-acetylpyridine adenine dinucleotide, myophosphoric acid, threonin, protein kinase A inhibitor, protease inhibitors mixture, monoclonal anti-GST tag antibody, and trifluoroacetic acid were purchased from Sigma. Monoclonal anti-His6 antibody was purchased from PerkinElmer Life Sciences. Glutamate dehydrogenase and ATP were purchased from Roche Applied Science. Glutathione-Sepharose 4B was purchased from Amersham Biosciences.

**Cell Culture**—BSC40 cells were grown in Dulbecco’s modified Eagle’s medium with 10% newborn calf serum (Invitrogen), 100 µg/ml penicillin, and 50 µg/ml gentamicin at 37 °C in a humidified incubator with 7.5% CO2.

**Cloning and Expression of Recombinant Mouse GST-GFAT2**—The 5′ end 769 base pairs of the mouse GFAT2 cDNA (accession number NM_013529) were amplified by PCR using oligonucleotides 5′-CGCTGCTGAAGCTATTGCTTGCTAC-3′ and 5′-TTCTATGATGCCATTTTGCC-3′. The product was inserted into the EcoRI site of pBS and sequenced. The 1280 base pairs of 3′ sequence of mouse GFAT2 were amplified by PCR using oligonucleotides 5′-GAAGGGAGGGGGGCCCTTGCTATC-3′ and 5′-GATGAGCAGAGGGGGGCTCTTCTTGC-3′. The mutated segment was cloned into mGFAT2 and sequenced to confirm placement of the mutation.

**Vaccinia Virus Expression and Purification of Recombinant Fusion Proteins**—Procedures for the generation of recombinant GST-mGFAT2 virus were as described using both mycophenolic acid and bromodeoxyuridine selections (19, 20). The vaccinia virus system was a kind gift from Dr. B. Moss (19). To express GST-mGFAT2, the BSC40 cells were infected with recombinant GST-mGFAT2 virus and VTF7-3 virus, which expresses T7 polymerase in infected mammalian cells; T7 polymerase can bind to the promoter of GST-mGFAT2, resulting in the overexpression of GST-mGFAT2 proteins. After 24 h of infection, the cells were collected and lysed in the extraction buffer containing 20 mM Tris (pH 7.5), 0.5 mM NaCl, 0.5% Nonidet P-40, 5 mM MgCl2, 5 mM EDTA, 20% glycerol, 5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 5 mM benzamidine, 2 mM fructose 6-phosphate, and protease inhibitor mixture. After standing on ice for 20–30 min, the supernatant was collected by centrifugation, and the GST-mGFAT2 was purified by incubation with a 50% slurry of glutathione-Sepharose beads at 4 °C for 30–60 min. The beads were collected by centrifugation and washed three times with the extraction buffer. The fusion protein bound to the glutathione beads was eluted with 20 mM reduced glutathione. To express GST-mGFAT2, the plasmid pTMGST-mGFAT2 was transfected into BSC40 cells by electroporation at 250 V and 500 microfarads. After overnight recovery, the cells were infected with recombinant vaccinia virus VTF7-3. After incubating for 24 h, the cells were lysed in the extraction buffer (without EDTA). The N-terminal His-tagged mGFAT2 was purified with His-tagged beads as directed by the manufacturer (Novagen).

**Enzyme Assay of mGFAT2**—The enzyme activity was determined by a spectrophotometric method (21, 22) in a 1-ml standard assay as described previously. The protein concentration was determined by SDS-PAGE. A blank calibration control consisted of the entire reaction mixture with the same volume of GST- or His-tagged bead elution buffer.

**Phosphorylation of mGFAT2**—The purified GST-mGFAT2 and His-mGFAT2 fusion proteins (0.1 µg) were phosphorylated in vitro by the catalytic subunit of PKA in phosphorylation buffer containing 50 mM Tris–Cl (pH 7.5), 100 µM ATP, 10 mM MgCl2, 5 mM dithiothreitol at 30 °C for 20 min. After the phosphorylation reaction, the GFAT activity was measured. Fructose 6-phosphate and glutamine were added to final concentrations of 10 mM and incubated at 37 °C for 1 h. The reactions were stopped by boiling for 2 min. After the temperature was lowered by sitting the reaction mixture on ice for 3 min, the supernatants were collected by centrifugation at 14,000 rpm for 5 min. Glutamate dehydrogenase (20 units) and 3-acetylpyridine adenine dinucleotide at a final concentration of 0.5 mM were added in an enzyme activity assay buffer to a final volume of 1 ml. After incubation at 30 °C for 30 min, the A295 was determined.

**[γ-32P]ATP Labeling of mGFAT2**—The GST-mGFAT2 or mutant GST-mGFAT2 (0.5 µg) was incubated with or without 100 units of the catalytic subunit of PKA in the phosphorylation buffer described above. Unlabeled ATP at a final concentration of 0.1 mM and 1 µl of [γ-32P]ATP (10 µCi/ml) were added to the reaction. After incubation for 20 min at 30 °C, the GST beads were added into the reaction mixture and incubated at room temperature for 10 min with gentle shaking, and then the beads were spun down and washed three times with phosphorylation buffer. The sample with the beads was then boiled, and the protein was separated by 8% SDS-PAGE followed by autoradiography of the dried gel.

**O-GlcNAc Modification of mGFAT2**—Prior to the O-GlcNAc modification assay, the purified GST-mGFAT2 still bound to glutathione beads was treated with protein phosphatase catalytic subunit at 30 °C for 20 min in the phosphatase reaction buffer (25 mM imidazole (pH 7.4), 0.1 mM bovine serum albumin, 1 mM dithiothreitol, 50 mM NaCl). After the reaction, the beads were spun down and washed, and the GST-mGFAT2 protein was eluted with 20 mM reduced glutathione. With UDP-GlcNAc at a final concentration of 5 µM, the eluted GST-mGFAT2 was mixed with the purified GST-OGT, which was expressed in BSC40 cells using the same recombinant vaccinia virus expression system (mGFAT2 (11)). The activity of the enzyme mixture was confirmed as described (10). After incubation at room temperature for 1 h, the reaction mixtures were loaded onto an 8% SDS polyacrylamide gel. The O-GlcNAc-acylated proteins were detected with RL2 antibody (23, 24) by Western blot, and the same blot was stripped and rebolted by anti-GST tag antibody to detect the GST-tagged proteins.
Fig. 1. Expression of recombinant mouse GFAT2 in mammalian cells. GST-mGFAT2 is approximately 110 kDa, and His-mGFAT2 is approximately 80 kDa. A, the proteins in the cell lysate were separated by 10% SDS-PAGE stained with Coomassie Blue. Lane 1, cell lysates from cells infected with VTF7-3 virus and GST-mGFAT2 recombinant virus; lane 2, control cell lysate proteins from cells infected with VTF7-3 virus only. B, the same cell lysates as in A were analyzed by Western blot with anti-GST tag antibody. C, GST-mGFAT2 was purified on glutathione beads, and purified protein was eluted with 20 mM reduced glutathione. Lane 1 shows the starting material was cell lysate from cells infected by both GST-mGFAT2 and VTF7-3 vaccinia viruses. The GST-mGFAT fusion protein was purified on glutathione beads followed by separation of the protein by 10% SDS-PAGE and staining with Coomassie Blue. Lane 2 shows the starting material was cell lysate from cells infected with only VTF7-3 vaccinia virus. Purification and analysis was the same as lane 1. D, to express His-mGFAT2, BSC40 cells were transfected with plasmid pTM3His-mGFAT2 (D, lane 1) or vector control pTM3 (D, lane 2) following infection by VTF7-3 virus. The cell lysates were analyzed by Western blot with anti-His tag antibody.

RESULTS

Expression of GST-mGFAT2 and His-mGFAT2 in BSC40 Cells—The GST-mGFAT2 was expressed in BSC40 cells co-infected with VTF7-3 virus and the recombinant vaccinia virus that encodes GST-mGFAT2. The control cell lysate from the cells infected only with VTF7-3 virus showed many bands (Fig. 1A, lane 2). When the cells were infected with VTF7-3 virus and the GST-mGFAT2 recombinant virus, a new band appeared at approximately 110 kDa (Fig. 1A, lane 1). Western blot analysis of the lysates with an anti-GST tag antibody showed that the 110-kDa band of the cell lysate programmed for expression of the recombinant GST-mGFAT2 was a GST-reactive band (Fig. 1B, lane 1). The fusion protein was purified on glutathione beads. The eluted GST-mGFAT2, seen by Coomassie Blue staining after purification on GST beads (Fig. 1C, lane 1), was used in the enzyme activity and phosphorylation assays. Similar purification and elution steps were performed on the control lysate, yielding a few bands of lower intensity background (Fig. 1C, lane 2). The cells were also programmed to express His-mGFAT2, in which the six histidines were substituted for the GST on the N terminus of mGFAT2. Compared with the control lysate, which was from cells that were transfected with vector pTM3 and infected with VTF7-3 virus (Fig. 1D, lane 2), the lysate of the cells transfected with pTM3His-mGFAT2 contained a new band, which was visible on analysis by Western blot, with anti-His tag antibody. The band was at 81 kDa (Fig. 1D, lane 1) as predicted from the size of the His-mGFAT2 cDNA. The nickel affinity-purified protein at the same molecular weight was barely visible on a Coomassie Blue-stained gel (data not shown).

Kinetics of mGFAT2 Enzymatic Activity—A spectrophotometric assay was used to determine whether the purified GST-mGFAT2 fusion protein showed GFAT activity. The concentration of both substrates, fructose 6-phosphate (Fig. 2A) and glutamine (Fig. 2B), was varied while the other substrate was kept at 10 mM (saturation concentration) and the initial rates of the reactions were determined. When the reciprocal of the rate of the reaction was plotted against the reciprocal of the substrate concentration (Fig. 2, lower panels), a linear plot was obtained (Lineweaver-Burke plot). From these linearized results, the $K_m$ values were obtained for both substrates. The result showed that the purified recombinant GST-mGFAT2 did have GFAT activity like GFAT1. The $K_m$ of GST-mGFAT2 for fructose 6-phosphate was 0.8 mM, and $K_m$ for glutamine was 1.2 mM (Fig. 2). Previous studies have measured the kinetic characteristics of GFAT1 by different assay methods and showed the $K_m$ values range between 0.2 and 1.56 mM for fructose 6-phosphate and 0.4 and 3.8 mM for glutamine (25, 26). The $K_m$ values of GST-mGFAT2 for both of its two substrates measured in this study fall within these ranges.

PKA Treatment Induces an Increase of mGFAT2 Activity in Vitro—From the previous studies on hGFAT1 (human GFAT1), we found that phosphorylation of recombinant protein GST-hGFAT1 by PKA at its serine 205 blocks the enzyme activity, and serine 235, the other potential phosphorylation site of hGFAT1, is neither necessary nor sufficient for the inhibition (22). Alignment of different GFATs shows this serine is very conserved from E. coli to human (Fig. 3A) and that the RRGS is a potential recognition site of PKA. The counterpart of serine 235 of GFAT1 is not present in GFAT2 (Fig. 3A). No other potential PKA phosphorylation sites were present in either protein. The presence of only a single site in GFAT2 made analysis simpler than for GFAT1. As for GFAT1, recombinant purified mGFAT2 fusion protein was tested to determine whether it can be phosphorylated and its activity regulated by PKA. Indeed, our results showed that GST-GFAT2 can be phosphorylated by the catalytic subunit of PKA (Fig. 3D), but unlike recombinant GST-GFAT1 that is inhibited by this phosphorylation, both purified fusion proteins GST-mGFAT2 and His-mGFAT2 were stimulated approximately 2.2-fold by this modification (Fig. 3, B and C). When a PKA inhibitor was added into the reaction with the catalytic subunit of PKA, the activation of mGFAT2 by PKA was substantially reduced (Fig. 3C). According to the homology around the single PKA phosphorylation site of mGFAT2, its serine 202 was replaced by an alanine. Not only did this mutation abrogate the ability of mGFAT2 to be phosphorylated (Fig. 3D), it also prevented the change in activity that was observed for the wild type enzyme (Fig. 3B), regardless of whether the N-terminal tag was GST or His. Thus, the two isoforms of GFAT can be phosphorylated by PKA at a comparable site; yet the enzymatic activity of one is inhibited, whereas the other is simulated.

UDP-GlCNAC Partially Inhibits the Activity of Recombinant mGFAT2—It has been reported that GFAT1 can be feedback-inhibited by UDP-GlCNAC, the final product of the hexosamine biosynthetic pathway (26, 27). GFAT2 was also found to be inhibited in a dose-dependent manner by UDP-GlCNAC, but the maximal inhibition of its activity was only 15% (Fig. 4). The inhibition appears specific to the sugar because neither UDP-galactose nor GlcNAc without a UDP moiety was able to inhibit the enzyme activity. GFAT1 appears to be more influenced by UDP-GlCNAC because its inhibition was observed to be greater at 51% (27) and 80% (26), respectively.

mGFAT2 Is Probably Not O-GlCNAC-modified—One mecha-
nism by which GFAT activity might be feedback-inhibited through increasing quantities of UDP-GlcNAc is by modification with \( O\)-GlcNAc. This post-translational modification is known to alter the activity of other proteins, such as Sp1, and it has been shown that OGT activity is substrate concentration-dependent. To test this idea, we attempted to modify GST-mGFAT2 with recombinant GST-OGT. Before treating GST-mGFAT2 with GST-OGT, the fusion protein, although bound to glutathione beads, was pretreated with a general phosphatase to preclude possible blockage of \( O\)-GlcNAc modification by a potential phosphorylation at the same site; however, no modification of GST-mGFAT2 could be detected with RL2 antibody either before or after exposure to OGT (Fig. 5). Furthermore, OGT exposure did not change GST-mGFAT2 activity (data not shown). Although it remains possible that mGFAT2 is modified by \( O\)-GlcNAc and that this modification is hidden from detection by the RL2 antibody, it is more likely that it is not modified by OGT. Thus, product inhibition by UDP-GlcNAc of mGFAT2 may be allosteric.

**DISCUSSION**

The GFAT2 cDNA was cloned and designated as GFAT solely on the basis of sequence homology to GFAT1 (18). Here, we demonstrate for the first time that GFAT2 has GFAT activity. Thus, there are now two enzymes that can sample some of the glucose presented into the cell and divert it through the hexosamine pathway (27). Because the tissue distribution of the two GFAT proteins differs, the enzymes may provide for the specific needs of the tissue. For example, we showed previously that GFAT1 is inhibited by phosphorylation by PKA. Antisense reduction of GFAT1 in the \( \beta \) cells of transgenic mice makes these cells resistant to streptozotocin apoptosis (5) and so does the incretin Glp-1 (28, 29), of which the signal to the \( \beta \) cell is transduced by cAMP. The isolated islets from these transgenic mice also secrete more insulin under glucose stimulation than the islets of their littermates (7), again mimicking the effect of the incretins on insulin secretion. Conversely, an increase in the O-GlcNAc content of \( \beta \) cells inhibits insulin secretion (7). Thus, in \( \beta \) cells, the natural down-regulation of GFAT1 activity by cAMP or the unnatural down-regulation of the enzyme by an antisense strategy both provide less substrate for O-GlcNAc transferase via the hexosamine pathway and may play some role in insulin secretion.

The GFAT2 homology to GFAT1 extends to a homologous PKA phosphorylation site in GFAT2. The putative serine 202 site in GFAT2 is near the serine 205 site observed in GFAT1. Because of our elucidation of the role of cAMP in GFAT1 activity, we investigated this potential phosphorylation site and found that GFAT2 is also modified by PKA, but instead of inhibiting enzymatic activity of GFAT2, phosphorylation by this kinase stimulated its activity. Mutagenesis of the putative phosphorylation site not only abrogated the modification but also blocked the ability of PKA to stimulate the enzymatic activity of GFAT2. This 2-fold stimulation effect of PKA was independent of the epitope tag on the N terminus because both GST-GFAT2 and His-GFAT2 behaved identically. Therefore, the potential dimerization that might occur with the GST tag and does not occur with the His tag rules out tag-induced dimerization as a cause of this effect on GFAT2. Interestingly, when GFAT1 was His-tagged, PKA still inhibited the enzymatic activity (data not shown) (22), also ruling out the potential role of the N-terminal tag in this isozyme. Because the tissue distribution of GFAT2 differs from that of GFAT1 (18), we believe this stimulation of activity by PKA could subserve a different function in these tissues of the hexosamine pathway under cAMP stimulation.

Although the GFAT enzymes used in these experiments were recombinant and tagged, studies of the enzyme purified from rat liver (17), Candida albicans (30), and Drosophila (31) all

![Fig. 2. Km determinations of recombinant purified GST-mGFAT2 for fructose 6-phosphate (A) and glutamine (B). The Km values were determined by measuring the enzyme activity (initial rate of enzyme reaction) at different concentrations of one substrate while holding the other substrate at a constant and saturating concentration (10 mM) (upper panels). The Km values were derived from linearized double reciprocal plots (lower panels). Each point represents the mean of three determinations ± S.D. The measured Km values of purified GST-mGFAT2 for fructose 6-phosphate (Fru-6-P) and glutamine (L-Gln) were 0.8 and 1.2 mM, respectively.](http://www.jbc.org/content/29991/33/29991/F2)

![A](http://www.jbc.org/content/29991/33/29991/F2a)

![B](http://www.jbc.org/content/29991/33/29991/F2b)
show stimulation of GFAT activity by PKA. Because the two mammalian GFATs are homologous and similar in size, it remains possible that GFAT2 was purified, thereby accounting for the stimulation by PKA that was identical to that which we observed for GFAT2. Interestingly, the same interchange of GFATs may have occurred in Drosophila, which also appears to have two isozymes. The only recombinant GFAT tested was highly expressed in the chitin-synthesizing organs, and it is stimulated by PKA. A careful examination shows that the tested GFAT is more similar to GFAT2 than GFAT1, despite
the title of the report (31). Studies on the other Drosophila GFAT would be helpful. The GFAT in fungus, which synthesizes chitin, unlike that in mammals, is also stimulated by PKA. The evolution of an isozyme that is inhibited by PKA must have come approximately in multicellular organisms in which the diversification of the tissues requires differential regulation of the hexosamine pathway by cAMP. Studies on how homologous enzymes containing homologous phosphorylation sites can have opposite regulation could provide insight into the regulatory mechanisms of GFAT.

Although the GFAT proteins differed with respect to the effects of PKA phosphorylation, they were much more similar with respect to product inhibition. Both enzymes were inhibited by UDP-GlcNAc, although the inhibition was partial and appeared greater for GFAT1 (27) than for GFAT2. Modification of GFAT2 by O-GlcNAc was not detected with the RL-2 antibody, suggesting that the inhibition by UDP-GlcNAc resulted from an allosteric change in GFAT2. This product inhibition and the inhibition of GFAT1 but not GFAT2 by cAMP provide strong caveats for the use of GFAT1 in transgenic animals. For example, overexpression of GFAT1 in the β cells of transgenic mice may result in increased flux through the hexosamine pathway of isolated β cells. When these cells are in vivo, however, where modulation of AMP by incretins like Glp-1 might occur, GFAT1 might be inhibited during meals at a time when both incretins and product inhibition turn off the presumptive conversion of glucose to glucosamine. The finding of a GFAT isoform that is oppositely regulated by cAMP and more modestly regulated by its product, UDP-GlcNAc, will provide the opportunity to create transgenes that much more effectively alter the hexosamine flux in these and other cells.

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