Dissection of the Bifunctional *Escherichia coli* N-Acetylglucosamine-1-phosphate Uridyltransferase Enzyme into Autonomously Functional Domains and Evidence That Trimerization Is Absolutely Required for Glucosamine-1-phosphate Acetyltransferase Activity and Cell Growth

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The bifunctional N-acetylglucosamine-1-phosphate uridyltransferase (GlmU) enzyme catalyzes both the acetylation of glucosamine 1-phosphate and the uridylation of N-acetylglucosamine 1-phosphate, two subsequent steps in the pathway for UDP-N-acetylglucosamine synthesis in bacteria. In our previous work describing its initial characterization in *Escherichia coli*, we proposed that the 456-amino acid (50.1 kDa) protein might possess separate uridyltransferase (N-terminal) and acetyltransferase (C-terminal) domains. In the present study, we confirm this hypothesis by expression of the two independently folding and functional domains. A fragment containing the N-terminal 331 amino acids (Tr331, 37.1 kDa) has uridyltransferase activity only, with steady-state kinetic parameters similar to the full-length protein. Further deletion of 80 amino acid residues at the C terminus results in a 250-amino acid fragment (28.6 kDa) still exhibiting significant uridyltransferase activity. Conversely, a fragment containing the 233 C-terminal amino acids (24.7 kDa) exhibits acetyltransferase activity exclusively. None of these individual domains could complement a chromosomal *glmU* mutation, indicating that each of the two activities is essential for cell viability. Analysis of truncated GlmU proteins by gel filtration further localizes regions of the protein involved in its trimeric organization. Interestingly, overproduction of the truncated Tr331 protein in a wild-type strain results in a rapid depletion of endogenous acetyltransferase activity, an arrest of peptidoglycan synthesis and cell lysis. It is shown that the acetyltransferase activity of the full-length protein is abolished once trapped within heterotrimers formed in presence of the truncated protein, suggesting that this enzyme activity absolutely requires a trimeric organization and that the catalytic site involves regions of contact between adjacent monomers. Data are discussed in connection with the recently obtained crystal structure of the truncated Tr331 protein.

**UDP-N-acetylglucosamine (UDP-GlcNAc), the nucleotide-activated form of N-acetylglucosamine, plays a very important role in the biochemistry of all living organisms. In bacteria, it is required for the biosynthesis of essential cell-envelope components, namely peptidoglycan (1), lipopolysaccharides (2, 3), and teichoic acids (4), and for the formation of the enterobacterial common antigen (5).**

**Conditional-lethal mutants of *Escherichia coli* altered in the biosynthesis of this essential precursor are characterized by a cell-lysis phenotype (6–10). The four-step formation of UDP-GlcNAc from fructose-6-P has been now completely elucidated in this bacterial species (6, 7, 9, 11–13). It involves the successive actions of GlcN-6-P synthase, phosphoglucomutase, GlcN-1-P acetyltransferase, and GlcNAc-1-P uridyltransferase (GlmU); also named UDP-GlcNAc pyrophosphorylase).** We showed earlier that the two latter activities were carried by a single 456-amino acid protein, the product of a gene we named *glmU* located just upstream from the GlcN-6-P synthase *glmS* gene at 84 min on the *E. coli* chromosome (7, 14). The *glmU* gene has been identified in some other bacterial species, in particular *Neisseria gonorrhoeae* (15) and *Bacillus subtilis* (16).

The bifunctional *E. coli* GlmU enzyme has been purified to homogeneity, and its kinetic parameters were determined (7, 13, 17). A complete loss of acetyltransferase activity was observed following incubation of the enzyme in the absence of reducing agent or treatment with thiol-specific reagents. Site-directed mutagenesis experiments further demonstrated the important role of two of the four cysteines of GlmU, namely residues Cys307 and Cys324, for acetyltransferase activity (17). The GlmU protein has been shown to exhibit a number of characteristics, which suggested that the acetyltransferase and uridytransferase activities may reside in separate catalytic domains: (i) the substrates, products, and effectors of the acetyltransferase reaction did not inhibit the uridytransferase activity and *vice versa* (13, 18); (ii) the intermediate GlcNAc-1-P was clearly released from the acetyltransferase domain prior to transformation by the uridytransferase domain (18); (iii) portions of the GlmU amino acid sequence showing simi-

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* This work was supported by CNRS Grant EP1088, Grant 97.C.0177 “Biotechnologies” from the Ministère de l’Education Nationale de la Recherche et de la Technologie, and by a grant-in-aid from Hoechst Marion Roussel (to F. P.). 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.

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† The abbreviations used are: GlmU, N-acetylglucosamine-1-phosphate uridyltransferase; IPTG, isopropyl-1-thio-galactopyranoside; del, deletion; Tr, truncated; Wt, wild-type; OD, optical density; HPLC, high performance liquid chromatography; NTA, nitrilotriacetic acid; PAGE, polyacrylamide gel electrophoresis.
larieties with that of other previously characterized XDP-sugar pyrophosphorylase and acetyltransferase activities were located in the N-terminal portion and the second third of the protein, respectively (13, 15, 19); (iv) the acetyltransferase but not the uridylytransferase was shown to be inactivated by thiol-specific reagents, and the two cysteine residues whose alteration resulted in dramatic decreases of acetyltransferase activity were identified in the second moiety of the protein sequence (17); (v) mutagenesis of residues that are important for uridylytransferase activity did not affect acetyltransferase activity at all (Ref. 20 and data not shown); (vi) the fusion of a His$_6$ tag at the C terminus of the protein resulted in a 20-fold decrease of acetyltransferase activity, without change in its uridylytransferase activity (17).

The crystal structure of a truncated form of the GlmU enzyme, GlmU-Tr331, was recently resolved at 2.25- and 2.3-Å resolution in the absence or presence of UDP-GlcNac, respectively (20). The crystal structure is composed of two distinct domains connected by a long α-helical arm: a N-terminal domain resembling the dinucleotide-binding Rossmann fold and a C-terminal domain adopting a left-handed parallel β-helix structure (LβH) that is also found in homologous bacterial acetyl- and acetyltransferases (21–24).

We here report the construction of truncated forms of this enzyme, which confirms that the bifunctional enzyme is composed of two autonomously folding and functional domains of roughly equivalent sizes, the N-terminal one exhibiting uridylytransferase activity and the C-terminal one acetyltransferase activity. It is also shown that trimer organization is essential for expression of the acetyltransferase activity and that the catalytic site of the latter should be formed by complementary regions from adjacent monomers.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions—**E. coli strains JM38 (ara Δ[lac-proAB] rpsL thi Φ80 dlacZ ΔM5) (25) and DH5α (supE44 ΔlacU169 hsdR17 recA1 endA1 gyrA96 thi-1 relA1) were used as hosts for plasmids and for the overproduction of wild-type and mutant GlmU enzymes. Strain UG886 (JM38 glmU::kan [pGM1]), which carries an inactivated copy of the glmU gene on the chromosome and a wild-type copy of glmU on a plasmid whose replication is thermosensitive, was used in complementation experiments (7). The plasmid vector pTrcHis30 for expression of proteins under a N-terminal histidine-tagged form has been described previously (26). 2YT (26) was used as culture medium, and growth was monitored at 600 nm with a Shimadzu UV-1601 spectrophotometer. For proteins under a N-terminal histidine-tagged form has been described previously (29). The different His$_6$-tagged enzymes were purified as reported recently (17), basically following the steps in the manufacturer’s (Qiagen) recommendations: binding of His$_6$-GlmU on Ni$^{2+}$-nitrilotriacetic-agarose (Ni$^{2+}$-NTA) and extensive washing with 20 mM potassium phosphate buffer, pH 7.4, containing 1% β-mercaptoethanol, 0.5 mM MgCl$_2$, 0.1% sodium dodecyl sulfate, and 0.1% Triton X-100, elution with 6 M guanidine hydrochloride, and elution with imidazole (100–300 mM) added to washing buffer; dialysis of His$_6$-GlmU eluate against 100 volumes of the same phosphate buffer supplemented with 10% glycerol. The His$_6$-tagged GlmU enzymes prepared in this manner were all at least 90% pure, as estimated by SDS-PAGE. Protein concentrations were determined by the method of Bradford, with bovine serum albumin as a standard (30).

**Extraction and Quantitation of Peptidoglycan Precursors—**Cells of JM38(pFP3-Tr331) were grown at 37 °C in 2YT medium (1-liter cultures). At OD = 0.1 (6 × 10$^6$ cells·ml$^{-1}$), IPTG was added to one culture at a final concentration of 1 mM of IPTG. As soon as the first effects on cell growth were observed in induced cells (2 h later, final OD (600 nm) = 0.7), cultures were stopped by rapid chilling to 4 °C, and cells were harvested in the cold. Cultures of JM35 cells carrying the pTrcHis30 vector were made in parallel as a control. The extraction of peptidoglycan nucleotide precursors as well as the analytical procedure used for their quantitation were as described previously (31). UDP-GlcNac was purified from cell extracts by first using the same two-step chromatographic procedure that is commonly used to purify the peptidoglycan nucleotide precursors: a gel filtration on Sephadex G-25 followed by HPLC on a column of AG-50W-X8 (5 × 10$^3$) (where it is eluted in mixture with UDP-GlcNac (31). The separation of UDP-GlcNac and UDP-GlcNac was then achieved by a second step of HPLC on the same column, using this time an elution with 50 mM triethylammonium formate, pH 4.75, at a flow rate of 3 ml·min$^{-1}$ (their retention times were 9 and 21 min, respectively).

**Methods for Enzyme Assays—**Enzyme activities of GlmU were performed as described previously (17), after appropriate dilutions of the enzyme in 20 mM potassium phosphate buffer, pH 7.4, containing 1 mg·ml$^{-1}$ BSA, 0.5 mM MgCl$_2$, and 0.1% β-mercaptoethanol. One unit of enzyme activity was defined as the amount that catalyzed the formation of 1 μmol of product/min. UDP-GlcN, which is used as an alternative substrate of GlmU in some acetyltransferase assays, was synthesized enzymatically by using UDP-glucose pyrophosphorylase, as described by Gehring et al. (18). The enzyme was purified by HPLC as described above, and its authenticity was confirmed by determination of its hexosamine content after acid hydrolysis.
Dissection of Bifunctional E. coli GlmU Enzyme

Fig. 1. Schematic structure of various truncations of the GlmU protein generated in the present study. Truncated proteins are represented by cross-hatched regions, and numbers indicate amino acid residue numbers within the wild-type protein sequence. All truncated proteins were expressed with a N-terminal His6 tag extension, as shown for the full-length protein.

RESULTS

Construction of Various Truncated Forms of GlmU and Their Activity—As attempts to crystallize the full-length GlmU protein remained unsuccessful, crystallization of individual domains was therefore envisaged. This prompted us to more precisely define the size of the two putative autonomous domains. Plasmids for high-level overexpression of GlmU proteins truncated either in the N- (del constructs) or in the C-terminal (Tr constructs) region were constructed (Fig. 1). All of these proteins were expressed in a His6-tagged form (N-terminal Met-His6-Gly-Ser extension), allowing their convenient one-step purification. Most of them were successfully overproduced in a soluble form and were purified to near homogeneity (Fig. 2). Others (del26, del182, del250, and Tr227) either appeared very poorly produced, due probably to structural instability and rapid intracellular degradation, or formed insoluble inclusion bodies.

However, none of these different plasmids could complement the thermosensitive glmU mutant strain UGS83, indicating that the engineered truncations had resulted in the loss of at least one of the two activities of GlmU. This was confirmed by assays of the pure proteins for acetyl- and uridylytransferase activities (Table I). The uridylytransferase activity of proteins truncated in the N-terminal region (del78, del130, del227, and del233) was decreased by a factor of at least 1000, but surprisingly a residual and almost invariant activity (kcat = 0.1–0.3 s⁻¹) was retained by all of them. As discussed below, this residual activity was due to contaminating wild-type GlmU enzyme (originating from chromosomal gene expression) that could form heterotrimers with these His6-tagged truncated proteins. The deletion of the first 130 N-terminal amino acid residues of GlmU resulted in only a 50% decrease of its acetyltransferase activity. Truncation of 100 more residues (del233) was accompanied by a more important decrease (98%) of activity, but the residual acetyltransferase activity remained relatively high with a kcat of 25 s⁻¹. Gehring et al. (18) also previously constructed a truncated form of GlmU (glutathione S-transferase fusion), deleted in that case of the first 179 residues. Its acetyltransferase activity was 150-fold reduced as compared with full-length GlmU with a kcat of 0.5 s⁻¹. As shown in Table I, an inverse pattern of activities was observed for proteins carrying deletions in the C-terminal region; Tr331 and Tr250 proteins lacking the last 125 and last 206 amino acid residues, respectively, had undetectable acetyltransferase activity but retained significant uridylytransferase activity (42% and 2.5% of wild-type enzyme activity, respectively).

Trimer Organization—As reported previously, chromatography of full-length GlmU protein on gel filtration was consistent with a homotrimer arrangement (13). The oligomerization state of the different truncated GlmU fragments generated here was then investigated (Table I). The ability of the proteins to trimerize was clearly correlated to the presence of at least the initial part of the C-terminal domain. The fact that the two del227 and Tr331 proteins were trimers suggested that residues involved in the oligomerization process might be located between these two sites of truncation. With the exception of the Tr250 protein, which turned out to be a monomer, all other truncated proteins generated in the present work consisted of trimers.

As shown above, a very low but detectable uridylytransferase activity of 0.1–0.3 s⁻¹ was consistently detected with all preparations of proteins carrying either partial or complete dele-
Overproduction of GlmU-Tr331 protein on E. coli cell growth. Cells of JM83(pFP3-Tr331) were grown at 37 °C in 2YT-ampicillin medium. At the time indicated by an arrow (optical density = 0.1), the overproduction of the Tr331 protein was induced with IPTG (1 mM) and growth of induced (open symbols) and not induced (filled symbols) cells was monitored at 600 nm. Growth of cells carrying as a control the plasmid vector pTrcHis30 was unaffected by IPTG and paralleled that of noninduced JM83(pFP3-Tr331) cells (data not shown).
Cells were grown in 2YT-ampicillin medium at 37 °C. At OD = 0.1, IPTG (1 mM) was eventually added and incubation was continued until the first effects on cell growth were observed, about 2 h later (Fig. 3). At this time, peptidoglycan and its precursors were extracted and quantitated as described in the text. Crude protein extracts were also prepared and were assayed for GlcN-1-P acetyltransferase and GlcNAc-1-P uridylytransferase activities. The values are means of determinations obtained in two independent experiments.

### TABLE II

<table>
<thead>
<tr>
<th>Strain</th>
<th>IPTG</th>
<th>Amount</th>
<th>Specific activity</th>
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<tr>
<td></td>
<td></td>
<td>UDP-GlcN</td>
<td>UDP-GlcNAc</td>
</tr>
<tr>
<td>JM83(pTrHis30)</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>JM83(pFP3-Tr311)</td>
<td>+</td>
<td>360</td>
<td>350</td>
</tr>
</tbody>
</table>

* The peptidoglycan content was expressed in terms of its uramic acid content.

* Not detected (< 10 nmol/g of bacteria, dry weight).

* No activity detected (< 0.0001 units × mg of protein⁻¹).

should be noted that the effects on cell morphology and peptidoglycan metabolism were observed only in the presence of IPTG. The fact that the 30-fold decrease of acetyltransferase activity detected in noninduced cells had no apparent effect on cell growth was consistent with the previous demonstration that this activity of GlmU was in great excess in E. coli cells as compared with its specific requirements (13).

Formation of a mixture of four different heterotrimers Wt(3), Wt(2)-Tr(1), Wt(1)-Tr(2), and Tr(3) is expected to occur in vivo, whose proportions theoretically reflect relative abundance of wild-type (Wt) and truncated (Tr) monomers. In conditions where the truncated monomer is largely predominant (at least a 200:1 ratio could be estimated in IPTG-induced cells from the increase of uridylyltransfer activity), Wt(3) and Wt(2)-Tr(1) species are most probably very rarely generated, and wild-type monomers should be exclusively in the Wt(1)-Tr(2) form (model shown in Fig. 4). The fact that the acetyltransferase activity of the wild-type enzyme was completely undetectable in these conditions therefore suggested that more than one full-length monomer per trimer was required for expression of the latter activity. However, the question of the acetyltransferase activity of the two Wt(2)-Tr(1) and Wt(1)-Tr(2) species remained. To generate significant amounts of these two heterotrimers, JM83 cells were transformed by two plasmids, pFP1kan and pFP3-Tr331, for concomitant expression of the wild-type (Wt) and the His₆-tagged truncated (Tr) proteins, respectively. Analysis of crude extracts prepared from these cells confirmed the large accumulation of the two proteins (data not shown). Purification on Ni²⁺-NTA was then performed as described above, using extensive washing steps for complete elimination of the non-His-tagged Wt(3) form. The resulting purified material appeared composed of both Wt and Tr proteins in roughly equivalent amounts, as judged by SDS-PAGE (data not shown). Unfortunately, attempts to separate the different His-tagged heterotrimers present in this mixture by gel filtration techniques failed, due to their very close molecular masses, 111, 123, and 135 kDa for Tr(3), Wt(1)-Tr(2), and Wt(2)-Tr(1), respectively. However, this mixture, which theoretically contains the two Wt(2)-Tr(1) and Wt(1)-Tr(2) heterotrimers, exhibited a high uridylyltransferase activity (120 s⁻¹) but had no detectable acetyltransferase activity, as observed for the pure Tr(3) homotrimer. This finding was thus consistent with the hypothesis (discussed below) that three full-length monomers should be present in the trimer for expression of the latter activity.

Overexpression of other truncated proteins described in the present work had no effects on cell growth. It should be noted that most of them exhibited significant acetyltransferase activity. In fact, the only other engineered protein with undetectable acetyltransferase activity, Tr250, appeared unable to oligomerize and consequently could not trap the full-length GlmU enzyme into inactive heterotrimers.

Interestingly, IPTG-induced JM83(pFP3-Tr331) cells were shown to accumulate large amounts of a compound, which was identified as UDP-GlcN (Table II). This unexpected finding was clearly correlated with the depletion of the acetyltransferase activity of GlmU in these cells. It suggested that in the absence of the latter enzyme activity GlcN-1-P molecules had been transformed into UDP-GlcN by the still present (and highly overproduced) uridylyltransferase activity of GlmU. GlmU-catalyzed uridylyltransfer to glucosamine-1-P was previously reported to be undetectable (< 0.0001 units × mg of protein⁻¹) (18). In our hands, however, the pure GlmU enzyme could catalyze the synthesis of UDP-GlcN from GlcN-1-P and UTP with a very good efficiency (k₉₉₉₉ = 23 s⁻¹, as compared with 330 s⁻¹ when GlcNAc-1-P is used as the substrate), a finding consistent with the in vivo accumulation of this compound.

**DISCUSSION**

Data obtained in the present study confirm that the bifunctional GlmU protein is organized in two autonomously folding and functional domains. As judged by the specific activities exhibited by the various truncated forms of GlmU described here, the size of the two individual domains might be roughly equivalent, each one representing about half of the protein. In the light of the recently established crystal structure of a truncated form of GlmU (Tr331), the connection between the two domains is achieved by a long α-helical arm located between residues Asn₂₂₈ and Ala²⁵₀ (20). It suggests that this bacterial
protein evolved by fusion of two uridylyltransferase and acetyltransferase fragments. In eukaryotes, the biosynthesis of UDP-GlcNAc occurs by a slightly different route (via GlcNAc-6-P) in which GlcN-6-P acetyltransferase and GlcNAc-1-P uridylyltransferase activities are carried by two distinct monofunctional enzymes (33–37). The selective advantage (if any) conferred to bacteria by this unique bifunctional enzyme remains an enigma. In particular, there is no apparent requirement for a common regulation of the component activities at the level of transcription or translation. It was previously shown that both activities were in great excess in cells as compared with specific requirements in UDP-GlcNAc molecules of the peptidoglycan and lipopolysaccharide pathways (7, 13). As demonstrated by Plumbridge et al. (38), the glmU gene is cotranscribed with the downstream GlcN-6-P synthase glmS gene in E. coli and seems to be expressed at a high constitutive level whatever the growth conditions used. The construction of various mutated forms of GlmU enzyme affected in either of the two activities also showed that the ratio of acetyltransferase and uridylyltransferase activities (which is about 5 for wild-type enzyme) could be greatly modified in vivo without detectable effect on the functioning of this pathway. Additionally, there is no apparent advantage for GlmU to be a bifunctional protein in terms of reaction mechanism. By using radiolabeled substrates, Gehring et al. (18) demonstrated that GlcN-1-P was released by the enzyme before being used as substrate by the second enzyme activity. In addition, the thermosensitive glmU mutant strain UGS83 was shown to accumulate large amounts of GlcNAc-1-P when grown at the restrictive temperature (7). These results were a priori not consistent with the hypothesis of a concerted action of the two enzyme activities.

Gehring et al. (18) reported previously that the GlmU enzyme was unable to catalyze uridylyltransfer from UTP to GlcN-1-P but could catalyze acetyltransfer from acetyl-CoA to UDP-GlcN, although at a 12-fold reduced rate. It was one of the arguments why these authors conclude that acetyltransfer precedes uridylyltransfer in the two-step formation of UDP-GlcNAc by GlmU. We effectively confirmed here that GlmU could catalyze acetyltransfer to UDP-GlcN and the $k_{cat}$ value we determined was 170 s$^{-1}$ (9-fold lower as compared with 1500 s$^{-1}$ for GlcN-1-P). However, we here observed that GlmU could also efficiently catalyze uridylyltransfer to GlcN-1-P, at a 15-fold reduced rate ($k_{cat} = 23$ s$^{-1}$) as compared with that with GlcN-1-P (350 s$^{-1}$). In our hands, the GlmU enzyme therefore appears theoretically capable to catalyze a two-step synthesis of UDP-GlcNAc in which uridylyltransfer precedes acetyltransfer but the greatly reduced kinetic parameters confirmed the previous assumption that these two reactions occur in the inverse order under normal physiological conditions (13, 18). As shown in the present report, a significant accumulation of UDP-GlcN was observed in cells in which the acetyltransferase activity of GlmU has been inhibited. This finding confirmed that GlmU could effectively catalyze the uridylyltransfer from UTP to GlcN-1-P in vivo, but it is clear that this only occurred because of very particular physiological conditions in which the availability of the preferred substrate GlcNAc-1-P was impaired.

GlmU and its truncated derivative Tr331 are trimeric proteins, as judged by their behavior on gel filtration. The recently obtained crystal structure of GlmU-Tr331 also showed a trimeric arrangement around the long dimension of the L6H prism (20). The trimeric association of the particular L6H domain is highly conserved between GlmU-Tr331 and other previously characterized bacterial acetyl- or acetyltransferases, namely LpxA, PaXAT, DapD, and Cam (20–24). However, it is not known whether a trimeric organization of these proteins is absolutely required for expression of their acetyl- or acyltransferase activities. This also raised the question of the activity of the various heterotrimeric proteins that could be generated from a mixture of wild-type and truncated monomers. To answer this question, we followed the growth of a wild-type E. coli strain during the overexpression of GlmU-Tr331, a truncated protein with null acetyltransferase activity but still able to associate in trimers. Its overproduction by a factor of about 200-fold as compared with the wild-type protein level resulted in the complete disappearance of acetyltransferase activity in cells, which was followed by deleterious effects on peptidoglycan biosynthesis and cell growth. This finding strongly suggested that the wild-type enzyme could not exhibit acetyltransferase activity under a monomer form in vivo. It also indicated that the activity of this wild-type protein originating from chromosomal expression could no more be detected once trapped within heterotrimers formed in presence of the predominantly expressed truncated protein (model shown in Fig. 4). An organization in trimer is therefore required for exhibition of the acetyltransferase activity but the data here obtained could be interpreted in several ways. (i) Each monomer carries a complete catalytic site, the active conformation of which is formed only during trimer assembly; (ii) each catalytic site is made of specific complementary regions belonging to more than one monomer, suggesting that one to three catalytic site(s) could exist per trimer unit. To date, only the crystal structure of the truncated form of GlmU has been determined (20) and the exact position and number of binding-sites of the substrate acetyl-CoA are not known. It should be noted that, in the homologous structures of acetyltransferases DapD and PaXAT (21, 22), three binding sites for the substrate acetyl-CoA were detected, each one being located between two subunits on the exterior face of the trimeric L6H domains. This positioning could also be adopted for GlmU. The finding that a mixture of Tr1(0), Wt1(2) Tr2(0), and Wt(5), Tr(1) heterotrimer has undetectable acetyltransferase activity further suggests that the catalytic site(s) of the active trimer W(0) should be formed by adjacent and complementary regions from three full-length monomers. The confirmation of this hypothesis now requires the elucidation of the three-dimensional structure of the entire GlmU protein. Trimerization is clearly not essential for expression of the uridylyltransferase activity of GlmU. The latter activity was retained by the Tr250 protein, which is unable to associate in trimers. However, the 40-fold reduced uridylyltransferase activity of this protein suggests that trimerization or at least interactions between regions of the two domains may participate in the folding and stability of the N-terminal domain. In the crystal structure, the only observed contacts between the two domains were van der Waals interactions between the surface loop Ala$^{31}$-Gly$^{32}$ in the N-terminal domain and the Arg$^{263}$ side chain in the C-terminal domain (20). Residues within the long $\alpha$-helical arm (Asn$^{228}$–Ala$^{250}$) also established numerous interactions with residues in the two domains (20). The abolishment of at least the van der Waals interactions in the Tr250 protein could partially account for the decreased activity of this protein. However, the positioning of the long $\alpha$-helical arm seems to play an important role for the uridylyltransferase activity with the helix dipole aligned with the position of the key positively charged Arg$^{18}$ residue. Whether an incorrect positioning of the $\alpha$-helical linker in the Tr250 protein could explain for its decreased activity remains to be elucidated.

As mentioned above, the pathway for UDP-GlcNAc biosynthesis appears significantly different in eukaryotes. In the latter, acetyltransfer occurs on GlcN-6-P and not on GlcN-1-P, and, most importantly, acetyltransferase and uridylyltransferase activities are carried by two distinct monofunctional
enzymes that show little sequence homology with GlmU (35–37). The GlmU enzyme, which is essential and specific of the bacterial world, should therefore be considered as an interesting target for the search of new antibiotics. Biochemical and crystallographic investigations are now developed to gain more information on active sites of this bifunctional enzyme. The present demonstration that a trimer organization is absolutely required for acetyltransferase activity of GlmU could also open the way for a search of inhibitors based on the inhibition of the oligomerization process.

REFERENCES


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doi: 10.1074/jbc.M004788200 originally published online November 17, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M004788200

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