Bacterial UMP kinases are essential enzymes involved in the multistep synthesis of UTP. They are hexamers regulated by GTP (allosteric activator) and UTP (inhibitor). We describe here the 2.8 Å crystal structure of Escherichia coli UMP kinase bound to GTP. The GTP-binding site, situated at 15 Å from the UMP-binding site and at 24 Å from the ATP-binding site, is delineated by two contiguous dimers. The overall structure, as compared with those bound to UMP, UDP, or UTP, shows a rearrangement of its quaternary structure: GTP induces an 11° opening of the UMP kinase dimer, resulting in a tighter dimer-dimer interaction. A nucleotide-free UMP kinase dimer has an intermediate opening. Superposition of our structure with that of archaeal UMP kinases, which are also hexamers, shows that a loop appears to hamper any GTP binding in archaeal enzymes. This would explain the absence of activating effect of GTP on this group of UMP kinases. Among GTP-binding residues, the Asp-93 is the most conserved in bacterial UMP kinases. In the previously published structures of E. coli UMP kinase, this residue was shown to be involved in hydrogen bonds between the subunits of a dimer. Its substitution by an alanine decreases the cooperativity for UTP binding and suppresses the reversal by GTP of UTP inhibition. This demonstrates that the previously described mutual exclusion of these two nucleotides is mediated by Asp-93.

Uridine 5'-monophosphate kinases (UMPKs) catalyze the reversible transfer of the γ-phosphoryl group from ATP to UMP, according to the reaction, Mg\textsubscript{2+}ATP + UMP ↔ Mg\textsubscript{2+}ADP + UDP. The resulting UDP is further phosphorylated by nucleoside diphosphate kinase to UDP, serving as a substrate for RNA polymerase or as a precursor for CTP. UDP can also be reduced by ribonucleoside diphosphate reductase to 2'-deoxy-UDP, which serves in the production of dTTP and dCTP used for DNA synthesis.

The eukaryotic UMP/CMP kinases phosphorylate with comparable efficiency both UMP and CMP. Conversely, bacteria possess two distinct enzymes, each specific for either UMP or CMP. Bacterial UMPKs exist in solution as stable homohexamers, whereas eukaryotic UMP/CMP kinases are monomers. We previously solved three crystal structures of UMPK from Escherichia coli (UMPK\textsubscript{eco}) in complex with UMP, UDP, or UTP (1), using an enzyme variant (D159N) with similar stability and kinetic properties (2) but with significantly higher solubility than the wild-type protein. These structures showed that the overall fold of the monomer is different from that of eukaryotic UMP/CMP kinases. Besides, the three-dimensional structure of the UMPK\textsubscript{eco} hexamer is closely related to all of the other bacterial UMPKs structures deposited in the Protein Data Bank.

Bacterial UMPKs are submitted to allosteric activation by GTP and to feedback inhibition by UTP, the major product of the reaction they catalyze (3). This contributes to balance the synthesis of purine versus pyrimidine nucleoside triphosphates. The crystal structure of UMPK\textsubscript{eco} in complex with UTP showed that the nucleotide, in its magnesium-free form, interacts with the phosphate acceptor site. Kinetic experiments showed indeed that magnesium-free UTP is a competitive cooperative inhibitor (4). GTP is the common allosteric effector for all known bacterial UMP kinases. In Gram-negative organisms, it suppresses the inhibition by excess of UMP and reverses the UTP binding and inhibition. In Gram-positive bacteria, GTP increases the affinity for ATP through lowering of cooperativity for this phosphate donor nucleotide.

For all bacterial species, UMPK appears as essential for growth (5–8). Bacterial UMPKs represent a unique target for the design of antibacterial agents as underlined by recently published papers (8, 9) and even a patent (10). An interesting path to follow is probably the design of inhibitors targeting the allosteric GTP-binding site. However, so far there is no published structure of this site allowing its detailed analysis.

Here we describe the crystal structure of UMPK\textsubscript{eco} bound to GTP. We discuss its implications for the enzyme regulation mechanism, with the help of site-directed mutagenesis experiments.
**The GTP-binding Site of E. coli UMP Kinase**

**EXPERIMENTAL PROCEDURES**

*Chemicals—Nucleotides, restriction enzymes, T4 DNA ligase, Tfu DNA polymerase, and coupling enzymes were from Roche Applied Science, Qbiogene Inc., or Sigma. NDP kinase from Dictyostelium discoideum (2000 units/mg protein) was kindly provided by I. Lascu (Institut de Biochimie et Génétique Cellulaire, Université de Bordeaux).

**Bacterial Strains, Plasmids, Growth Conditions, and DNA Manipulations—**General DNA manipulations were performed as described by Sambrook et al. (11). The construct of UMPKeco D159N (considered as the reference form) with an N-terminal His tag was previously reported (1). The single mutants N72A and D93A were constructed by the one-tube PCR-based mutagenesis method (12) using the plasmid pLA2.1.1 harboring the UMP kinase gene with D159N mutation as template, Tfu DNA polymerase, the deoxyribonucleoside triphosphates, and the following mutagenic primers: 3′-UMPK N72A, 5′-GTCGCCACAAAGCGTGCTGACCCCGTTGCGC-3′; 3′-UMPK D93A, 5′-GGGCCGCTGAGTGCGTCGCCGAT-TGCGACGCC-3′. The PCR product was cloned at the Ndel and Xhol restriction sites of the vector pET28a, giving the plasmids pCE1.1.1 and pCE1.1.2, respectively. The double mutant N72A/D93A was constructed in the same way but used pCE1.1.2 as a template and 3′-UMPK N72A as a mutagenic primer, giving the plasmid pCE1.1.5. These plasmids were sequenced to verify either integrity or incorporation of the desired modifications. They were introduced into *E. coli* strain BL21(DE3)pDia17 (13). The recombinant strains were grown in 2× yeast extract tryptone medium supplemented with kanamycin (70 μg/ml) and chloramphenicol (30 μg/ml) at 37 °C to overproduce the UMP kinase. When the optical density reached 1.3 at 600 nm, 1 mM isopropyl-β-d-thiogalactopyranoside was added to the medium to induce the expression of recombinant proteins, and the growth was carried on for an additional 3 h. The cells were then harvested by centrifugation and served as source for protein purification.

**Purification of UMPK and Activity Assay—**Each N-terminal His-tagged variant of UMPKeco, overproduced in *E. coli*, was purified by nickel-nitritoltriacetic acid affinity chromatography using the QIA express system (14) after sonication of the pellet and purification by nickel–nitrilotriacetic acid affinity chromatography followed by nickel-nitritoltriacetic acid elution buffer containing 50 mM Na2HPO4, 150 mM imidazole, and 10 mM EDTA (pH 8.0). The eluted protein was dialyzed against a buffer containing 50 mM Tris-HCl (pH 8.5) containing 100 mM NaCl at a temperature of 4 °C for 3 h and was then frozen at -80 °C for storage. The protein concentration was determined by the Bradford method (15). SDS-PAGE was performed as described by Laemmli (16). Mass spectra of purified proteins were recorded on a quadrupole mass spectrometer API365 (PerkinElmer Life Sciences) equipped with an ion spray (nebulizer-assisted electrospray) source.

The UMP kinase activity was determined at 30 °C (0.5-ml final volume) using a coupled spectrophotometric assay (17). The reaction medium contained 50 mM Tris-HCl, pH 7.4, 1 mM phosphoenolpyruvate, 0.2 mM NADH, 2 units each of lactate dehydrogenase, pyruvate kinase, and NDP kinase, and various concentrations of ATP, MgCl2, and UMP. A 2 mM excess of MgCl2 over the concentration of NTPs was used, as described previously (4).

The reaction was triggered with appropriately diluted UMPKeco in 50 mM Tris-HCl, 100 mM NaCl, pH 8.0, and the decrease of absorbance at 340 nm was recorded. One unit of UMPK corresponds to 1 nmol of product formed/min.

The kinetic results were fitted to one of the following two equations, using the nonlinear least squares fitting analysis of Kaleidagraph software,

\[
v = V_m[S]/(K_m + [S])
\]

(Eq. 1)

\[
v = V_m[S]/(K_m + [S]^2/K_i)
\]

(Eq. 2)

where \(v\) is the steady-state velocity, \(V_m\) the maximal rate, \([S]\) is the substrate concentration (i.e. ATP or UMP in the forward reaction), \(K_m\) is the Michaelis-Menten constant, and \(K_i\) the inhibition constant. The cooperativity of inhibition by UTP of UMP kinases was expressed by the equation \(K_m^{	ext{UTP}} = K_m^{	ext{ATP}}(1 + [UTP]^{n} / K_{UTP}^{n})\), where \(K_m^{	ext{UTP}}\) and \(K_m^{	ext{ATP}}\) are the apparent \(K_m^{	ext{ATP}}\) in the absence and presence of a given concentration of UTP, \(n\) is the cooperative index, and \(K_{UTP}\) is the concentration of UTP doubling the apparent \(K_m^{	ext{ATP}}\) (4).

The thermal stability of the different variants of UMPK was tested by incubating the purified enzyme (1 mg/ml) in 50 mM Tris-HCl (pH 8.5) containing 100 mM NaCl at a temperature between 30 and 80 °C for 10 min in the absence or presence of various nucleotides (UTP or GTP). The results were expressed as a percentage of residual activity as compared with nonincu-
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RESULTS AND DISCUSSION

Structure of E. coli UMP Kinase in Complex with GTP

The Overall Hexameric Structure—The three previously published structures of UMPKeco bound either to the substrate (UMP), reaction product (UDP), or inhibitor (UTP) were obtained from R3 rhomboedral crystals. The asymmetric unit contained a dimer of two subunits (each monomer contains 241 residues) related by noncrystallographic symmetry. The crystallographic 3-fold symmetry axis reconstituted the biological hexamer. Conversely, the complex of UMPKeco with GTP crystallized in the orthorhombic P2₁2₁2₁ space group (Table 1) with an equivalent biological hexamer in the asymmetric unit. The six subunits are related by noncrystallographic 3-fold and 2-fold axes (Fig. 1A). The six monomers that constitute the asymmetric unit were refined as different models, using individual B factors. In the final steps, we placed water molecules in residual density above 2.5 S.D. values. The final model contains six polypeptide chains, A–F, six GTP molecules, G–L, and 36 water molecules. A summary of the refinement results together with model statistics is given in Table 1. Models were superposed with the procedure implemented in O (24) with defaults, and the relevant root mean square deviations for Ca atoms were calculated. Buried surface in protein-protein interaction was calculated using the Protein-Protein Interaction server (25). Relative movements of the monomers in a dimer were calculated using the protein domain motion program DynDom (26). The Protein Data Bank code of the UMPKeco-GTP complex is 2v4y.

Structure Solution, Model Building, and Refinement—The structure of the UMPKeco-GTP complex was solved by molecular replacement performed at 3.0 Å resolution with PHASER (19), using only the protein part of the UMPKeco-UTP model. Since no solution was found using the dimer of the asymmetric unit as a model, we used a monomer as a model. Three monomers were found automatically by the program (resulting in a 48.4% R factor); the remaining three were then hand-constructed. A clear density immediately appeared for the nucleotide in all six molecules of the asymmetric unit. Models were built using TURBO (20). CNS (21) version 1.1 and, in final steps, PHENIX (22) version 1.3 were used for refinement, which was monitored using a free R factor (23). Simulated annealing was initially used, as well as noncrystallographic restraints. The latter applied to two domains comprising residues 5–171 and 183–241 and were progressively loosened during the first 10 refinements. Then the six molecules of the asymmetric unit were refined as different models, using individual B factors. In the final steps, we placed water molecules in residual density above 2.5 S.D. values. The final model contains six polypeptide chains, A–F, six GTP molecules, G–L, and 36 water molecules. A summary of the refinement results together with model statistics is given in Table 1. Models were superposed with the procedure implemented in O (24) with defaults, and the relevant root mean square deviations for Ca atoms were calculated. Buried surface in protein-protein interaction was calculated using the Protein-Protein Interaction server (25). Relative movements of the monomers in a dimer were calculated using the protein domain motion program DynDom (26). The Protein Data Bank code of the UMPKeco-GTP complex is 2v4y.

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**A)**

![Image of UMPKeco-GTP structure](image)

**B)**

![Image of GTP binding](image)

**C)**

![Image of UMPKeco-GTP structure](image)

**D)**

![Image of UMPKeco-GTP structure](image)

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(Extreme values 0.28 and 0.55 Å) on 236 Ca atoms. In the previously published structures in complex with UMP, UDP, or UTP (hereafter referred to as UXP), the dimer assembly buried 11% of the total surface, whereas interactions between two neighboring dimers buried only 6.5% of the surface. In the UMPKeco-GTP structure, the mean value of the buried surface between two monomers in a position homologous to that of a UMPKeco-UMP dimer is 11.8% of the accessible surface. This value is higher than that calculated between two neighboring monomers from contiguous dimers, 8.0%. Since the buried surface is larger inside a dimer than between contiguous ones, the enzyme complexed to its allosteric effector can be considered as a trimer of dimers (colored blue, green, and red in Fig. 1), as for the UXP-bound structures. Also like those structures, the interactions between two monomers of a dimer (e.g. the cyan and dark blue ones in the right part of Fig. 1A) mainly involve the α3 helix, the longest secondary structure of the enzyme.

The GTP Binding Sites in the Hexamer—Whereas the substrates, UMP and ATP, are engulfed in a unique monomer (Fig. 1B), the positive regulator is inserted between two dimers of the enzyme hexamer. For instance, in the right part of Fig. 1A, the allosteric sites can be distant by more than 30 Å (27).

The Movements Induced by Binding GTP or Uridine Nucleotides—The UMPKeco-GTP hexamer closely resembles that of the ligand-free enzyme from the Gram-negative bacterium *Haemophilus influenzae*, which shares 75% sequence identity with UMPKeco. The root mean square deviation value between the monomers of each enzyme is 0.82 Å (on 229 Ca atoms), and that between the two hexamers is only 1.01 Å (on 1375 Ca atoms). These values are even lower than the root mean square deviation between the UMPKeco-GTP and UMPKeco-UTP hexamers (1.64 Å on 1278 Ca atoms). As compared with the previously published structures in complex with UXP (see Fig. 2A for UMPKeco-UTP, enzyme green), there is a rotation of the two monomers inside a dimer that makes it more open (Fig. 2A, GTP-bound enzyme red). This opening move of the dimers induced by GTP binding, and the opposite dimer closure induced by UDP binding are represented diagrammatically on Fig. 2B. When compared with the UTP-bound form, GTP binding induces an 11° rotation within the UMPK dimer (Fig. 2C) along an axis going through α3 helices and almost parallel to the hexamer 3-fold axis. This results in a movement...
of 6.7 Å of the Glu-194 α carbon from helix α6, the most distant residue from the rotation axis. In the structure of the nucleotide-free enzyme from H. influenzae, the dimer position is inter-
mediary between those of UMPKeco-GTP and UMPKeco-U TP. Thus, opposite rotations of the two monomers in a dimer are induced by GTP (opening) and UTP (closure). The GTP dimer opening results in a tighter dimer-dimer interaction, which is reflected by the increase (from 6.5 to 8%) in the per-
diment interaction results in a tighter dimer-dimer interaction, which is reflected by the increase (from 6.5 to 8%) in the percentage of the surface buried, as mentioned above.

At the monomer level, when comparing UMPKeco-GTP monomer to the previously published structures of the UXP-
bound enzyme, the most noticeable difference is the move of the α2 helix, opening the UMP-binding cavity (Fig. 2D). The largest Cα move is that of Gly-63 (6.3 Å induced by UMP bind-
ing), the N-terminal residue of α2, which can make a hydrogen bond with the ribose from the bound nucleotide. Kinetic exper-
iments have shown that 2’-deoxy-
UMP is not a substrate but a com-
petitive inhibitor (28). With this ligand, there is no induced fit, related to the 2’-hydroxyl of the nucleotide, that closes the active site, explaining why the catalysis cannot proceed. The superposition of UMPKeco-GTP monomer with that of the free enzyme from H. influenzae indicates no essential backbone difference in the phos-
phate acceptor binding site. Such a similarity is also observed for the more distant ATP-binding site. This is in accordance with the particular activation mechanism observed with GTP in the E. coli enzyme; GTP does not significantly change the $K_m$ or $V_m$ for either substrate (ATP or UMP), its actual effect being to reverse the inhibition by UTP or by excess of UMP.

**Residues Involved in GTP Binding**

The positive regulator is entrapped by enzyme subunits (cyan and pink in Fig. 3A) from two neighboring dimers (blue and red in Fig. 1A). The base moiety is also close to the N extremity of the α3 helix from the subunit dark blue in Fig. 1A. No direct hydrogen bond is established in that case, but there is a Van der Waals contact (the closest distance being 3.4 Å) between the guanine and Asn-72 side chain (indicated in Fig. 3A). As shown on Fig. 3B, the GTP-binding domain is restricted to several residues from 92 to 130, belonging mainly to the α3 helix, β3 strand, α4 helix, and β5 strand. For all six monomers (with minor dif-
ferences among monomers in the number of hydrogen bonds estab-
lished between the terminal amino groups of arginine and the atoms of the base), Arg-92 and Asp-93 are the residues interacting with the purine moiety of GTP. In addition, for all subunits the ring from Trp-119 is close from the guanine base and sandwiches it with the help of the His-96 imidazole ring. The only residue that interacts with the ribose is Arg-127, hydrogen-bonded to the 3’-OH. This is consistent with the observation that 2’-deoxy-GTP is still able to activate UMP (Fig. 3A). As shown on Fig. 3A, the GTP-binding domain is restricted to several residues from 92 to 130, belonging mainly to the α3 helix, β3 strand, α4 helix, and β5 strand. For all six monomers (with minor dif-
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The GTP-binding Site of E. coli UMP Kinase

acts with the β phosphate. Only few of the GTP-binding residues are conserved among various UMPKs, and the low conservation among Gram-negative and Gram-positive bacterial enzymes is noteworthy. Asp-93 appears as the most conserved residue, followed by Arg-103 and Arg-130. Furthermore, residues from UMPKeco that interact with GTP are not conserved in archaeal UMPKs.

Interaction between GTP and UTP Binding Sites as Revealed by Site-directed Mutagenesis

We previously showed that Thr-138 and Asn-140, which participate in the binding by UMPKeco of UMP as well as of UDP or UTP, are involved in a “cross-talk” between vicinal dimers (1). The substitution of Asn-140 by Ala suppressed the cooperativity of the inhibition caused by UTP but not its reversal by GTP (4). To further elucidate the structural basis of enzyme activity regulation by the two antagonist nucleotides, we examined the role of the hydrogen bonds implied in the formation of the dimer itself. Helix α3 (pink in Fig. 4A) forms most of the dimer interface. In the structures complexed with any of three uridine nucleotides, the two head-and-tail α3 helices from facing monomers are connected through hydrogen bonds between Asp-93 and Asn-72 at each extremity of these helices. As compared with the UTP-bound form of UMPKeco (green in Fig. 4B), binding of GTP induces a flipping of the Asn-72 side chain, which can no longer interact with the side chain of Asp-93 from the facing subunit (GTP-bound UMPKeco is red and superposes well with the nucleotide-free UMPKhi (gray)). This is correlated with a slightly different position of the two neighboring α3 helices, which appear farther apart from each other. The distance between α-carbons from the two terminal residues Arg-73 and Arg-97 of α3 (white labels in Fig. 4B) increases by 0.6 Å in UMPKeco-GTP as compared with UMPKeco-UTP. The substitution of Asn-72 and Asp-93 with Ala obtained by site-directed mutagenesis moderately altered the thermodynamic stability of UMPKeco. The Tm of the N72A variant was close to that of the reference UMPKeco. The Tm of the D93A variant was 10 °C lower than that of the reference enzyme, a shift comparable with that of the previously described N140A variant (1), and the Tm of the N72A/D93A variant was 15 °C lower. The kinetic constants of the modified variants (Table 2) indicated two significant differences as compared with the reference enzyme (D159N): (i) a loss of inhibition by excess of UMP of modified enzymes and, as a corollary,

### Table 2

| Kinetic parameters of three modified forms of UMPKeco obtained by site-directed mutagenesis |
|---------------------------------------------------------------|
| **UMPKeco** | Reference | N72A | D93A | N72A/D93A |
| UMP as fixed substrate (0.3 mM), no GTP | | | | |
| V_0 (units/mg) | 61.6 ± 1.9 | 95.6 ± 2.3 | 90.7 ± 1.3 | 78.7 ± 2.3 |
| K_m (mM) | 0.19 ± 0.03 | 0.59 ± 0.06 | 0.36 ± 0.03 | 0.59 ± 0.10 |
| UMP as fixed substrate (1 mM), 0.5 mM GTP | | | | |
| V_0 (units/mg) | 84.2 ± 2.8 | 105.4 ± 2.1 | 93.9 ± 2.5 | 76.1 ± 1.9 |
| K_m (mM) | 0.20 ± 0.02 | 0.40 ± 0.04 | 0.41 ± 0.05 | 0.66 ± 0.06 |
| ATP as fixed substrate (2 mM), no GTP | | | | |
| V_0 (units/mg) | 92.5 ± 1.9 | 67.2 ± 2.2 | 77.1 ± 2.1 | 52.7 ± 1.9 |
| K_m (mM) | 46.0 ± 4.2 | 32.1 ± 5.0 | 23.8 ± 3.2 | 24.0 ± 2.1 |
| K_I (mM) | 0.44 ± 0.03 | No inhibition | No inhibition | 10.9 ± 3.6 |
| ATP as fixed substrate (2 mM), 0.5 mM GTP | | | | |
| V_0 (units/mg) | 100.3 ± 2.2 | 95.8 ± 1.8 | 83.4 ± 2.2 | 51.4 ± 0.6 |
| K_m (mM) | 51.0 ± 4.4 | 47.8 ± 4.7 | 24.4 ± 4.0 | 24.6 ± 1.7 |
| K_I (mM) | No inhibition | No inhibition | No inhibition | No inhibition |
the absence of activation by GTP; (ii) a 2–3-fold increase in the apparent $K_m$ for ATP of modified UMPKs versus the reference enzyme. This relatively limited increase suggests that the overall structure of each individual monomer is conserved.

Then we explored the inhibition of the activity of N72A, D93A, and N72A/D93A mutants by UTP. The apparent $K_m$ for UMP of each variant was determined at constant concentrations of ATP and several constant concentrations of UTP (between 0.1 and 1.5 mM). The resulting values were then fitted to the equation, $K_m' = K_m (1 + [UTP]^{n_H}/K_{UTP})$. All three modified variants exhibited a 2 to 3 times higher $K_{UTP}$ than the reference UMPKeco, whereas the cooperativity of the inhibition by UTP appeared significantly altered in the case of the D93A mutant as compared with the reference enzyme (Table 3). A similar loss of the cooperativity of the inhibition by UTP was previously noticed with the N140A variant of UMPKeco (4).

Therefore, the D93A variant was tested for the reversal by GTP of the inhibition provoked by UTP (Table 4). The increase of the apparent $K_{ATP}$ and $K_{ATP}$ induced by UTP, was reversed by GTP in both reference UMPKeco and its N140A variant but not in the case of the D93A variant. This striking difference suggests that the binding sites for UTP and GTP are tightly “coupled” in both reference enzyme and N140A variant and “uncoupled” in the D93A mutant. Asp-93 appears as one of the key amino acids in the transfer of information from the regulatory site to the active site. This result also suggests that the communication between the GTP and the UTP sites is related primarily to the contacts between the subunits forming the dimer. The N72A mutation has less severe effects on enzyme activity regulation than the D93A substitution; this can be explained, since it still allows a hydrogen bond between its main chain and Asp-93, whereas the D93A substitution completely suppresses the related subunit-subunit hydrogen bonds (Fig. 4A).

What is the molecular mechanism of mutual exclusion between GTP and UTP? As inferred from kinetic experiments, GTP should hamper the interactions of the enzyme with the $\beta$- and $\gamma$-phosphates of UTP. The residues involved are Lys-15, Ser-17, Gly-18, Gly-58, and Arg-62. When comparing the structures of UMPKeco-GTP, UMPKeco-UTP, and the nucleotide-free UMPKhi (homologous residues Lys-12, Ser-14, Gly-15, Gly-55, and Arg-59), these residues have nearly identical positions, except Arg-62 (Fig. 5A). In the presence of GTP, Arg-62 points in a direction opposite to the one adopted when the enzyme binds UTP and also opposite to that of Arg-59 from H. influenzae enzyme. The position of the arginine in the complex with GTP is stabilized by a hydrogen bond with Gly-26. In the case of UMPKhi, the residue stabilizing Arg-62 is Glu-16. Taking in account that these two Gly and Glu residues are well conserved in bacterial UMPKs, we suggest that some structural rearrangement induced by GTP and probably involving Asp-93 would favor the particular orientation of Arg-62 side chain that is not compatible with an efficient binding of UTP.
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of Arg-62 in UMPKeco by a histidine resulted in a less active enzyme, which was no more inhibited by excess of UMP (29).

In addition, the dimer-dimer contacts induced by GTP could also intervene in UTP release. In particular, the side chains of Thr-144 and Asp-201 (residues conserved in bacterial UMP kinases) have a common orientation in the UMPKeco structures with UMP, UDP, or GTP but flip to a different orientation in the UMPKeco-UTP complex. Asp-201 variants of the enzyme have been studied in two Gram-negative bacteria: a D201N variant in E. coli (29) and a D201G variant in Salmonella typhimurium (30). Both variants were still inhibited by UTP but no more activated by GTP.

Differences in Regulatory Mechanisms between Gram-negative Bacteria, Gram-positive Bacteria, and Archaeal Organisms

The fold of bacterial UMPKs is related to that of the hexameric UMPKs of archaean origin (31, 32). Therefore, bacterial and archaean UMPKs represent a structurally homogeneous family. However, comparison of the kinetic properties of recombinant UMPKs from Gram-negative bacteria (4), Gram-positive bacteria (4, 9), and archaean (31, 32) indicated significant differences in the regulation of their activity by nucleotides. (i) With respect to ATP, UMPKs from Gram-positive organisms exhibit cooperative kinetics, whereas UMPKs from Gram-negative bacteria or archaean exhibited noncooperative kinetics. (ii) UTP is a common negative regulator for Gram-negative bacteria, Gram-positive bacteria, and archaean. However, whereas in Gram-negative organisms UTP interacts cooperatively with the UMP site, in Gram-positive organisms, this nucleotide seems to interact with a site that overlaps the GTP-binding site (4). Crystals of a Gram-positive UMP kinase complexed to UTP, the next challenge, should give interesting information on the allosteric site of this group. (iii) GTP appears to be the common positive effector for all investigated bacterial UMPKs from either Gram-positive or Gram-negative bacteria. However, UMPK from archaean are insensitive to activation by GTP, which they cannot bind (32). In order to better understand this difference at the structural level, we superposed the known crystal structures from several organisms. In the archaean enzyme (cyan in Fig. 5B), the C-terminal part of the α3 helix is one turn shorter than in bacterial UMPKs, and there is no equivalent of the β3 strand. Instead, there is a loop whose position is not compatible with the presence of the phosphates from a bound GTP. We suggest that this is the reason why GTP cannot bind to archaean enzymes. Therefore, the hexameric state of UMP kinases is not necessarily related to allosteric regulation by GTP. However, it is certainly important for regulation, as UTP is a competitive-cooperative inhibitor of UMPK from the archaean organism S. solfataricus (32).

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