End-Product Regulation and Kinetic Mechanism of Guanosine-Inosine Kinase from *Escherichia coli*

Hisashi KAWASAKI, Megumi SHIMAOKA, Yoshihiro USUDA, and Takashi UTAGAWA

Applied Microbiology Laboratory, Fermentation and Biotechnology Laboratories, Ajinomoto Co., Inc.,
1-1 Suzuki-cho, Kawasaki-ku, Kawasaki-shi 210-8681, Japan

Received November 10, 1999; Accepted January 19, 2000

*Escherichia coli* guanosine-inosine kinase was overproduced, purified, and characterized. The native and subunit molecular weights were 85,000 and 45,000, respectively, indicating that the enzyme was a dimer. A pI of 6.0 was obtained by isoelectric focusing. In addition to ATP, it was found that deoxyadenosine 5'-triphosphate, UTP, and CTP could serve as phosphate donors. The phosphate acceptors were guanosine, inosine, deoxyguanosine and xanthosine, but not adenosine, cytidine, uridine, or deoxycytidine. Maximum activity was attained at an ATP/Mg2+ concentration ratio of 0.5. In the presence of pyrimidine nucleotides, enzyme activity was slightly increased, while it was markedly inhibited by GDP and GTP. Initial velocity and product inhibition studies support an ordered Bi Bi mechanism in which guanosine was the first substrate to bind and GMP was the last product to be released. Guanosine kinase may be a regulatory enzyme that has a role in modulating nucleotide levels.

**Key words:** end-product regulation; kinetic mechanism; guanosine-inosine kinase; purine nucleotide; *Escherichia coli*

Guanosine-inosine kinase (ATP: guanosine 5'-phosphotransferase [E.C. 2.7.1.73]) catalyzes the phosphorylation of guanosine and inosine to their corresponding monophosphates by the following reaction:

\[ \text{Guanosine, Inosine} + \text{ATP} \rightarrow \text{GMP, IMP} + \text{ADP} \]

Eukaryotic and prokaryotic cells usually contain two ribonucleoside kinases, one specific for adenosine and the other specific for uridine and cytidine. *Escherichia coli*, *Salmonella typhimurium* and *Brevibacterium acetylicum* are exceptions to this rule because they have guanosine-inosine kinases instead of adenosine kinase.1,2,3

The use of purine nucleosides occurs through two pathways. One pathway involves purine nucleoside phosphorylase, which catalyzes the phosphorylation of ribonucleosides to the corresponding nucleobase and ribose 1-phosphate. The nucleobase is subsequently phosphorylated to its corresponding nucleoside monophosphate by purine phosphoribosyltransferase. The other pathway involves purine nucleoside kinase, which catalyzes the direct phosphorylation of purine nucleosides to the corresponding nucleoside 5'-monophosphate.

The guanosine-inosine kinase of *Brevibacterium acetylicum* was recently purified and characterized.3 This enzyme was activated by pyrimidine nucleotides and was inhibited by purine nucleotides. Therefore, regulation of guanosine-inosine kinase activity, if it occurred in vivo, could modulate intracellular nucleotide levels.

The gene encoding the *E. coli* guanosine-inosine kinase (gsk) has been cloned and its gene product, expressed in a recombinant strain, has been analyzed.4,5 Recent data have shown that a guanosine kinase feedback-insensitive mutant was extremely sensitive to imbalances in nucleotide pools,6 although studies have yet to be done with the purified enzyme. *B. acetylicum* guanosine-inosine kinase has only low sequence similarity with *E. coli* guanosine-inosine kinase in spite of their identity in the phosphate acceptor specificity.7 Therefore, we have compared their enzymatic properties in preparation for investigating their structure-function relationship, their physiological role, and their evolution.

**Materials and methods**

**Chemicals.** [14C]guanosine and [14C]inosine were purchased from Moravec Biochemicals (Brea, CA, USA). [γ-32P]ATP was from Amersham (Little Chalfont, Buckinghamshire, United Kingdom). The other nucleosides, nucleotides and acyclovir; 9-(2-hydroxyethyl)methyl-guanine (ACV) were obtained from

To whom correspondence should be addressed. Hisashi KAWASAKI, Tel: +81-44-244-7138; Fax: +81-44-244-4757; E-mail: hisashi_kawasaki@ajinomoto.com

**Abbreviations:** Guo, guanosine; Ino, inosine; dGuo, deoxyguanosine; Xia, xanthosine; Ado, adenosine; Cyd, cytidine; Urd, uridine; dThd; deoxythymidine; dATP, deoxyadenosine 5'-triphosphate; dGDP, deoxyguanosine 5'-diphosphate; dGTP, deoxyguanosine 5'-triphosphate; Gua, guanine; PAGE, polyacrylamide gel electrophoresis; IEF, isoelectric focusing; DTT, dithiothreitol
Sigma (St. Louis, MO, USA). DEAE-Toyopearl and Butyl-Toyopearl were obtained from Tosoh (Tokyo, Japan). Superdex 200pg and gel filtration calibration kit were from Pharmacia (Uppsala, Sweden).

**Bacterial strain, plasmid, medium, and buffers.** *E. coli* JM109 and plasmid pUC18 were used expressing the gsk gene. LB medium was used for culturing *E. coli*. The following buffers were used: 50 mM Tris-HCl (pH 8.0), 1 mM DTT (Buffer A); 50 mM Tris-HCl (pH 7.8), 1 mM DTT, 100 mM KCl, 20% (v/v) glycerol (Buffer B).

**Enzyme assay and measurement of protein.** The standard reaction mixture contained 100 mM Tris-HCl (pH 7.4), 300 mM KCl, 10 mM MgSO4, 5 mM ATP, 0.2 mM [32P]inosine or 0.02 mM [14C]guanosine and an enzyme preparation. The enzyme concentration was 35.6 mg of protein per ml when guanosine was used as the phosphate acceptor and 3.56 g protein per ml when inosine was the phosphate acceptor. The reaction mixture was incubated for 2 min at 30°C. Then 2 g of the reaction mixture was spotted onto an Art 5715 silica gel plate (Merck, Whitehouse, NJ, USA), developed in an eluent of 1-butanol-ethanol-water (2:1:1), and air-dried. To identify the phosphorylation site, [γ-32P]ATP was used as a phosphate donor. The reaction mixture was spotted onto a PEl-cellulose plate (Merck, Art 5725) and developed in an eluent that contained 0.5 M LiCl and 1 M formic acid. Radio-labeled compounds were detected and measured using a BAS2000 Bio-Image analyzer (Fujiﬁlm, Tokyo, Japan). The protein concentration was measured with a protein assay kit (Bio-Rad, Hercules, CA, USA) using bovine serum albumin as the standard.

**Overproduction of *E. coli* guanosine-inosine kinase.** The *E. coli* gsk gene was amplified by PCR using 5′-GGCTGCAGCCATGAATTTCGCCGTAACCG-3′ as the upstream primer (5′-flanking Pst I restriction site) and 5′-GGAGCTTAACGATCCCAGTAGACTCTTC-3′ as the downstream primer (5′-flanking Hind III restriction site). The PCR was done as described by Sambrook *et al.* The PCR product was digested with *Pst* I and *Hind* III, and cloned into pUC18 digested with *Pst* I and *Hind* III to generate the plasmid pUCgsk. The synthetic *trp* operon promoter was inserted between the *BamH I* site and the *Pst* I site of pUCgsk. This promoter contained the *trp* operon Shine-Dalgarno sequence. *E. coli* JM 109 transformed with the resulting plasmid was cultured in 50 ml of LB medium containing 100 µg/ml of ampicillin for 12 h at 37°C. The culture broth was transferred to 11 of LB medium containing 100 µg/ml of ampicillin in a jar fermentor and was cultured for 8 h at 37°C, with the pH kept at 6.5.

**Purification procedure.** Unless otherwise stated, all the operations were done at 4°C. Fifty grams (wet weight) of frozen cell pellet was suspended in Buffer A containing 100 mM KCl and disrupted with glass beads using a bead beater (Biospec, Bartlesville, OK, USA). Cell debris was removed by centrifugation. Solid ammonium sulfate was added to the supernatant to give 50% saturation and the precipitate was collected by centrifugation. The precipitate was dissolved in Buffer A containing 100 mM KCl and put on a DEAE-Toyopearl column (5 × 35 cm) equilibrated with Buffer A containing 100 mM KCl. Elution was done with a linear gradient of KCl (100 mM to 400 mM) in Buffer A. Sodium ammonium sulfate was added to the active fractions to give 20% saturation and the precipitate was removed by centrifugation. The supernatant was put onto a Butyl-Toyopearl column (2.5 × 50 cm) equilibrated with Buffer A containing 100 mM KCl and 20% ammonium sulfate. To elute the enzyme, a linear gradient from 20% to 0% ammonium sulfate in Buffer A containing 100 mM KCl was used. Solid ammonium sulfate was added to the active fractions at a saturation of 50% and the precipitate was collected by centrifugation. The precipitate was dissolved in Buffer B and dialyzed against the same buffer.

**Gel filtration column chromatography.** To estimate the apparent native molecular weight, gel filtration was done on a Superdex 200pg column (1.6 × 60 cm) equilibrated with Buffer A containing 300 mM KCl. Two hundred microliters of purified protein solution (12 mg/ml) was put on the column and developed with the same buffer. The molecular weight markers were aldolase, albumin, ovalbumin, chymotrypsinogen A, and ribonuclease A.

**Electrophoresis and isoelectric focusing.** SDS-PAGE was done essentially as described by Laemmli on 4–20% polyacrylamide gradient gel. The isoelectric point was estimated by isoelectric focusing (IEF) under non-denaturing conditions (pH range 3–10). Carbonic anhydrase II, carbonic anhydrase I, and lentinlectin were used as the pl markers. Gels were stained with Coomassie brilliant blue or with silver nitrate.

**N-terminal sequencing.** The N-terminal amino acid sequence of the purified protein was analyzed by automated Edman degradation using a gas-phase protein sequencer (model 477A protein sequencer; Perkin-Elmer, Foster City, CA, USA)

**Results**

**Overproduction, purification, and characterization of *E. coli* guanosine-inosine kinase**

The high level of overproduction of guanosine-inosine kinase...
sine kinase enabled us to purify 300 mg from 50 g of wet cells using the procedure summarized in Table 1. A highly purified protein was obtained, as shown in Fig. 1A and B. SDS-PAGE indicated a molecular weight of around 45,000, which was approximately the same as that calculated from the sequence, but gel filtration yielded a molecular weight of about 85,000 (data not shown). The first five N-terminal amino acid residues of the purified protein were MKFPQ by automated Edman degradation sequencing. This sequence corresponds to the deduced N-terminal amino acid sequence. These results indicate that the enzyme was a dimer. The isoelectric point was 6.0 by IEF (Fig. 1B). Initial velocity studies were done using the purified enzyme. As shown in Fig. 2, A and B, measurements of initial velocity as a function of the concentration of Mg-ATP or guanosine (at various fixed concentrations of guanosine and Mg-ATP) generated parallel line patterns. For a two substrate reaction, this pattern is characteristic of a ping-pong mechanism. However, product inhibition studies reported below rule out a ping-pong mechanism and support instead an ordered Bi Bi mechanism (The cause of this discrepancy is discussed in later.). The $K_m$ for guanosine (Guo), inosine (Ino) and ATP were 7.8 $\mu$M, 1.5 $\mu$M and 0.49 $\mu$M respectively. These values agree well with those reported previously. The $K_m$ for deoxyguanosine (dGuo) was 4.3 $\mu$M. ATP, deoxyadenosine 5'-triphosphate (dATP), UTP, and CTP could all serve as phosphate donors for this enzyme, while GTP, ADP, AMP, and $p$-nitrophenyl phosphate could not (Table 2). Phosphate acceptor specificity was examined using $[\gamma^{32}P]ATP$ as the phosphate donor. In addition to Guo, dGuo, and Ino, xanthosine (Xao) could also serve as a phosphate acceptor, but its efficiency was only about 30% that of inosine. Adenosine (Ado), uridine (Urd), cytidine (Cyd), and deoxycytidylic acid (dThd) could not act as phosphate acceptors. Furthermore, a nucleoside analogue, ACV, which has a guanine (Gua) moiety, could not act as a phosphate acceptor.

**Effect of ATP concentration on the activity of E. coli guanosine-inosine kinase at several fixed concentrations of Mg$^{2+}$**

Guanosine-inosine kinase of *Brevibacterium acetyllicum* has maximal activity when the ATP/Mg$^{2+}$ concentration ratio is close to 1. The effects of changes in the ATP concentration on the activity of the *E. coli* enzyme were examined in the presence of Mg$^{2+}$ (1 mM to 20 mM). When the Mg$^{2+}$ concentration was 1 mM, phosphorylation of inosine was not observed. The maximum activity was attained when the ATP/Mg$^{2+}$ concentration ratio was 0.5 (Fig. 3). Figure 3 also shows that Mg$^{2+}$ did not inhibit the enzyme while free ATP inhibited it.

**Effects of nucleotides on the activity of E. coli guanosine-inosine kinase**

*Brevibacterium acetyllicum* guanosine-inosine kinase was activated by pyridine nucleotides, especially CMP and CTP, while it was inhibited by AMP, ADP, and GMP. *E. coli* guanosine-inosine kinase was also slightly activated by pyridine nucleotides and inhibited by ADP (Table 3). Guanine nucleotides, particularly GDP and GTP which are distal

![Fig. 1. SDS-PAGE and IEF of Purified Guanosine-inosine Kinase from *E. coli.*](image)

(A) SDS-PAGE of purified *E. coli* guanosine-inosine kinase 0.54 micrograms of purified enzyme was run on 4–20% gradient polycrylamide gel and stained with silver nitrate (lane 2). Molecular weight markers (lane 1) are myosin (200 k), $\beta$-galactosidase (116 k), phosphorylase B (97.4 k), bovine serum albumin (66.3 k), glutamic dehydrogenase (55.4 k), lactate dehydrogenase (36.5 k), carbonic anhydrase (31.0 k), trypsin inhibitor (21.5 k), lysozyme (14.4 k), aprotinin (6.0 k).

(B) IEF of purified *E. coli* guanosine-inosine kinase A 4.7-microgram sample of purified enzyme was put on the gel at a pH range of 3 to 10 and stained with Coomasie brilliant blue (lane 1). The pI markers (lane 2) are carbonic anhydrase I (5.9), carbonic anhydrase I (6.6), and lentil lectin (7.2, 8.2, 8.8).

**Table 1. Summary of the Purification of the Protein from Recombinant**

<table>
<thead>
<tr>
<th>Purification procedure</th>
<th>Total amount of protein (mg)</th>
<th>Specific activity ($\mu$mol min$^{-1}$ (mg of protein)$^{-1}$)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>4590</td>
<td>13.3</td>
<td>100</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>3440</td>
<td>16.8</td>
<td>95</td>
</tr>
<tr>
<td>fractionation</td>
<td>936</td>
<td>30.6</td>
<td>47</td>
</tr>
<tr>
<td>DEAE-Toyopearl</td>
<td>302</td>
<td>40.3</td>
<td>20</td>
</tr>
</tbody>
</table>


Fig. 2. Initial Velocity Studies in the Absence of Products.

(A) Effects of Guo concentration on the reaction rate, with various fixed concentrations of ATP. The assay was done as described in Materials and methods. ATP concentrations: ○, 0.25 mM; ●, 0.5 mM; ◆, 1.0 mM; ▲, 2.0 mM. (B) Effects of ATP concentration on the reaction rate, with various fixed concentrations of Guo. Guo concentrations: ○, 2 μM; ◆, 5 μM; ○, 10 μM; ▲, 20 μM.

Table 2. Phosphate Donor Specificity

<table>
<thead>
<tr>
<th>Phosphate Donor</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>ATP</td>
<td>100</td>
</tr>
<tr>
<td>GTP</td>
<td>19</td>
</tr>
<tr>
<td>CTP</td>
<td>12</td>
</tr>
<tr>
<td>dATP</td>
<td>34</td>
</tr>
<tr>
<td>ADP</td>
<td>0</td>
</tr>
<tr>
<td>AMP</td>
<td>0</td>
</tr>
<tr>
<td>p-Nitrophenyl phosphate</td>
<td>0</td>
</tr>
</tbody>
</table>

Kinase activity was measured as described in Materials and methods using guanosine as the phosphate acceptor and with the purified enzyme preparation at a concentration of 35.6 ng/ml.

Table 3. Effects of Nucleotides on the Activity of E. coli Guanosine-inosine Kinase

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>AMP</td>
<td>100</td>
</tr>
<tr>
<td>ADP</td>
<td>88</td>
</tr>
<tr>
<td>GMP</td>
<td>80</td>
</tr>
<tr>
<td>GDP</td>
<td>4</td>
</tr>
<tr>
<td>GTP</td>
<td>12</td>
</tr>
<tr>
<td>CMP</td>
<td>121</td>
</tr>
<tr>
<td>UMP</td>
<td>118</td>
</tr>
<tr>
<td>dTMP</td>
<td>117</td>
</tr>
</tbody>
</table>

The assay was done as described in Materials and methods except for adding each nucleotide to a final concentration of 2 mM.

end-products, inhibited the E. coli enzyme (Table 3) despite having little effect on the guanosine-inosine kinase of B. acetylicum.

Fig. 3. Effects of ATP Concentration on the Activity of E. coli Guanosine-inosine Kinase at Several Fixed Concentrations of Mg²⁺.

Inosine was used as a phosphate acceptor. The assay was done as described in Materials and methods. The Mg²⁺ concentration was 1 mM (▲), 5 mM (○), 10 mM (◇), or 20 mM (△).

Product inhibition studies

Product inhibition studies were done in which either substrate was varied while holding the second substrate at a non-saturating concentration. Inhibition of guanosine-inosine kinase by GMP, while guanosine concentration was maintained constant at variable concentrations of Mg-ATP, produced a mixed inhibition pattern. Inhibition of guanosine-inosine kinase by GMP, when Mg-ATP concentration was maintained constant while guanosine was varied,
showed a competitive inhibition pattern. Inhibition of guanosine-inosine kinase by ADP, when guanosine concentration was maintained constant while Mg-ATP was varied, also showed mixed inhibition. Inhibition of guanosine-inosine kinase by ADP, when Mg-ATP concentration was maintained constant while guanosine was varied generated mixed inhibition. The absence of competitive inhibition between guanosine and ADP indicated that the enzyme does not follow a ping-pong mechanism.

The inhibition patterns and kinetic constants are summarized in Table 4. These data suggest an ordered Bi Bi mechanism in which Guo is the first substrate to bind and GMP is the last product to be released (Scheme 1).

**Effects of distal end-products on enzyme activity**

As noted above, the distal end-products GDP and GTP both inhibited the enzyme. The effects of changes in the GDP and GTP concentration on inhibition were examined. As shown in Fig. 4, inhibition by GDP was stronger than that by GTP. Since GDP is a precursor of GTP, deoxyguanosine 5'-diphosphate (dGDP), and deoxyguanosine 5'-triphosphate (dGTP), it is likely that GDP is an important distal end-product in the regulation of guanine nucleotide biosynthesis. Kinetic studies showed that inhibition by GDP was non-competitive with Mg-ATP at the non-saturating Guo concentrations (data not shown). As was the case for GMP, GDP may behave as a bisubstrate analog. In contrast to this, inhibition by GTP was competitive with ATP at non-saturating Guo concentrations (data not shown), suggesting that GTP may only bind to the ATP-binding site.

**Discussion**

The high level of overproduction of *E. coli* guanosine-inosine kinase allowed us to purify 300 mg from 50 g of wet cells. With our purification procedure, the yield of activity was about 20% and enzyme activity was stable under the conditions used. Therefore, this enzyme accounted for 30% of the total cellular protein, considering that the yield of protein was 6.5% after purification. The purified enzyme showed a single band on SDS-PAGE and IEF. Moreover, N-terminal amino acid sequencing gave a single sequence. Accordingly, we concluded that a highly purified protein was obtained. SDS-PAGE indicated a molecular weight of around 45,000, which was approximately the same as that calculated from its sequence, but gel filtration gave a molecular weight of about 85,000 (data not shown). These results indicate that the enzyme is a homodimer.

Guo, dGuo, Ino, and Xia acted as phosphate acceptors, while Ado, Cyd, Urd, and dThd did not. Consequently, the hydroxyl group at the 6-position of the purine base appears to be essential for substrate recognition by the enzyme. Since there was a large difference between the $K_m$ for Guo, dGuo, and Ino, the hydroxyl group at the 2'-position of ribose and the amino group at the 2-position of the purine were presumed to interact with the enzyme. The nucleoside analogue ACV, which had a Guo moiety, could not serve as a phosphate acceptor. Therefore, the ribose part of the nucleoside is required for phosphotransferase activity. These data suggest that Guo is the physiological substrate of the enzyme.

The maximum activity was achieved when the
ATP/Mg\(^{2+}\) concentration ratio was 0.5. When the Mg\(^{2+}\) concentration was 1 mM, phosphorylation of inosine was not observed. As the Mg\(^{2+}\) concentration was increased in the presence of a constant ATP concentration, the velocity of the reaction also increased. This result was opposite to that for guanosine-inosine kinase from plant mitochondria\(^{10}\) and adenosine kinase from several sources,\(^{12,13,14,15}\) which were inhibited by excess Mg\(^{2+}\). Although the reason for the difference in the effects of Mg\(^{2+}\) among several purine nucleoside kinases is unknown, these data suggest that the Mg-ATP complex is probably the substrate and that Mg\(^{2+}\) was required for enzyme activity in addition to its role in forming the Mg-ATP complex.

Pyrimidine nucleotides increased the enzyme activity but had less effect compared with their influence on the guanosine kinase of \textit{B. acetylicum}.\(^{11}\) This difference may have been caused by a difference in the effector concentrations. The guanosine kinase of \textit{B. acetylicum} was stimulated at a low concentration (0.1 mM) of CMP and CTP, and was only slightly stimulated at the concentration of 1 mM.

Our studies of initial velocities of \textit{E. coli} guanosine-inosine kinase as a function of the concentration of one substrate, at fixed concentrations of the other substrate generated a pattern of parallel lines (Fig. 2), which is considered to be characteristic for a ping-pong mechanism.\(^{17}\) However, as discussed previously,\(^{18}\) parallel or near parallel patterns could be explained by the loss of a constant term in the rate equation for a Bi Bi sequential mechanism, because of a very low dissociation constant of the first substrate to bond. Indeed, several cases were reported in which parallel patterns had been observed in spite of acting via a sequential mechanism.\(^{17,18,19,20}\) In the kinetic mechanism of this enzyme shown in Scheme I, this substrate, which has a very low dissociation constant, should be Guo.

Product inhibition studies showed that this enzyme operates via an ordered sequential Bi Bi mechanism in which Guo is the first substrate to bind and GMP is the last product to be released. This conclusion suggests that the enzyme has two substrate binding sites, a phosphate acceptor binding site and a phosphate donor site. Since the phosphate donor and phosphate acceptor substrates have a common structure, GMP could not only bind to the phosphate acceptor site but also to the phosphate donor site.

There are several reports on the kinetic mechanism of nucleoside kinases. Adenosine kinase from several sources followed an ordered Bi Bi mechanism\(^{12,14,20,21,22,23}\) except for one which followed a ping-pong mechanism.\(^{13}\) Guanosine kinase from \textit{Trichomonas vaginalis} was reported to follow a ping-pong mechanism.\(^{20}\) Deoxyguanosine kinase from mammalian mitochondria and from \textit{Lactobacillus acidophilus} had been shown to follow a random Bi Bi mechanism,\(^{25,26}\) while the deoxyadenosine kinase from \textit{Lactobacillus acidophilus} has an ordered mechanism.\(^{28}\) Why various reaction mechanisms have been reported for purine nucleoside kinase is not apparent. There is a complexity in the kinetics of purine nucleoside kinase that is caused by the fact that the reaction that it catalyzes involves closely similar substrates and products.

Guanosine-inosine kinase is proposed to be a member of the ribokinase family based on sequence alignments.\(^{27}\) Recently, the three-dimensional structure of \textit{E. coli} ribokinase was analyzed.\(^{28}\) Ribokinase has been shown to have two substrate binding sites, one for phosphate acceptor, ribose and the other for the phosphate donor, ATP. Recently, the three-dimensional structure of human adenosine kinase has been analyzed.\(^{29}\) This is the first three-dimensional structure of a purine nucleoside kinase. Interestingly, the overall structure of human adenosine kinase is similar to the structure of ribokinase from \textit{E. coli} in spite of the low amino acid sequence identity between them (only 22%). Adenosine kinase has also been suggested to have two substrate binding sites. These two sugar kinases have a consensus sequence which is found in all adenosine kinases, ribokinase, fructokinase, deoxyglucokinase, and guanosine kinase: DT(A,S,P)T(N,I)G(A)AGD. The second Asp residue interacts with Mg\(^{2+}\) ion that exists in a trough between the substrate binding sites. The guanosine-inosine kinase from \textit{E. coli} may have a similar three-dimensional structure to human adenosine kinase and \textit{E. coli} ribokinase, and if so, it has two substrate binding sites. Our kinetic data obtained in this study suggested guanosine-inosine kinase from \textit{E. coli} has two substrate binding sites and forms a ternary complex comprising a phosphate acceptor, enzyme, and phosphate donor.

Recently, \textit{E. coli} guanosine-inosine kinase was suggested to be subject to inhibition by GTP and a feedback-insensitive mutant was extremely sensitive to imbalances in nucleotide pools, although studies have yet to be done with purified enzyme.\(^{30}\) The data shown in this paper which was obtained with purified enzyme support these results. However, the enzyme is inhibited not only by GTP but also by GDP. Guanosine-inosine kinase from plant mitochondria,\(^{11}\) deoxyadenosine / deoxyxycytidine kinase from \textit{Bacillus subtilis} \(^{30}\) and deoxyguanosine kinase from mammalian mitochondria\(^{30}\) are also inhibited by their distal end-product, nucleoside triphosphate. In contrast to these enzymes, the nucleoside diphosphate GDP was found to be the most powerful inhibitor of \textit{E. coli} guanosine-inosine kinase among those we tested. GDP at a concentration of 1 mM reduced enzyme activity to 14% of the control level. Such a concentration of GDP is physiologically possible \textit{in vivo}.\(^{31}\) Since GDP is a precursor of GTP, dGDP, and dGTP, it is likely that GDP is a major distal end-product involved in the regulation of gua-
nine nucleotide biosynthesis in *E. coli*. Our kinetic study showed that inhibition by GDP was non-competitive with Mg-ATP in the presence of non-saturating Guo concentrations. As was the case for GMP, GDP may behave as a bisubstrate analog. The nucleoside moiety of this nucleotide could bind to the nucleoside site on the enzyme, while its phosphate groups could overlap and bind to the phosphate donor site. In contrast, inhibition by GTP was competitive with ATP even at the non-saturating Guo concentration. Though this suggests that GTP may only bind to the ATP binding site, GTP might also bind to the nucleoside site via its nucleoside moiety.

Considering the data obtained in this study, such as the low $K_a$ for Guo, the ordered sequential Bi Bi mechanism in which Guo is the first substrate to bind, and the distal end-product inhibition, it seems likely that guanosine kinase might be regulated *in vivo* by changes of nucleotide concentrations and may modulate the nucleotide levels efficiently.

**Acknowledgments**

We thank O. Hasegawa for operation of the jar fermentor. We thank M. Asano for operation of the protein sequencer.

**References**


