SCO5059, encoded in *Streptomyces coelicolor* A3(2), was identified as a polyphosphate glucokinase. The \( K_m \) values of SCO5059 for glucose and polyphosphate (poly(P)) were estimated to be 12 and 4 \( \mu M \), respectively, and the \( k_{cat} \) value was 0.3 s\(^{-1}\) at pH 7.7 at 28°C. SCO5059 homologs are highly conserved among *Streptomyces*, and can work as polyphosphate glucokinase as well.

**Key words:** polyphosphate glucokinase; SCO5059; *Streptomyces coelicolor* A3(2); glucose 6-phosphate

The glucokinase (Glk) catalyzes the phosphorylation of glucose to yield glucose 6-phosphate (G6P) with a phosphoryl donor such as ATP and/or inorganic polyphosphate (poly(P)). Because Glk is an important initiation enzyme in glucose catabolism (glycolysis), regulation of the expression of glk genes and the biochemical properties of Glks have important effects on the metabolism of a living system. In *Streptomyces*, Glks are believed to be key proteins in carbon catabolite repression (CCR), an important regulatory system that responds to environmental carbon sources. The *Streptomyces* are Gram-positive soil-dwelling filamentous eubacteria with high GC-content genomes. They are industrially important as producers of antibiotics, antitumor agents, immune-suppressants, and insecticides. For these reasons, the regulation of glk gene expression and the biochemistry studies of Streptomycete Glks, have been extensively investigated.

ATP-dependent glucokinases (ATP-Glk, GlkA) from *Streptomyces coelicolor* and *Streptomyces peucetius* have been characterized biochemically, including detailed kinetics. In addition, the crystal structure of ATP-Glk derived from the streptomycin producer *Streptomyces griseus* (SgGlkA, SGR\_5377) has been solved, revealing that its substrate recognition mechanism and conformational changes depend on the substrates glucose and ATP. The expression of the glkA gene in *S. coelicolor* has been extensively analyzed, revealing its significant effect on the regulation of many other genes. Less attention, however, has been paid to the polyphosphate glucokinases (PP-Glks) derived from *Streptomyces*. To date, only one investigation of *Streptomyces aureofaciens*, a chlorotetraycline-producing strain, has indicated that the roles of ATP-Glk and PP-Glk are physiologically important. PP-Glk activity was found to correlate with the production of chlorotetraycline, but PP-Glk derived from *Streptomyces* has never been purified or biochemically characterized.

Here, we report on a characterization of SCO5059, derived from *S. coelicolor* A3(2) as a PP-Glk (Scheme 1). Its homologous genes are completely conserved in all genomic sequences reported thus far for *Streptomyces*. Examples include SAV\_3209 (84% identity, 91% positives), and SGR\_2471 (84% identity, 88% positives).

We cloned the sco5059 gene by PCR. The chromosomal DNA of *S. coelicolor* A3(2) served as template, and we expressed the gene in *Escherichia coli* (see experimental detail, in Supplemental Information; *Biosci. Biotechnol. Biochem*. Web site). The recombinant N-terminal His-tagged SCO5059 was purified by Talon metal affinity chromatography, followed by PD-10 gel-filtration for removal of imidazole (Supplemental Fig. 1). Glucose (15.0 mM) and 2 mM of (NaPO\_4)\(_2\) served as template, and the reaction mixture was treated with 0.5 mM of NAD\(^+\) and 1 U of glucose 6-phosphate dehydrogenase, in order to observe NADH formation (\( \varepsilon_{340} = 6220 \text{ M}^{-1} \text{ cm}^{-1} \)), which is dependent on G6P generation by SCO5059. As expected, we found that SCO5059 catalyzed the phosphorylation of glucose with polyphosphate as phosphoryl donor. To optimize...
Characterization of Polyphosphate Glucokinase SCO5059

![Scheme 1. Polyphosphate Glucokinase Reaction Catalyzed by SCO5059.](image)

The state of association for SCO5059 in solution was analyzed by size-exclusion chromatography (Superdex™ 200). Under standard chromatography conditions (50 mM Tris buffer pH 7.5, containing 150 mM of KCl and 5% glycerol), a broad peak was observed around 200 kDa (Supplemental Fig. 3). As the size of the monomer for the N-His tagged SCO5059 was only 28 kDa, it appeared that the protein was oligomerized. Although the precise mechanism of oligomerization is unclear, the quaternary structural changes and conformational changes in each unit in the presence of ATP, glucose, and polyphosphate, might have an effect on Glk activity. Further structural analysis of this important class of Glks is necessary to determine their biochemical behavior.

Multiple sequence alignments indicated that SCO5059 is homologous to a family of PP-Glks including RV2702 and Tfu1811 and that it has the same conserved amino acid residues, which have been found to be both substrate recognition and catalytic motifs (Supplemental Fig. 4). The crystal structure of a PP-Glk derived from Arthrobacter sp. strain KM (Polyphosphate/ATP-glucomannokinase) has confirmed the catalytic importance of these amino acid residues. 18)

In summary, we characterized SCO5059 as a PP-Glk derived from S. coelicolor A3(2) for the first time. SCO5059 had kinetic values comparable to well-known ATP-Glks, highlighting the importance of this family of enzymes in the Streptomyces genus. Additional detailed biochemical studies with longer polyphosphates and genetic analysis of the SCO5059 family of PP-Glks in Streptomyces are necessary for a greater understanding of the processes involved in glycolysis and carbon catabolite repression.

### Table 1. Kinetic Parameters of SCO5059 at pH 7.7 at 28°C

<table>
<thead>
<tr>
<th>Glucose</th>
<th>(NaPO₄)₆h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kₘ [mM]</td>
<td>(1.24 ± 0.038) × 10⁻²</td>
</tr>
<tr>
<td>kₚ [s⁻¹]</td>
<td>0.25 ± 0.04</td>
</tr>
</tbody>
</table>

The reaction conditions, various pH values and reaction temperatures were examined (Fig. 1). Maximum PP-Glk activity was found at pH 9.5–10 at 28 °C. At higher temperatures, SCO5059 showed signs of aggregation and was less active. The high pH preferred by SCO5059 has been observed for other PP-Glks. 14) We conducted kinetic experiments using various concentrations of glucose or (NaPO₄)₆ under standard reaction conditions (pH 7.7, 28 °C) (Table 1). The Kₘ value of SCO5059 with glucose, 12.4 μM, was found to be smaller than those of other PP-Glks such as SgGlkA (SGR5377), for which Kₘ = 207 μM has been reported. 13) On the other hand, the kₚ value of SCO5059 (0.3 s⁻¹, pH 7.7, 28 °C) was similar to that of SgGlkA, kₚ = 0.5 s⁻¹ (pH 8.0, 30 °C). 11,12) These kinetic values indicate that SCO5059 can use low concentrations of glucose throughout the stationary phase of growth (during which secondary metabolites such as antibiotics are produced) in Streptomyces. Thus the kinetic behavior of SCO5059 as a PP-Glk corresponds to earlier observations made of PP-Glk activity in S. aureofaciens. 13) It is possible, therefore, that the PP-Glk activity of SCO5059, in combination with an ATP-Glk such as SCO2126, a well-characterized Glk in S. coelicolor A3(2), contributes to CCR.

We also tested the ATP-Glk activity of SCO5059 with various concentrations of ATP and a fixed concentration of glucose. As shown in Supplemental Fig. 2, sigmoidal kinetic behavior dependent on the ATP concentration was observed. At lower concentrations of ATP (<1.5 mM), ATP-Glk activity disappeared. This suggests that the ATP-Glk activity of SCO5059 is positively controlled by ATP through allosteric interaction. Thus this enzyme might be a key player regulating the concentrations of ATP and polyphosphate through glucose.

![Fig. 1. Effects of pH and Temperature on the PP-Glk Activity of SCO5059.](image)
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References