Sequence of the Gene Encoding Argininosuccinate Synthetase in *Streptomyces coelicolor* A3(2)

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Argininosuccinate synthetase (E.C.6.3.4.5) catalyzes the penultimate step of the arginine biosynthetic pathway. In *Streptomyces*, the enzyme is encoded by argG gene of which mutation occurs at high frequencies spontaneously or by mutagenesis. *argG* mutation has been known to involve the deletion of a DNA sequence spanning over 10 kb which results in pleiotropic phenotype changes such as loss of aerial mycelium formation, deprivation of carbon and nitrogen catabolite repression and increase in chloramphenicol sensitivity. *Streptomyces* species, thus, have tendency to require arginine or argininosuccinate for their growth, and this auxotrophy should not return to prototrophy by mutation.

We have been studying *argG* mutation in *Streptomyces* and have cloned genes, which complemented the *argG* mutation of *S. lividans*, from *S. coelicolor* A3(2) M130 and *S. lividans* 1326. In this paper, we describe the nucleotide sequence of a 2.2 kb fragment containing *argG* gene cloned from *S. coelicolor* A3(2) in order to characterize *argG* gene in comparison with those from other bacteria, yeast and human.

To determine nucleotide sequence, the 2.2 kb fragment in a plasmid pMCP25 was subcloned into pUC18 (Takara Shuzo Co., Kyoto, Japan). Plasmid DNAs were prepared by alkaline lysis method. Nucleotide sequence was determined for both strands by the methods of Sanger using a reaction kit and reverse primer DNAs (Takara Shuzo Co.) and Maxam and Gilbert.

Figure 1 shows the nucleotide sequence of the 2.2 kb fragment as well as the deduced amino acid sequence of the putative argininosuccinate synthetase. A potential protein-coding sequence was predicted by the computer-aided "FRAME" analysis developed by Bamb et al. The analysis revealed only one large ORF extending 1461 nucleotides (nt317-1777). This ORF turned out to contain five possible translational start codons; ATG at nt317-319, 320-322, 335-337 and 425-427, and GTG at nt500-502. In any case, however, no complementary sequence to the 3' end of the 16S rRNA of *S. coelicolor* A3(2) was found. We, therefore, assumed ATG at nt317-319 as the translational start codon and the resulting largest peptide of 487 amino acid residues (53,615 Da) as the *Streptomyces* argininosuccinate synthetase. In the upstream region, direct and inverted repeat sequences were found as indicated by the arrows. In the downstream of the stop codon at nt1778-1780, there is an inverted repeat (ΔG = -6.81 kcal/mol) which may possibly serve as a rho-independent transcriptional terminator. The sequence was deposited in DDBJ, Genbank and EMBL Databases as an accession number of D00799.

The putative argininosuccinate synthetase was then analyzed for its similarity to the corresponding enzymes of different origins by using the algorithm of Needleman and Wunsch (GAP program). As shown in Fig. 2, significantly high levels of similarity were recognized among them at amino acid level. The *S. coelicolor* argininosuccinate synthetase showed similarities of 77.2% with *E. coli* (44.2% with *Methanosarcina barkeri*, 44.3% with *Methanococcus vannielii*, 48.4% with *human* and 47.0% with *Saccharomyces cerevisiae*), respectively. It was thus obvious that the *S. coelicolor* enzyme is most similar to the *E. coli* enzyme. Over the whole amino acid sequences, three boxed regions in Fig. 2 were recognized to be highly conserved as assumed to be involved in the substrate binding. It seems likely that these six enzymes have been evolved from a common ancestor.

The protein encoded by *argG* gene was produced in *E. coli* using mini cells and its size was estimated by SDS-polyacrylamide gel electrophoresis followed by autoradiography (Fig. 3). The mini cells were prepared by the method described and the proteins were synthesized in the presence of [35S]methionine. Consequently, a protein with Mr of 57,000 ± 4,000 Da was detected when *E. coli* mini...
Fig. 1. Nucleotide sequence of the argG gene of *Streptomyces coelicolor* and the deduced amino acid sequence of *A* -aspartate:inosinate synthetase. The arrows in the upstream region indicate direct and inverted repeat sequences. The *heavy* arrows (nt1796-1812) downstream of the stop codon mark an inverted repeat (ΔG = -6.81 kcal/mol).
Fig. 2. Comparison of argininosuccinate synthetases from different sources. The entire amino acid sequences of S. coelicolor (this paper), E. coli, M. barkeri, M. vanillielii, human and S. cerevisiae argininosuccinate synthetase are aligned by introducing gaps as indicated by dot. Numbers refer to the position of the corresponding amino acid residues in each enzyme. Symbols · · · · above the sequences indicate that comparison values are the same, greater or equal to 0.5 and 0.11, respectively.

A: S. coelicolor, B: E. coli, C: M. barkeri, D: M. vanillielii, E: human, F: S. cerevisiae.
Fig. 3. The autoradiogram of the SDS-polyacrylamide gel electrophoresis of the proteins produced in E. coli mini-cells. Lane 1, pUC18; and lane 2, pMCP25a.

cells contained pMCP25a (lane 2), whereas no corresponding protein was produced in a detectable amount when the mini cells contained pUC18. pMCP25a was constructed by inserting the 2.2 kb BglIII fragment of pMCP25 into the BamHI site of pUC18. The size of the protein produced with pMCP25a agreed reasonably well with that (53,615 Da) of the protein deduced from the nucleotide sequence of argG gene.

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