THE AMINO ACID SEQUENCE OF ACTINOXANTHIN APOPROTEIN DEDUCED FROM THE BASE SEQUENCE OF THE GENE

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Actinoxanthin, produced by *Streptomyces globisporus* 1131, is a member of the chromoprotein family of antibiotics. The amino acid sequence of the protein component (apoprotein) of actinoxanthin, based on biochemical analysis of the purified protein[1], is very similar to, but not identical with, that of C-1027, another chromoprotein antibiotic[2]. Since the two antibiotics differ significantly in biological activities[1,3~5], we suspected that minor differences in the apoprotein structure could be a reason. We therefore wanted to confirm the amino acid sequence of actinoxanthin apoprotein by analyzing the base sequence of the gene. The similarity between the amino acid sequences of the two proteins suggested the possibility of cloning the actinoxanthin apoprotein gene by using as a probe the C-1027 apoprotein gene (*cagA*) which we previously cloned and sequenced[6]. Total DNA from *S. globisporus* 1131 was digested with *Sma* I, yielding 2.2 Kb fragments strongly hybridizable to a segment of *cagA* (corresponding to the amino acid sequence of Y^{31} to D^{89}, which had been excised from pC27a[6] and labeled by nick-translation, 1.6×10^8 cpm/μg), as shown in Fig. 1. DNA fragments recovered from the 2.2 Kb area of the gel were ligated to pUC119, shot-gun cloned using *E. coli* DH5α as a recipient, and colonies were hybridized to the same probe described above. A transformant strongly hybridizable to the probe was found to harbor a 5.4 Kb plasmid, pAX1 (Fig. 2). Using this plasmid as a template, the 193 bp DNA segment spanning the Y^{31} to D^{89} sequence was amplified by PCR. The amino acid sequence deduced from the base sequence of this amplified DNA segment differed from the reported one at 3 residues however. Starting with the base sequence of this DNA segment, a series of primers walking along pAX1 was conducted as long as 869 bp, which turned out to include an open reading frame for the gene (*axnA*) directing a precursor for the actinoxanthin apoprotein, as shown in Fig. 3. The amino acid sequence deduced from the base sequence of the DNA indicated: 1) the precursor protein had a signal peptide of 33 amino acid residues ahead of the apoprotein sequence, 2) the amino acid sequence determined here corrected the previously reported one at 6 residues in total which shown by underlined letters

Fig. 1. Southern blot analysis.

A: Total DNA from *Streptomyces globisporus* 1131 was digested with appropriate restriction endonucleases and electrophoresed. B: Autoradiogram of Southern hybridization with a 188 bp fragment of *cagA* as a probe. The arrow indicates 2.2 Kb *Sma* I fragments.

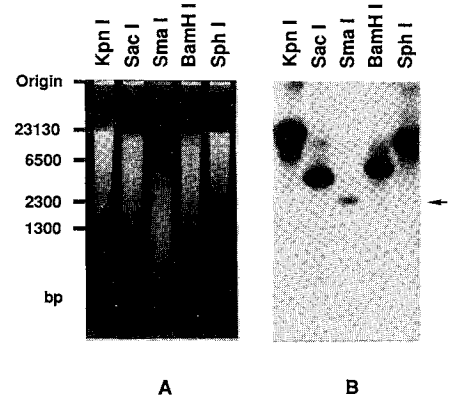


Fig. 2. A 5.4 Kb plasmid, pAX1 including a restriction endnuclease map of the cloned 2.2 Kb *Sma* I fragment.

The solid box indicates the coding region for actinoxanthin apoprotein.

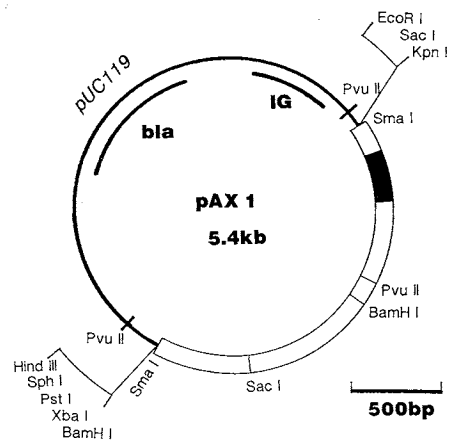
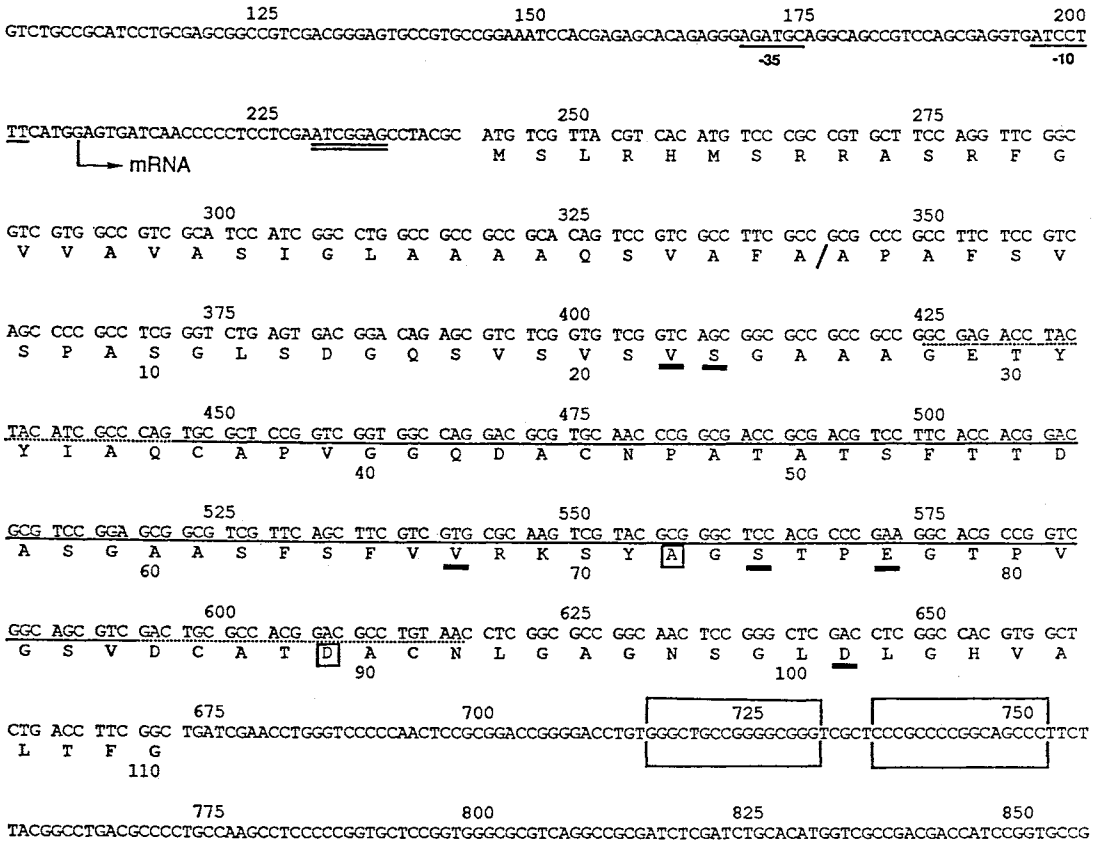


Fig. 3. Nucleotide sequence of *axnA*.



The bent arrow indicates transcriptional start point. The Shine-Dalgarno (SD) sequence is double-underlined. The slash indicates the splitting site for leader peptide and apoprotein. The amino acid residues behind the slash (apoprotein region) are numbered. The boxed amino acid residues are those different between apoprotein of actinoxanthin and C-1027. The DNA sequence amplified by PCR is underlined. The Dotted underlines correspond to PCR primers. The boxes behind ORF represent the stem of rho-like factor independent terminator. The nucleotide sequence data of *axnA* appeared in the DDBJ, EMBL and GeneBank Nucleotide Sequence Databases under accession number D11457.

in Fig. 3, 3) the difference between actinoxanthin apoprotein and C-1027 apoprotein was only at 2 amino acid residues, Ala^{72} (GCG) vs Thr^{72} (ACG) and Asp^{89} (GAC) vs Ala^{89} (GCC), although there were three more silent mutation differences between the two genes.

X-ray crystallography of actinoxanthin[7] indicated that a segment including Ala^{72} to Asp^{89} was localized near the chromophore binding cleft. The presence or absence of either a hydroxyl at the 72nd residue, or a negative charge at the 89th residue, or both, could affect binding between the apoprotein and the chromophore and, accordingly, the biological activity of the two holoantibiotics.

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