Cloning and Characterization of the Gene (farA) Encoding the Receptor for an Extracellular Regulatory Factor (IM-2) from Streptomyces sp. Strain FRI-5

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IM-2 is a butyrolactone autoregulator that controls production of blue pigment and nucleoside antibiotics in Streptomyces sp. strain FRI-5. An IM-2-specific receptor gene, farA, was cloned from strain FRI-5, and nucleotide sequencing revealed that the farA gene consists of 666 bp encoding a 221-amino-acid protein of 24.3 kDa with an NH₂-terminal amino acid sequence identical to that of purified native receptor. Another gene, farX, encoding a homolog of AfsA of Streptomyces griseus, was present upstream of farA. The monocistronic nature of the farA transcript was shown by Northern blot hybridization, and the transcript level increased upon addition of IM-2. Recombinant FarA expressed in and purified from E. coli showed clear ligand specificity toward IM-2, with a dissociation constant (Kd) for [³H]IM-2-C₃ of 18.2 nM. FarA showed high overall homology to BarA (virginiae butanolide receptor from S. virginiae) and ArpA (A-factor receptor from S. griseus). Sequence alignment of the three receptor proteins revealed that the NH₂-terminal region containing a helix-turn-helix DNA binding motif was highly conserved. The DNA binding motif is common in procaryotic repressors of the TetR family, suggesting that all the Streptomyces autoregulator receptors may act as transcriptional repressors.

IM-2 [(2R,3R,1'R)-2-(1'-hydroxybutyl)-3-(hydroxymethyl)butanolide] of Streptomyces sp. strain FRI-5 is one of the butyrolactone autoregulators of Streptomyces species and triggers production of blue pigment as well as the nucleoside antibiotics showdomycin and minimycin at a concentration of 0.6 ng/ml (6, 17, 27, 34). Butyrolactone autoregulators have been regarded as a kind of Streptomyces hormone which switches on morphological differentiation, such as aerial mycelium formation, and/or physiological differentiation, such as the production of antibiotics. They have a 2,3-disubstituted y-butyrolactone skeleton in common, and the 10 butyrolactone autoregulators identified to date are classified into the following three groups, on the basis of their minor structural differences: (i) A-factor type, possessing the 1'-keto group, to which only A-factor of S. griseus belongs (13, 18); (ii) virginiae butanolide (VB) type, possessing the 1'-α-hydroxyl group, to which VB-A through VB-E (14, 24, 33) and Grafe's three factors (3) belong; and (iii) IM-2 type, possessing the 1'-β-hydroxyl group, to which IM-2 and factor I (4) belong (Fig. 1).

Although the three groups of butyrolactone autoregulators differ in only minor structural detail, almost no cross-reactivity was observed among the responsive strains; i.e., induction by VB of aerial mycelium formation or streptomycin production by A-factor in Streptomyces griseus was not observed (16), while virginiamycin production in Streptomyces virginiae triggered by VBs requires a 1.7 × 10³-fold higher concentration of A-factor or IM-2 (7, 19). Blue pigment production in strain FRI-5 triggered by IM-2 requires 170- and 1.7 × 10³-fold-higher concentrations of the corresponding VB- and A-factor-type compounds, respectively (our unpublished data). In addition to the strict requirement for IM-2 in strain FRI-5, IM-2 is unique in that it can modify the antibiotic production profile. While Streptomyces sp. strain FRI-5 produces 1-cycloserine in the absence of IM-2, addition of IM-2 at 5 h of cultivation results in termination of d-cycloserine production and instead triggers production of blue pigment (17, 34) and the nucleoside antibiotics showdomycin and minimycin (6). The ability to switch from production of one kind of antibiotic to that of another was observed only in the case of IM-2 in Streptomyces sp. strain FRI-5.

The strict ligand specificity and the unique antibiotic-switching ability of IM-2 likely reflect the characteristics of the corresponding receptor protein. Previously we purified an IM-2-specific receptor protein from Streptomyces sp. FRI-5 (23) and showed that this IM-2 receptor is a homodimer of 27-kDa subunits, although its detailed characterization was impeded due to the very small amount of the purified protein available.

To clarify the basis of the strict ligand specificity among autoregulator receptors and the unique signal-transducing mechanism of IM-2 leading to antibiotic production switching, we have cloned and sequenced the gene for the IM-2 receptor. The recombinant receptor was overexpressed in Escherichia coli, purified, and characterized in more detail.

MATERIALS AND METHODS

Bacteria and plasmids. Streptomyces sp. strain FRI-5 (MAFF10-06015; National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan) (23) was used as a source of native IM-2 receptor, genomic DNA, and total RNA. The strain was grown at 28°C as described previously (6) in a medium containing, per liter, 7.5 g of yeast extract (Difco), 7.5 g of glycerol, and 1.25 g of NaCl (pH 6.5). For genetic manipulation in E. coli, strain DH5α (5) was used. For expression of the cloned genes in E. coli and Streptomyces, E. coli BL21(DE3)pLysS (29) and Streptomyces lividans TK21 (9), respectively, were used as hosts. pUC19 was used for the construction of a genomic library and for DNA sequencing. pET-td (30) was used for construction of the expression plasmids. Streptomyces plasmid pIJ486 (31) was kindly provided by D. A. Hopwood (John Innes Institute, Norwich, England). DNA manipulations in E. coli and in Streptomyces were performed as described by Sambrook et al. (25) and Hopwood et al. (8), respectively.
FIG. 1. Structures of butyrolactone autoregulators from *Streptomyces* species. Absolute configurations of A-factor (13, 18), VBs (14, 24), and IM-2 (17) have been assigned to (5R), (2R,3R,6S), and (2R,3S,6R), respectively, as shown. Absolute configurations of factor I and three factors from *S. bikinensis* and *S. cyaneofuscatus* are not yet determined (3, 4).

Chemicals. All chemicals were of reagent or high-performance liquid chromatography (HPLC) grade and were purchased from either Nacali Tesque, Inc. (Osaka, Japan), Takara Shuzo Co., Ltd. (Shiga, Japan), or Wako Pure Chemical Industrial, Ltd. (Osaka, Japan). Marker proteins for molecular sieve HPLC and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) were purchased from Oriental Yeast Co., Ltd. (Osaka, Japan), and Pharmacia Biotech K.K. (Osaka, Japan), respectively.

**Southern blot hybridization and molecular cloning of farA.** Total DNA of *Streptomyces* sp. strain FR1-5 was obtained by the method of Rao et al. (22). Genomic DNA (5 μg/lane) was digested to completion with restriction endonucleases and electrophoresed on a 1.0% agarose gel prior to blotting onto a nylon membrane (Hybond-N; Amersham). The membrane was hybridized with an *S. virginiae* barX probe (1.2-kbp BamHI-EcoT22I fragment [20]) labeled with [α-32P]dCTP (3,000 Ci/mmol; ICN Biomedical Inc.) and a random primer labeling kit (Takara Shuzo). Hybridization was performed in 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH2PO4, and 1 mM EDTA [pH 7.7]) containing 0.5% SDS, 5× Denhardt’s solution, and 0.02 mg of salmon sperm DNA per ml for 18 h at 65°C, followed by washing twice with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.1% SDS for 5 min each at room temperature, with 1× SSC containing 0.1% SDS for 10 min at room temperature and once at 65°C, with 0.5× SSC containing 0.1% SDS for 10 min at 65°C, and finally with 0.1× SSC containing 0.1% SDS for 10 min at 65°C. The blot was exposed to X-ray film (Fuji RX—80°C, 3.5 h).

A partial genomic library was constructed with size-fractionated PstI fragments (ca. 8.0 kbp) and pUC19, using *E. coli* DH5α as a host, and screened by colony hybridization with the 32P-labeled barX probe. The DNA sequence was determined by the dideoxy-chain termination method (26) for both strands, using double-stranded templates of pUC19 clones with a Thermo Sequenase cycle sequencing kit (Amersham) and Cy5-labeled primers on a fluorescence DNA sequencer (ALFred; Pharmacia Biotech).

Sequence analyses and homology comparisons were done on a personal computer with the GENETYX software package (Software Development Co., Ltd., Tokyo, Japan).

**Northern hybridization.** Total RNA was isolated by a modification of the procedure of Chomczynski and Sacchi (1), and the amount was calculated from the 260. Sixteen micrograms of RNA was loaded on each lane, electrophoresed on a 1.0% agarose gel prior to blotting onto a nitrocellulose membrane (Hybond-N; Amersham). The membrane was hybridized with an *S. virginiae* barX probe (1.2-kbp BamHI-EcoT22I fragment [20]) labeled with [α-32P]dCTP (3,000 Ci/mmol; ICN Biomedical Inc.) and a random primer labeling kit (Takara Shuzo). Hybridization was performed in 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH2PO4, and 1 mM EDTA [pH 7.7]) containing 0.5% SDS, 5× Denhardt’s solution, and 0.02 mg of salmon sperm DNA per ml for 18 h at 65°C, followed by washing twice with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.1% SDS for 5 min each at room temperature, with 1× SSC containing 0.1% SDS for 10 min at room temperature and once at 65°C, with 0.5× SSC containing 0.1% SDS for 10 min at 65°C, and finally with 0.1× SSC containing 0.1% SDS for 10 min at 65°C. The blot was exposed to X-ray film (Fuji RX—80°C, 3.5 h).

An A-factor and an I-factor probe was used in Northern hybridization. The amount of A-factor, I-factor, and barX probe is 1.2-kbp BamHI-EcoT22I fragment labeled with [α-32P]dCTP (3,000 Ci/mmol; ICN Biomedical Inc.) and a random primer labeling kit (Takara Shuzo). Hybridization was performed in 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH2PO4, and 1 mM EDTA [pH 7.7]) containing 0.5% SDS, 5× Denhardt’s solution, and 0.02 mg of salmon sperm DNA per ml for 18 h at 65°C, followed by washing twice with 2× SSC containing 0.1% (wt/vol) SDS for 10 min each at 50°C.

**Construction of pET-farA and preparation of recombinant FarA (rFarA).** A SauI-NraI (967 bp) fragment carrying farA was used as a template in the PCR. PCR was performed with primer 1 (5′-AACATGGCCCGAACAAATGCGG) and primer 2 (5′-AAGGTATCCGTGGTCGCGGG GCGGCTCAG-3′) to generate an NcoI site and a BamHI site at the 5′ and 3′ ends of the farA coding sequence, respectively (underlined). After partial digestion with NcoI due to the presence of another NcoI site in the middle of farA, a 683-bp NcoI-BamHI fragment was recovered and cloned into NcoI- and BamHI-digested pET-3d, resulting in pET-farA. The nucleotide sequence around the NcoI junction was confirmed by DNA sequencing.

For preparing rFarA, *E. coli* BL21(DE3) pLysS harboring pET-farA was grown overnight at 37°C in LB medium containing 0.2% glucose, 25 μg of ampicillin per ml, and 2 μg of chloramphenicol per ml. Two hundred milliliters of fresh medium in a 500-ml Sakaguchi flask was inoculated with 5 ml of the preculture and cultivated at 27°C for 3 to 4 h until the A590 reached 0.4, followed by addition of 0.1 mM isopropyl-β-D-thiogalactopyranoside and 1 h of induction at 27°C. Cells were harvested and resuspended (1 g [wt weight] of cells per 10 ml of buffer) in buffer A (0.02 M triethanolamine (TEA)-HCl (pH 7) containing 20% glycerol, 0.5 mM diithiothreitol (DTT), and 0.1 mM (p-amidinophenylethanesulfonylfluoride hydrochloride (pAPMSF)) plus 0.1 M KCl and disrupted by sonication. Cell extracts were used for SDS-PAGE analysis and the assay of IM-2 binding activity. For purification of rFarA, the supernatant after centrifugation was absorbed on a DEAE HPLC column (TSKgel DEAE-5PW, 0.75 by 7.5 cm; Tosho) preequilibrated with buffer A containing 0.1 M KCl. After being washed with the same buffer, the bound proteins were eluted with a linear gradient of 0.1 to 0.35 M KCl (10 mM/min) in buffer A, and fractions showing a single band on SDS-PAGE eluting at around 0.23 M KCl were stored at −80°C until use.

**IM-2 binding assay.** IM-2 binding activity was assayed as described elsewhere (23) by measuring the difference between binding of [3H]IM-2-C2 (10 pmol, 40 Ci/mmol) in the absence and presence of nonlabeled IM-2-C2 (15 nmol, 1,500-fold molar excess) in a total volume of 100 μl by equilibrium dialysis against 1 ml of buffer A containing 0.1 M KCl for 4 h. When dilution was necessary, hemoglobin was added to a final concentration of 100 μg/ml to prevent rapid inactivation of rFarA due to a low protein concentration. For determining ligand specificity, nonlabeled IM-2-C2 was replaced with the ligand specified at different molar excesses. More effective ligand can give a larger difference between the extent of [3H]IM-2-C2 binding in the absence and that in the presence of the nonlabeled ligand. All of the nonlabeled ligands were chemically synthesized as previously described (11). For determining pH dependence of the IM-2 binding activity, the following buffers containing 0.1 M KCl and 20% glycerol were used for diluting the protein sample and for equilibrium dialysis: pH 5 to 9, 0.2 M TEA-HCl; pH 7 to 9, 0.02 M TEA-HCl; and pH 8 to 11, 0.02 M glycine-KOH. The radioactivity in the solution was measured with a liquid scintillation counter (LS6000; Beckman).

**Preparation of crude cell extracts from *S. lividans*.** *S. lividans* TK21 harboring the indicated plasmid was grown for 70 h in YEPE as described by Hopwood et al. (8). One gram of mycelia was suspended in 4 ml of 0.05 M TEA-HCl (pH 7) containing 0.5 M KCl, 5 mM DTT, and 0.1 mM pAPMSF and disrupted by
sonication. Cell debris was removed by centrifugation (28,000 g, 20 min, 4°C), and the supernatant was stored at −80°C until use.

Determination of molecular weight. SDS-PAGE was performed with a precast 10 to 20% linear gradient gel (Daiichi Pure Chemical Co. Ltd., Tokyo, Japan) by using a minigel apparatus (Daiichi Pure Chemical Co.) and stained with Coomassie brilliant blue G-250.

The molecular weight of purified rFarA under nondenaturing conditions was estimated as described elsewhere (23) by gel filtration HPLC (TSK-G2000SWXL, Mr, 160,000; Tosoh) with buffer A containing 0.3 M KCl and 5 mM DTT.

Protein assay. Protein concentration was determined by a dye binding assay (Bio-Rad protein assay kit), using bovine serum albumin as a standard.

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been submitted to the GenBank/EMBL data bank with accession no. AB001683.

RESULTS AND DISCUSSION

Southern hybridization analyses. To clone a gene for the IM-2 receptor protein, we first performed a series of Southern blot hybridizations against genomic DNA of strain FRI-5. However, with barA encoding a VB-specific receptor of S. virginiae as a probe, no clear signal was obtained; with synthetic degenerate oligonucleotides designed from the N-terminal Ala-Glu-Gln-Val-Arg-Ala-Ile-Arg determined from the purified native IM-2 receptor, many ambiguous signals were detected, due probably to the high redundancy of the oligonucleotide probe (data not shown). Then we used barX (accession no. AB001608), which is present 259 bp upstream of barA oriented in the opposite transcriptional direction and seems to encode a homolog of AfsA postulated to catalyze A-factor synthesis in S. griseus (10), in the hope that the gene arrangement of the afsA homolog-receptor gene is conserved in strain FRI-5. Among the several clear signals detected by the barX probe, we selected an 8.0-kbp PstI fragment, constructed a partial genomic library from size-fractionated PstI fragments, and screened it by colony hybridization using the barX probe. After isolating the 8.0-kbp PstI fragment, we localized the barX homolog to a 1.3-kbp BamHI-SacI fragment by subsequent Southern hybridizations (Fig. 2a).

To confirm the existence of and further localize the region containing the IM-2 receptor gene, we transformed S. lividans TK21 with the multicopy vector pIJ486 carrying the 8.0-kbp PstI-PstI fragment, 3.4-kbp PstI-SacI fragment A, or 4.5-kbp BamHI-PstI fragment B. In IM-2 binding assays of cell extracts prepared from the corresponding transformants, although the one containing fragment B reproducibly showed lower IM-2 binding activity than the one containing the 8.0-kbp fragment, due probably to a lower level of expression, both the entire 8.0-kbp PstI fragment and the right-hand BamHI-PstI fragment B were suggested to encode a protein with strong IM-2 binding activity, which in turn suggested that the IM-2 receptor gene is located on fragment B (Fig. 2b).

Nucleotide sequence of the IM-2 receptor gene and barX homolog. Nucleotide sequencing of the 1.3-kbp BamHI-SacI fragment and the adjacent 967 bp of fragment B revealed two open reading frames (ORFs) transcribed in the same direction.

FIG. 2. Restriction endonuclease maps of the 8.0-kbp PstI-PstI fragment and 2.2-kbp BamHI-NaeI fragment containing the farX and farA genes (a), and IM-2 binding activities of cell extracts prepared from S. lividans TK21 harboring plasmids carrying each fragment (b). The region homologous to barX localized by Southern hybridization is indicated as a dotted bar. Arrows indicate the coding regions of farX and farA. Fragment A (PstI-SacI) and fragment B (BamHI-PstI) indicate regions used to transform S. lividans TK21.

FIG. 3. Nucleotide and deduced amino acid (one-letter notation) sequences of farA and farX. The nucleotide numbering begins at the 5′-most BamHI site of the 2,234-bp BamHI-NaeI fragment and the adjacent 967 bp of fragment B revealed two open reading frames (ORFs) transcribed in the same direction.

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(Fig. 3). The first ORF has three possible initiation sites, ATG at nucleotide (nt) 317, TTG at nt 440, and GTG at nt 512, of which ATG at nt 317 and TTG at nt 440 were preceded by probable ribosome binding sites. From the alignment with BarX and AfsA as described below, TTG at nt 440 was chosen as the most probable initiation site. The resulting ORF consisting of 876 bp is predicted to encode a 291-amino-acid protein of 32.1 kDa which shows significant sequence homology to AfsA (40.6% identity and 78.8% similarity) and BarX (53.2% identity and 84.3% similarity) (Fig. 4a), and the ORF was designated farX (FRI-5 barX homolog). No other proteins with significant sequence homology were found in database searches. The second ORF was concluded to start from GTG at nt 1500 due to the presence of the preceding ribosome binding sequence (AAGGAG) and to the complete match of the amino acid sequence deduced from the ORF to the N-terminal sequence of the purified IM-2 receptor (AEQVRAIR) except for the initiation methionine residue. This ORF was designated farA (FRI-5 autoregulator receptor) and predicted to encode a 221-amino-acid protein of 24,250 Da, which agrees moderately well with the estimated molecular weight of the purified native IM-2 receptor (27,000) (23).

In a computer-aided homology search, FarA was found to have high overall homology to only BarA, specifying a VB-specific receptor from S. virginiae (20), with 48.5% identity and 86.1% similarity, and ArpA, specifying an A-factor-specific receptor from S. griseus (21), with 38.5% identity and 80.7% similarity (Fig. 4b). The NH2-terminal one-fourth of FarA containing a helix-turn-helix DNA binding motif was highly conserved among them, but the rest of the sequences showed much less homology. Because a very similar DNA binding motif is conserved among procaryotic repressor-type transcrip-
tional factors such as TcmR, regulating the tetracenomycin resistance gene in *Streptomyces glaucescens*, and TetR, controlling the tetracycline resistance gene of Tn1721 in *E. coli* (32), FarA may act as a transcriptional repressor by binding directly to a specific DNA sequence(s) with the conserved helix-turn-helix sequence. Although regions other than the N-terminal one-fourth should participate in recognition and discrimination of the corresponding ligand, we are at present unable to delimit the region. Creation of several chimeric receptors is under way in our laboratory, and their analysis will clarify the regions essential for ligand specificity.

**Northern hybridization analysis of farA.** To study transcription of farA during cultivation, we performed Northern blot hybridization with total RNA of *Streptomyces* sp. strain FRI-5, using a SacI-NaeI fragment as a farA probe (Fig. 5). We analyzed the transcripts in the mycelia after a 5-h cultivation and an 8-h cultivation with or without addition of IM-2 (20 ng/ml) at 5 h, because exogenous addition of IM-2 at 5 h caused the production of blue pigment at 8 h (6, 34). In all samples, only a 0.7-kbp band was observed, suggesting the monocistronic nature of farA. The addition of IM-2 caused a slight but reproducible increase (1.5- to 1.7-fold) in the level of farA transcription. Although the significance and the mechanism of this phenomenon are unclear at present, gel shift analyses using recombinant FarA toward possible target sequences, including those in the farA promoter region, are under way in our laboratory and may clarify the basis of this phenomenon.

**Expression of recombinant FarA in E. coli and its characterization.** To examine the farA product in more detail, we expressed farA in *E. coli* by means of the T7 expression vector pET-3d. The coding region of farA was amplified by PCR and placed under the control of the T7 RNA polymerase promoter as described in Materials and Methods. SDS-PAGE analysis (Fig. 6a) indicated that isopropylthiogalactopyranoside (IPTG)-induced *E. coli* BL21(DE3)/pLysS harboring pET-farA significantly overproduced a 27-kDa protein, the identity of
which was confirmed by analysis of its N-terminal amino acid sequence. Furthermore, cell extracts prepared from IPTG-induced cells harboring pET-farA showed an extremely high IM-2 binding activity (7.1 \times 10^7 \text{ dpm/mg of protein}), while cell extracts from the control cells harboring pET-3d showed no activity (data not shown). The overexpressed rFarA was purified to homogeneity by DEAE-5PW HPLC (Fig. 6a), with an apparent molecular weight of 27,000 on SDS-PAGE. Under native conditions by molecular sieve HPLC, purified rFarA was eluted as a trimer at a high protein concentration (3.9 mg/ml), but the elution position shifted to that of a dimer at a lower protein concentration (0.98 mg/ml), indicating that rFarA tends to aggregate at high protein concentrations. This phenomenon agrees well with the instability of rFarA in a buffer containing no glycerol, in which rFarA lost its IM-2 binding activity rapidly upon forming high-molecular-weight aggregates (data not shown). Because the native IM-2 receptor exists solely as a cytoplasmic soluble protein (23) and we have observed no sign of its association with membrane under several conditions, this aggregation of rFarA should be an artificial event at high protein concentrations. IM-2, a rather hydrophobic and noncharged ligand, seems to diffuse freely across the membrane and bind with the IM-2 receptor in the cytoplasm.

To study the ligand specificity of rFarA, we performed a series of competitive binding assays in which we used several autoregulators, i.e., IM-2-C5, to IM-2-C6, VB-C5, and A-factor-C5, as competitive nonlabeled ligands against [3H]IM-2-C5. The experiments were conducted at 1.5-, 15-, and 150-fold molar excesses of unlabeled ligands (Fig. 6b). IM-2-type compounds were shown to be the most effective ligands at each concentration, with IM-2-C5 and IM-2-C6 being the most effective. As the chain length at C-2 deviated from the optimum, the effectiveness decreased sharply. VB-C5 showed less affinity than did IM-2-C5, and A-factor-C5 showed very poor binding affinity, confirming that FarA is actually the IM-2-specific receptor. The concentration dependence of IM-2 binding activity was studied by Scatchard plotting (Fig. 7b). The plot gave a straight line, and the slope of the line indicates a dissociation constant (Kd) of 18.2 nM and Bmax (binding maximum) of 0.38 mol of [3H]IM-2-C5/mol of rFarA monomer. The Kd value was 14-fold higher than that for a partially purified native IM-2 receptor from Streptomyces sp. strain FRI-5 (1.5 nM) (23). A similar discrepancy of Kd values between the native receptor in the crude state and a recombinant receptor in the purified state is observed for the VB receptor (20), the Kd of which is 8.6 nM for the crude native receptor and 30 to 130 nM for the purified recombinant receptor, due mainly to the highly unstable nature of the purified receptor, although we cannot exclude the possibility of the existence of some kind of an accessory protein which stabilizes the corresponding receptor in Streptomyces.

Figure 7a shows the pH dependence of the IM-2 binding activity of rFarA, demonstrating clearly the pH optimum at 9. Although this alkaline optimum might suggest that the true ligand is in the free-acid form rather than the lactone form, based on the well-known fact that an ester bond is unstable at alkaline pH, this possibility is unlikely for the following two reasons. (i) In the reverse-phase HPLC, less than 0.5%, if not at all, of the IM-2-C5 was found to exist in the free-acid form when the pH of the running buffer was 9. Despite this large increase in the amount in the free-acid form, the IM-2 binding activity of the rFarA increased only 1.4-fold when the pH of the running buffer was 7, while 28% was in the free-acid form when the pH of the running buffer was 9. (ii) When the free-acid form of IM-2-C5 instead of the lactone form, was used in the standard assay at pH 7, at which a small fraction of the free-acid form was instantaneously converted to the lactone form but the majority remained in the free-acid form, the IM-2 binding activity was 1.7% of that with the lactone form of [3H]IM-2-C5, excluding the possibility that the free-acid form of IM-2 is the true ligand form.

More than 10 butyrolactone autoregulators have been identified in Streptomyces species, and they comprise a family of Streptomyces hormones which regulate cytodifferentiation and/or secondary metabolism. Since corresponding receptors have been identified for all three classes of butyrolactone autoregulators from Streptomyces species—the IM-2, VB, and A-factor receptors—the receptor proteins most likely participate in autoregulator-dependent signal transduction. Successful cloning and analysis of the gene encoding the IM-2 receptor, together with already cloned genes for VB and A-factor receptors, will facilitate the identification of specific regulatory roles at the molecular level.

**ACKNOWLEDGMENTS**

This work was supported in part by a grant from the Sankyo Foundation of Life Science and by a grant from the Research for the Future program of the Japan Society for the Promotion of Science.
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