

FK-506-Binding Proteins from Streptomyces Producing Immunosuppressive Macrolactones of the FK-506 Type

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FK-506-binding proteins (FKBPs), which in T cells are supposed to mediate the immunosuppressive effects of the compounds FK-506 and rapamycin, have been isolated from *Streptomyces chrysomallus*, *S. hygroscopicus* subsp. *ascomyceticus*, and *S. hygroscopicus*. The latter two strains are producers of ascomycin (the ethyl analog of FK-506) and rapamycin, respectively. Like the 12-kDa FKBP in eukaryotic organisms such as humans, bovines, and *Saccharomyces cerevisiae*, or the FKBP from gram-positive streptomyces are peptidyl-prolyl-*cis-trans* isomerases. Inhibition studies using FK-506, rapamycin, or ascomycin, revealed inhibition of the peptidyl-prolyl *cis-trans* isomerase activity of the proteins at the nanomolar level, which is in the same range as with eukaryotic FKBP. The M_r s of the various FKBP were 13,500 to 15,000, and they had the same pI of approximately 4.5. The N-terminal sequences of the three FKBP were nearly identical in the first 20 amino acids. The amino acid sequence deduced from the gene sequence of *S. chrysomallus* gave a polypeptide of 124 amino acids. The homologies to FKBP from humans, *S. cerevisiae*, and *Neurospora crassa* were 38, 39, and 50% identity in relevant positions, respectively. Significant homology of 38% was also seen with the C-terminal halves of bacterial protein surface antigens like the Mip protein of *Legionella pneumophila* and the 27-kDa Mip-like protein of *Chlamydia trachomatis*. In addition, two more open reading frames in *Pseudomonas aeruginosa* and *Neisseria meningitidis* of unknown function show regions of homology to the *S. chrysomallus* FKBP. In contrast to fungi, streptomyces are resistant to macrolactones. Ascomycin-producing *S. hygroscopicus* subsp. *ascomyceticus* excretes the compound almost quantitatively into medium, which indicates that the organism has an efficient self-protection mechanism against its own secondary metabolite.

The compound FK-506 is an immunosuppressive macrolactone that, like the cyclic peptide cyclosporin A, inhibits early events in T-cell activation (2, 8, 37). It exerts its effect by forming a complex with a specific cytosolic 12-kDa FK-506-binding protein (FKBP) that was shown to be a peptidyl-prolyl *cis-trans* isomerase (PPIase) (12, 33). Although binding of FK-506 inhibits the isomerase activity, detailed biochemical studies revealed that the immunosuppressive effect of the compound stems from the intrinsic structural properties of the complex rather than from inhibition of the enzyme (3, 16, 23, 37). Accordingly, rapamycin, another immunosuppressive macrolactone which has some structural elements in common with FK-506, is highly effective in binding to FKBP and inhibits isomerase activity but antagonizes the effect of FK-506 and is effective at a later stage in T-cell activation (2, 9, 21, 26). Recent data suggest that calcineurin, a mammalian protein phosphatase, is the target of the FK-506-FKBP complex. Interestingly, the complex between cyclosporin A and its cellular receptor cyclophilin (17.5 kDa) also binds to calcineurin (23). Cyclophilin is also a PPIase. On the basis of its amino acid sequence and drug-binding specificity, cyclophilin is clearly distinct from FKBP. Therefore, the two different drug-receptor complexes must share some similarity in at least one portion of their structures that mediates their interaction with calcineurin. Accordingly, the complex between FKBP and rapamycin does not bind to the protein phosphatase.

FKBP, like cyclophilin, has been reported to be present in

abundance in a variety of species and different cell types (up to 0.5% of the total cellular protein) (31). This suggests a fundamental role of the FKBP in the cell. Its isomerase activity has led to the suggestion that the enzyme is involved in the catalysis of a rate-determining step in protein folding (38).

FKBP is also present in lower eucaryotes, such as *Saccharomyces cerevisiae* (34) and *Neurospora crassa* (38), where it is presumed to mediate the cytotoxic effect of FK-506 against these microorganisms. By contrast, nothing is known on the presence of functionally active FKBP in prokaryotes. Several bacterial gene sequences which contain regions of homology to eucaryotic FKBP have been published (5, 17, 24, 29). PPIase activity has been demonstrated in an outer membrane protein of *Legionella pneumophila* (Mip) and a fusion protein of *Neisseria meningitidis* expressed in vitro (10, 30). The study of bacterial FKBP is interesting because FK-506 and its analogs do not interfere with bacterial growth. In addition, the simpler genetics of prokaryotes would eventually enable one to find out the true role of FKBP in the cell.

In this report, we describe the isolation and characterization of FKBP from streptomyces. These are filamentous gram-positive bacteria with a complex life cycle involving differentiation from vegetative mycelium to spores (15). They produce most of the known antibiotics, among them FK-506 and rapamycin (20, 32). We show that strains of *S. hygroscopicus* that produce these immunosuppressive drugs contain 15- to 15.5-kDa FKBP which are inhibited at the nanomolar level by FK-506 or rapamycin and thus strongly resemble FKBP from eucaryotic cells.

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MATERIALS AND METHODS

Chemicals and biochemicals. FK-506, rapamycin, and ascomycin were a gift of J.-J. Sanglier, Sandoz AG, Basel, Switzerland. Succ-Ala-Leu-Pro-Phe-4-nitroanilide and Succ-Ala-Ala-Pro-Phe-nitroanilide were purchased from Bachem, Heidelberg, Germany. All other chemicals were of the highest grade commercially available. The following oligonucleotides corresponding to amino acid sequences of FKBP from *S. chrysomallus* were used to screen a cosmid gene library of *S. chrysomallus* ATCC 11523 DNA in *Escherichia coli*: ATCGA(AG)AAGCC(CG)GA(AG)GT(CG)GACTTC CC(CG)GGIGGIGA(AG)CC(CG)CC(CG)GC(CG)GAC and GC(CG)ATCAAGGACATCTGGGA(AG)GGIGACGGIC C(CG)GT(CG)GC(CG)CAGGC(CG)GG. These oligonucleotides were purchased from TIB Molbiol (Berlin, Germany).

Strains and cultures. *S. chrysomallus* ATCC 11523, *S. hygroscopicus* ATCC 29253, and *S. hygroscopicus* subsp. *ascomyceticus* ATCC 14891 were from the American Type Culture Collection. *S. chrysomallus* X2-18 is a derivative of *S. chrysomallus* ATCC 11523 (19). All strains were kept on slants of a yeast extract-malt extract-maltose agar (CM) as previously described (18). Liquid cultures of the different strains mentioned in this work were grown in liquid CM essentially as described previously (18).

Plasmids, cloning, and sequencing procedures. For isolation of the FKBP gene, a cosmid gene library of *S. chrysomallus* DNA in *E. coli* 1400 was used (28). After transfection of packaged cosmids into *E. coli* DH5 α , clones were detected by hybridization screening using radioactively labelled oligonucleotides in accordance with previously published procedures (25). Techniques used for DNA isolation and manipulations were previously described (25). The plasmid used for subcloning and preparation of single-stranded DNA was phagemid pTZ18U (27).

Analytical methods. PPIase activity was tested essentially as previously described (11), by using Succ-Ala-Leu-Pro-Phe-4-nitroanilide in a coupled assay with chymotrypsin, except that the enzyme concentration was between 30 and 60 nM. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was done as described in reference 22. Protein determinations were done as described in reference 4. Antibodies against FKBP from *S. chrysomallus* were raised in rabbits by injecting them three times with 100 μ g of pure protein. The serum was used without further purification. Western blot (immunoblot) analysis was performed by standard techniques using a 2,000-fold dilution of primary antibody, phosphatase-conjugated goat anti-rabbit antibody (Sigma), and nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate toluidinium as reagents. DNA sequencing was done on single-stranded DNA by a dideoxy method offered by United States Biochemicals (Sequenase kit version 2).

Enzyme purification. All operations were performed at 2 to 4°C. For purification of FKBP from *S. chrysomallus*, some 60 g (wet weight) of *S. chrysomallus* X2-18 mycelium from 2-day-old cultures was suspended in 50 mM potassium phosphate (pH 6.8)–10% glycerol–1 mM dithiothreitol and passed through a French press at 10,000 lb/in². After DNase digestion and removal of cellular debris by centrifugation, the resultant crude extract was applied onto a DEAE-cellulose column (200-ml bed volume) equilibrated with the same buffer. After washing with buffer, enzyme was eluted from the column with buffer containing 0.3 M KCl. Fractions containing FKBP-related PPIase activity were pooled, and saturated (NH₄)₂SO₄ solution was added to give a final

saturation of 70%. The precipitate was dissolved in 50 mM Tris-HCl (pH 8)–10% glycerol–1 mM dithiothreitol–0.5 mM phenylmethylsulfonyl fluoride and fractionated by gel filtration on a Ultrogel Aca-54 column (60 by 4 cm) previously equilibrated with the same buffer. Active fractions were pooled and applied onto a Mono Q HR 5/5 column which previously had been equilibrated with 50 mM MOPS (morpholinepropanesulfonic acid) (pH 6.75)–5% glycerol–1 mM dithiothreitol–0.5 mM phenylmethylsulfonyl fluoride. The enzyme was eluted by a linear gradient from 0 to 0.3 M NaCl in the same buffer (3 h; flow rate, 0.5 ml min⁻¹) and appeared at 0.14 to 0.16 M NaCl. Fractions containing PPIase activity were pooled, diluted with 2 volumes of water, and concentrated by adsorption to a small DEAE-cellulose column (4 by 0.6 cm) equilibrated with the above-mentioned Tris buffer and subsequent elution with 0.3 M KCl. The enzyme was then purified to homogeneity by gel filtration on Superdex TM⁷⁵ equilibrated with 100 mM Tris-HCl (pH 8)–1 mM dithiothreitol. When necessary, the enzyme was concentrated by means of Centricon 10 microconcentrators (Amicon). The enzyme was stored frozen in buffer containing 15% glycerol at –80°C for at least 6 months without loss of activity.

Purification of FKBP from *S. hygroscopicus* ATCC 29253 and *S. hygroscopicus* subsp. *ascomyceticus* ATCC 14891 was done essentially by the same procedure as for FKBP from *S. chrysomallus*, except that 2.5- to 3-day-old mycelium was used when ascomycin or rapamycin synthesis in the cultures had started, as was measured by isolation and quantitation of the products by thin-layer chromatography along with the authentic products. Thin-layer chromatography was done on silica gel sheets (Merck, Darmstadt, Germany) by using ethyl acetate-methanol-water (100:2.5:1, by volume) as the solvent system.

Localization of ascomycin cultures of *S. hygroscopicus* subsp. *ascomyceticus* ATCC 14891. Three-hundred-milliliter portions of 3-day-old cultures of *S. hygroscopicus* subsp. *ascomyceticus* ATCC 14891 were harvested by suction filtration, and the mycelium was washed with 500 ml of distilled water. The combined filtrate and washings were extracted twice with 800-ml portions of ethyl acetate. After being dried with solid sodium sulfate, the ethyl acetate phase was evaporated to dryness under reduced pressure. The amount of ascomycin in the residue was determined as described above. The mycelium cake (ca. 6 g) was suspended in 200 ml of acetone and homogenized in a Waring blender. After suction filtration, extraction of the residue with acetone was repeated. Combined acetone extracts were evaporated to dryness, and ascomycin was determined. In control measurements, whole cultures (i.e., medium plus cells, 100 ml) were extracted with five 100-ml portions of ethyl acetate.

Nucleotide sequence accession number. Sequence data for *S. chrysomallus* FKBP have been deposited in the GenBank data base under accession no. M98428.

RESULTS

Enzyme purification. Cell extracts of *S. chrysomallus*, a streptomycete that does not produce immunosuppressive macrolactones, were tested for the presence of FKBP-related PPIase activity. This strain is currently being subjected to various biochemical investigations in our laboratory, and a genetic system is available (18, 19). PPIase activity related to FKBP was detected in extracts of cells derived from all stages of cultivation (data not shown). The

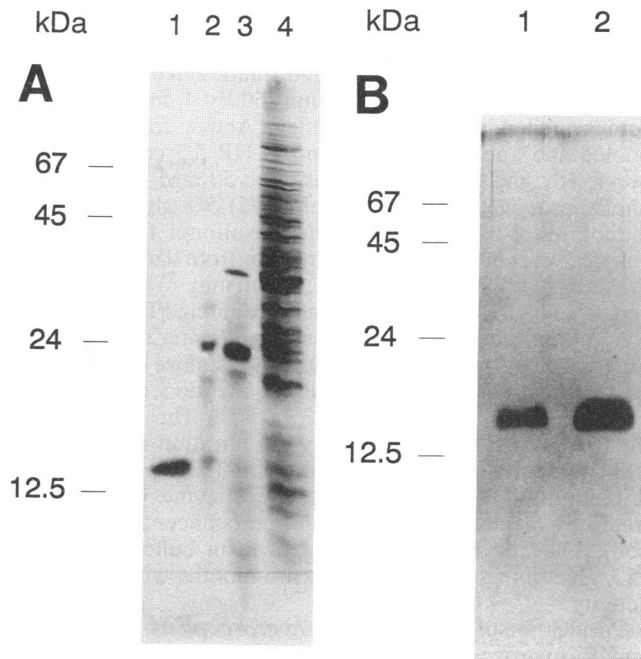


FIG. 1. (A) Purification of FKBP from *S. chrysomallus*. Lanes 1, 3.5 μ g of purified FKBP of *S. chrysomallus*; 2, 50 μ l (5 μ g of protein) of material from the Mono Q chromatographic step (MOPS, pH 6.75); 3, 50 μ l (15 μ g of protein) of material from the Ultrogel Aca-54 gel filtration step; 4, 50 μ l (100 μ g of protein) of material from the DEAE-cellulose chromatography step. The various steps are explained in Materials and Methods. Molecular weight markers are bovine serum albumin (67 kDa), ovalbumin (45 kDa), trypsinogen (24 kDa), and cytochrome *c* (12.5 kDa). Staining of the 15% slab gel was done with Coomassie brilliant blue. (B) Purification of FKBP from strains of *S. hygroscopicus*. Purified FKBP from *S. hygroscopicus* ATCC 29253 (lane 1, 0.75 μ g) and *S. hygroscopicus* subsp. *ascomyceticus* ATCC 14891 (lane 2, 1 μ g). The sodium dodecyl sulfate-polyacrylamide gel was silver stained.

enzyme activity was inhibited by FK-506 or rapamycin but not by cyclosporin A. In these initial experiments, the assays always contained cyclosporin A to suppress the action of cyclophilin, another PPIase present in streptomycetes (28). Typically, the FKBP isomerized the peptide substrate Succ-Ala-Leu-Pro-Phe-4-nitroanilide much faster than Succ-Ala-Ala-Pro-Phe-4-nitroanilide. The latter compound is a standard substrate for cyclophilin (11) and is a poor one for FKBP (13). Purification of FKBP from the crude extract of *S. chrysomallus* is described in Materials and Methods. In the first step, DEAE-cellulose chromatography afforded quantitative separation of FKBP from cyclophilin. Later steps involved gel exclusion chromatography, ion-exchange chromatography on Mono Q, and gel filtration on Superdex TM⁷⁵. Overall purification was 1,630-fold, and the yield was 21%. The process of purification is illustrated in Fig. 1A. Gel electrophoretic analysis of the last step of purification showed a single band with an M_r of 13,500 to 14,000, which is significantly higher than the M_r s of FKBP from eucaryotes (12, 33, 34).

Isolation of FKBP from ascomycin-producing *S. hygroscopicus*. It was of interest to see whether an FK-506- or rapamycin-producing *Streptomyces* strain contains an FKBP. Therefore, we next isolated the FKBP from *S. hygroscopicus* subsp. *ascomyceticus* ATCC 14891. This strain produces

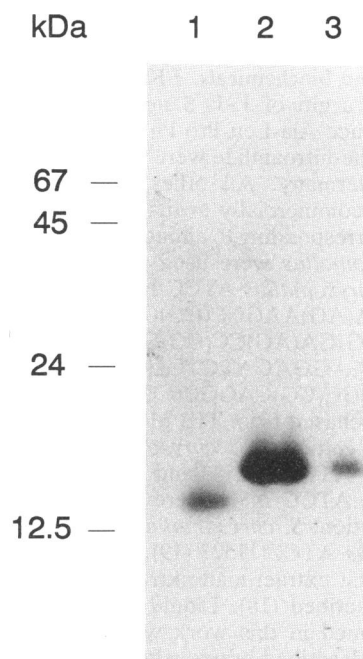


FIG. 2. Western blot analysis of an electropherogram with the FKBP of the different streptomycetes. FKBP were from *S. chrysomallus* (lane 1, 0.1 μ g), *S. hygroscopicus* ATCC 29253 (lane 2, 0.5 μ g), and *S. hygroscopicus* subsp. *ascomyceticus* ATCC 14891 (lane 3, 0.1 μ g).

ascomycin (1), an ethyl analog of FK-506. The reason for choosing this strain was that no FK-506 producing strain was available in our laboratory. Ascomycin (identical to FR-900520) has been reported to be active in inhibiting T-cell proliferation (14), as is FK-506 (although at 3- to 4-fold reduced efficiency) and is an effective inhibitor of FKBP in the nanomolar range (see Fig. 3). A functionally active FKBP from *S. hygroscopicus* cells, harvested at the peak of ascomycin production, was purified to homogeneity by the purification protocol used for *S. chrysomallus* FKBP (Fig. 1B, lane 2). Interestingly, the FKBP from *S. hygroscopicus* is bigger than that of *S. chrysomallus*. The denatured protein displays an M_r of 15,000 to 15,500 (Fig. 2, lane 3).

Isolation of FKBP from rapamycin-producing *S. hygroscopicus*. Rapamycin has been reported to interfere with T-cell receptor-induced cell activation at a later step than FK-506, although it binds to the same PPIase as FK-506 in humans and *S. cerevisiae* (21). We purified FKBP from actively rapamycin-synthesizing *S. hygroscopicus* ATCC 29253 by the protocol used for *S. chrysomallus*. This strain also contains a functionally active FKBP that in its denatured form has the same M_r of 15,000 to 15,500 as that of *S. hygroscopicus* subsp. *ascomyceticus* (Fig. 1B, lane 1).

Characterization of FKBP. Inhibition curves for the three immunophilins from the various streptomycetes are shown in Fig. 3. The PPIase activities of all three enzymes were inhibited by FK-506 at the nanomolar level. The 50% inhibitory concentrations were 30 to 60 nM and thus in the same range as those for FKBP from eucaryotes, e.g., *neurospora* (38). Accordingly, the 50% inhibitory concentration of rapamycin was 10 to 15 nM for each of the three FKBP, which is consistent with data concerning rapamycin action on isomerase activity of FKBP from *S. cerevisiae* and humans. These data indicate that rapamycin has a twofold

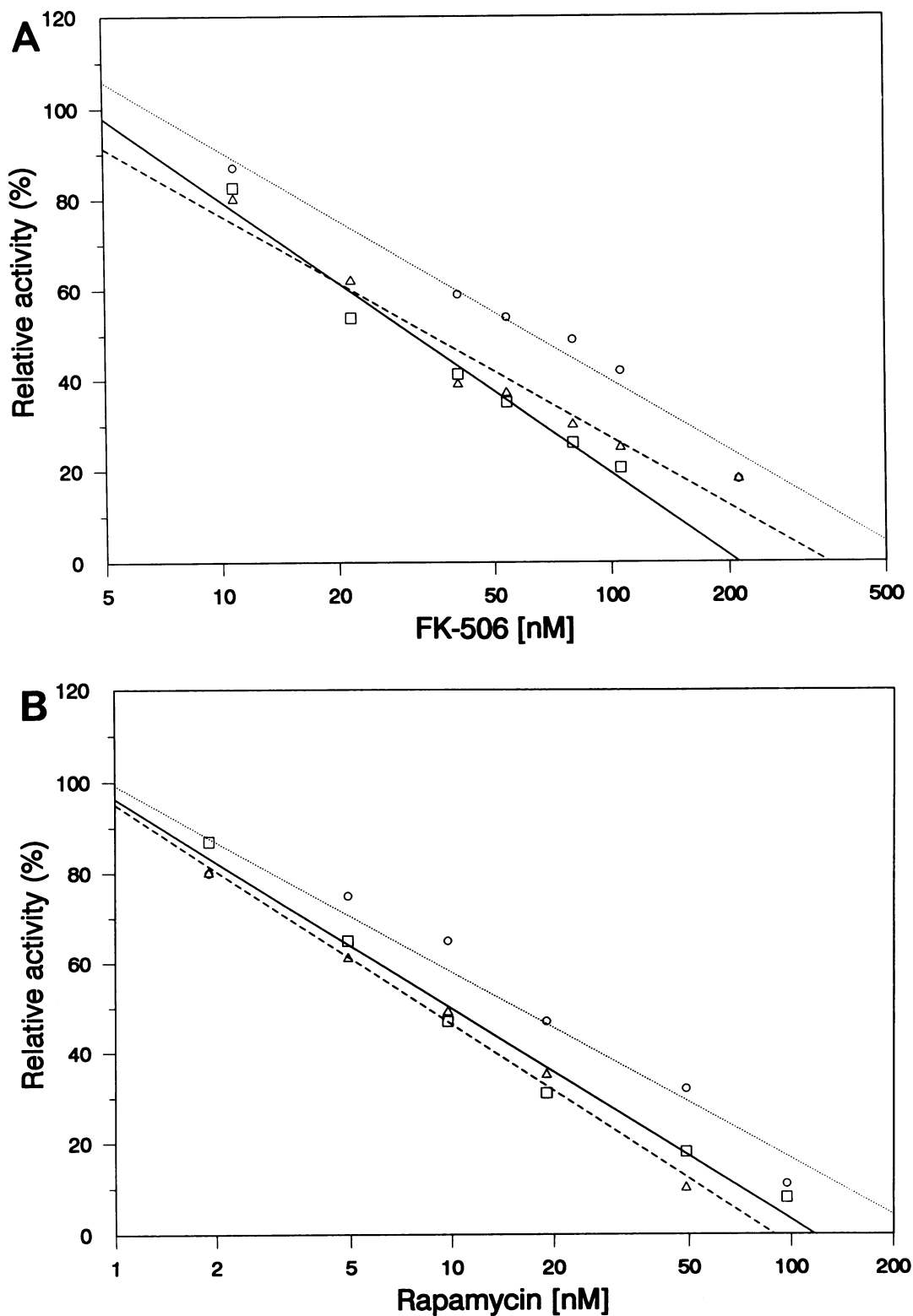


FIG. 3. Inhibition of the PPIase activities of FKBP from different streptomycetes by FK-506 (A) and rapamycin (B). *S. chrysomallus* (□), *S. hygroscopicus* subsp. *ascomyceticus* ATCC 14891 (○), and *S. hygroscopicus* ATCC 29253 (△) were tested. PPIase activity was determined by measuring isomerization of *N*-succinyl-Ala-Leu-Pro-Phe-4-nitroanilide (Bachem) in a coupled assay with chymotrypsin as previously described (11). The inhibition curves were constructed by incubating the enzymes from the different streptomycetes in the presence of various concentrations of FK-506 or rapamycin and measuring the first-order rate constants (11). The concentration of the enzyme was 35 to 40 nM in all cases. The inhibition of the three different FKBP by rapamycin was essentially the same as with FK-506.

higher affinity than FK-506 in binding to FKBP (2). In addition, ascomycin inhibited the three PPIases to the same extent as did FK-506. All three FKBP were abundant in the cells and were estimated to make up 0.2 to 0.5% of the total soluble protein. Also, they were present at any time during cultivation. Remarkably, the pI of the enzymes was the same in each case, with approximately 4.5 indicating an acidic protein in contrast to the pI of 8.9 for human FKBP (12). Antibodies raised against the FKBP from *S. chrysomallus* showed strong cross-reactivity with those from the two *S. hygroscopicus* strains that produce ascomycin and rapamycin, respectively (Fig. 2).

Microsequencing of the three FKBP revealed absolute amino acid sequence identity between FKBP of *S. chrysomallus* and *S. hygroscopicus* subsp. *ascomyceticus* in the first 20 amino acids. The corresponding sequence of *S. hygroscopicus* ATCC 29253 differed in two positions from that sequence (data not shown). These data indicate that the three enzymes are highly homologous to each other and also that the different sizes of the enzymes are not due to sequence extensions at their amino termini.

Cloning and sequencing of the FKBP gene. We decided to clone the gene from *S. chrysomallus* because a gene bank for this organism was available. Screening of a cosmid gene library in *E. coli* with two nonoverlapping 48-oligomers derived from the amino acid sequence obtained by microsequencing yielded a cosmid, designated pAP2000, approximately 45 kb long. Southern hybridization analyses of restriction digests enabled localization of the FKBP gene on a 600-bp DNA segment between the second *StuI* site and the fourth *SalI* site (Fig. 4A). The gene sequence, along with the deduced amino acid sequence, is shown in Fig. 4B. It is an open reading frame of 372 bp corresponding to a protein of 124 amino acids. The calculated M_r of 12,900 and the pI of 4.2 are in agreement with the protein data. The codon usage of the gene is typical for streptomycetes with a strong bias for codons having G or C in position 3. The G+C content in position 3 is 96%, whereas the overall G+C content is 72%.

Sequence analysis. The alignment of the *S. chrysomallus* FKBP amino acid sequence with the corresponding sequences from humans, *S. cerevisiae*, and *N. crassa* is shown in Fig. 5. There are regions of strong sequence conservation in the various FKBP (38, 39, and 50% identity with humans, *S. cerevisiae*, and neurospora, respectively) which indicate strong selective pressure on this enzyme during evolution. Interestingly, significant homology was also observed between the *Streptomyces* FKBP sequence and portions of the sequences of two protein surface antigens of *L. pneumophila* (38% identity) and *Chlamydia trachomatis* (31% identity). Furthermore, portions of two open reading frames from *N. meningitidis* and *Pseudomonas aeruginosa* share similarity with the *Streptomyces* protein.

Localization of the macrolactone produced in streptomycetes cultures. It is not known whether the FKBP has a vital function in streptomycetes. It is therefore of interest to see whether there is a protection mechanism in these organisms which helps to avoid contact between the enzyme inhibitor and the enzyme. Measurement of the macrolactone content of a culture of the ascomycin producer *S. hygroscopicus* subsp. *ascomyceticus* revealed that more than 99.5% of the immunosuppressant was in the medium whereas only traces were associated with the mycelium. The latter finding may be due to nonspecific adsorption to the cells. Thus, *S. hygroscopicus* subsp. *ascomyceticus* appears to protect itself by excreting the PPIase inhibitor into the medium, and most probably this is also the case for rapamycin-producing

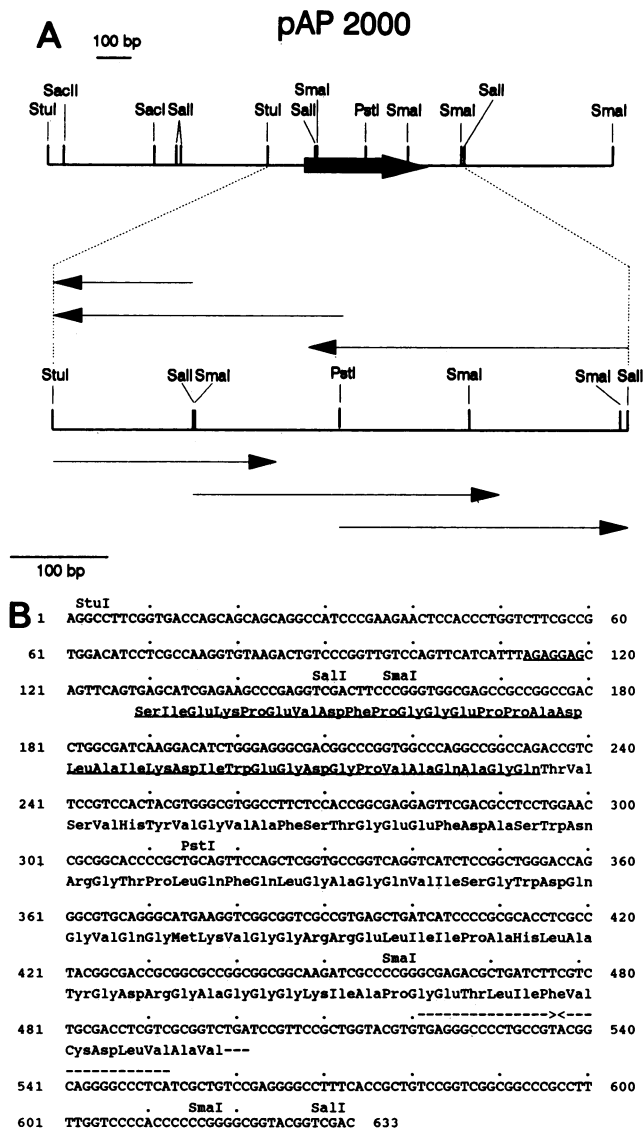


FIG. 4. (A) Restriction map of the environment of the *S. chrysomallus* FKBP gene on the cosmid pAP2000. The thick arrow depicts the position and orientation of the gene. Thin arrows illustrate the sequencing strategy from the second *StuI* site to the fourth *SalI* site. (B) Sequence of the FKBP gene and deduced amino acid sequence. The putative ribosomal binding site in the nucleotide sequence is underlined. The amino acid sequence obtained by microsequencing of the FKBP in the deduced amino acid sequence is also underlined. The dashed arrows downstream of the 3' end of the gene denote an inverted repeat structure which may be involved in transcription termination.

S. hygroscopicus. Evidently, these strains have a permeability barrier like that described for other streptomycetes that produce antibiotics or enzyme inhibitors (7) which may be harmful to the producing organism.

Resistance of streptomycetes to FK-506 and rapamycin. The *Streptomyces* strains used in this study were tested for sensitivity to FK-506 and rapamycin. Neither *S. chrysomallus* nor the two *S. hygroscopicus* strains were inhibited by the two compounds in disc diffusion tests on solid medium with amounts of up to 200 μ g per disc, regardless of whether

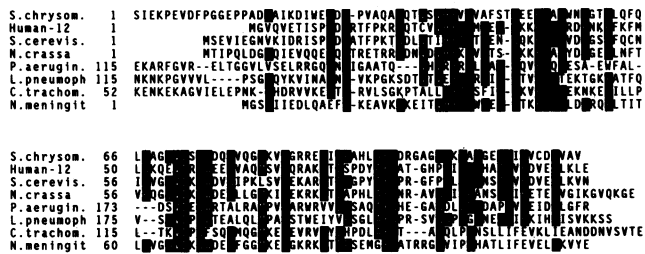


FIG. 5. Alignment of various FKBP amino acid sequences of eukaryotic origin and bacterial FKBP-like sequences with the corresponding sequence of *S. chrysomallus*. The sequences were taken from humans (36), *S. cerevisiae* (21), *N. crassa* (38), *L. pneumophila* (5), *C. trachomatis* (24), *P. aeruginosa* (17), and *N. meningitidis* (29). The numbers to the left of the sequences indicate amino acid position relative to the start of each protein sequence.

growing mycelium, germinating spores, or regenerating protoplasts were used.

DISCUSSION

The data presented here show that bacteria such as streptomycetes harbor FKBP that are functionally active PPIases. Likewise, in eucaryotes their PPIase activity can be inhibited by FK-506, rapamycin, or related compounds at the nanomolar level. Also, they appear to make up a considerable portion of the total cellular protein, and this indicates an important function of this class of enzymes in all living cells (34). It is remarkable that streptomycetes that produce rapamycin or ascomycin, the ethyl analog of FK-506, harbor FKBP that can be inhibited by these compounds. This suggests an efficient protection mechanism which apparently is based on an efficient excretion system in these two streptomycetes. In contrast to fungi, all of the streptomycetes tested are resistant to rapamycin or ascomycin, which indicates either that there is a permeability barrier or that elimination of this PPIase activity is not lethal to the organism. Experiments are under way to test this hypothesis by disruption of the gene in *S. chrysomallus*.

Ascomycin and rapamycin have been discovered to be antifungal compounds (1, 32) and may, like FK-506 and many other antibiotics, be important for the producing organism as part of its defense system against fungal competitors in soil, the natural habitat of nearly all streptomycetes.

The amino acid sequence of the *Streptomyces* FKBP shares extensive regions of strong sequence conservation with eukaryotic FKBP (Fig. 5). Identities of up to 50% (neurospora) were seen. When compared to the bacterial sequences, less homology was found. Their role is poorly understood. Nothing is known about the possible function of the sequence in *P. aeruginosa*. In vitro expression of the homologous region of *N. meningitidis* as a fusion protein revealed PPIase activity, which can be inhibited by FK-506 (30). Furthermore, two bacterial open reading frames with homologies to the FKBP gene differed from the eukaryotic ones in that only the C-terminal halves displayed the similarity to FKBP, indicating a specialized function of the encoded proteins. These proteins are bigger than FKBP (up to 27 kDa), and in fact one of these is the Mip protein, which has been previously identified as a virulence factor in *L. pneumophila* (5). Recently it was shown that the Mip protein has PPIase activity which is inhibitable in the nanomolar

range (10). In this context, it might be pointed out that even the *ninaA* gene product of drosophila shares, in part of its sequence, some homology with cyclophilin. This protein is assumed to have a specialized function in the protein folding of rhodopsin and the mechanism of its secretion (6, 35).

On the basis of their structure, function, and inhibition behavior, the *Streptomyces* FKBP are the first true FKBP from bacteria and stand close to the eukaryotic enzymes. A similar relationship was seen with *Streptomyces* cyclophilin, which shows high homology to the eucaryotic cyclophilins. By contrast, enterobacterial cyclophilin sequences shared much less homology with the *Streptomyces* cyclophilin (28).

The cellular functions of the PPIases are still unknown. We believe that the functions of FKBP and also cyclophilins in eucaryotes and streptomycetes might be very similar because of the similar properties and structures of the enzymes. Experiments are under way to test the possible role of PPIases in streptomycetes by gene disruptions and expression of the genes in heterologous systems.

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