Chemistry & Biology, Vol. 9, 367–373, March, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S1074-5521(02)00108-4

Hexaene Derivatives of Nystatin Produced as a Result of an Induced Rearrangement within the *nysC* Polyketide Synthase Gene in *S. noursei* ATCC 11455

Trygve Brautaset,[1] Per Bruheim,[1] Håvard Sletta,[2] Lars Hagen,[3] Trond E. Ellingsen,[2] Arne R. Strøm,[1] Svein Valla,[1] and Sergey B. Zotchev[1,4]
[1]Department of Biotechnology
Norwegian University of Science and Technology
N-7491 Trondheim
[2]SINTEF Applied Chemistry
SINTEF
N-7034 Trondheim
[3]Institute for Cancer Research
and Molecular Biology
Norwegian University of Science and Technology
N-7489 Trondheim
Norway

Summary

Genetic manipulation of the polyketide synthase (PKS) gene *nysC* involved in the biosynthesis of the tetraene antifungal antibiotic nystatin yielded a recombinant strain producing hexaene nystatin derivatives. Analysis of one such compound, S48HX, by LC-MS/MS suggested that it comprises a 36-membered macrolactone ring completely decorated by the post-PKS modification enzymes. Further characterization by bioassay has shown that S48HX exhibits antifungal activity. Genetic analysis of the hexaene-producing mutant revealed an in-frame deletion within the *nysC* gene via recombination between two homologous ketoreductase domain-encoding sequences. Apparently, this event resulted in the elimination of one complete module from NysC PKS, subsequently leading to the production of the nystatin derivative with a contracted macrolactone ring. These results represent the first example of manipulation of a PKS gene for the biosynthesis of a polyene antibiotic.

Introduction

The number of antifungal antibiotics currently available for human therapy is severely limited. The polyene macrolides represent a subgroup of polyketide antibiotics with potent antifungal activity due to their ability to interact with ergosterol in the fungal membranes, leading to the formation of transmembrane channels, ion leakage, and cell lysis [1]. Polyene antibiotics have large 20- to 40-membered macrolactone rings with chromophores composed of sets of 3–8 conjugated double bonds. It is suggested that the conjugated double bonds play a crucial role in the polyene-sterol interactions and thus are important for the antifungal activity [2].

Most of the polyene macrolide antibiotics are produced by the Gram-positive bacteria *Streptomyces* via biosynthetic routes that involve modular polyketide synthases (PKSs). The latter enzymes perform successive condensations of the simple carboxylic acids, resulting in the assembly of a polyketide chain, which is cyclized to form a macrolactone ring [3]. The conjugated double bonds on the macrolactone rings of polyenes apparently arise due to the reductive activities of specific domains embedded in the PKSs. Further modification of the polyene macrolactone ring via the action of monooxygenases and glycosyltransferases leads to the formation of a fully biologically active molecule [4]. Recently, a first report on the engineered biosynthesis of a novel derivative of the polyene antibiotic pimaricin has been published [5]. The data presented in this work confirm the importance of post-PKS modifications for the biological activity of polyenes and provide further insight into the structure-function relationship of the polyene antibiotics.

The properties of PKSs involved in the biosynthesis of macrolide antibiotics make it possible to change their structure and embedded enzymatic activities in such a way that new compounds with predictable chemical structures can be produced [6]. From this point of view, the PKSs involved in the biosynthesis of polyene macrolide antibiotics are excellent targets for manipulation that may provide novel antifungal agents with improved pharmacological properties.

The antibiotic nystatin, which is commonly used as an antifungal agent for the treatment of mycoses, is a member of the polyene macrolide group. This antibiotic is a rather unique tetraene in the sense that it has sets of two and four conjugated double bonds (the former not being considered a polyene feature) on its macrolactone ring that are separated by a fully saturated bond (Figure 1). We have recently cloned and sequenced the nystatin biosynthetic gene cluster from the producing organism *S. noursei* ATCC 11455 and proposed the complete biosynthetic pathway for this antibiotic [7]. Analysis of the gene cluster revealed that six polyketide synthase proteins are responsible for the formation of the nystatin macrolactone ring and the appearance of the conjugated double bonds. Taking advantage of this knowledge, we decided to genetically manipulate the nystatin PKS in order to produce novel antifungal antibiotics. In the current report, we describe an integration-induced rearrangement within the nystatin PKS gene in *S. noursei* that leads to the production of a hexaene nystatin derivative with antifungal activity.

Results

Inactivation of the ER Domain in Module 5 of the NysC PKS

The enoyl reductase (ER) domain in module 5 of the NysC PKS is apparently responsible for the appearance of a fully saturated bond between C-28 and C-29 on the nystatin molecule (Figure 1). Thus, by inactivating this particular domain, it is theoretically possible to obtain a compound with a double bond between C-28 and C-29, thus joining two sets of conjugated double bonds in the nystatin molecule and creating a heptaene macro-

[4]Correspondence: sergey.zotchev@chembio.ntnu.no

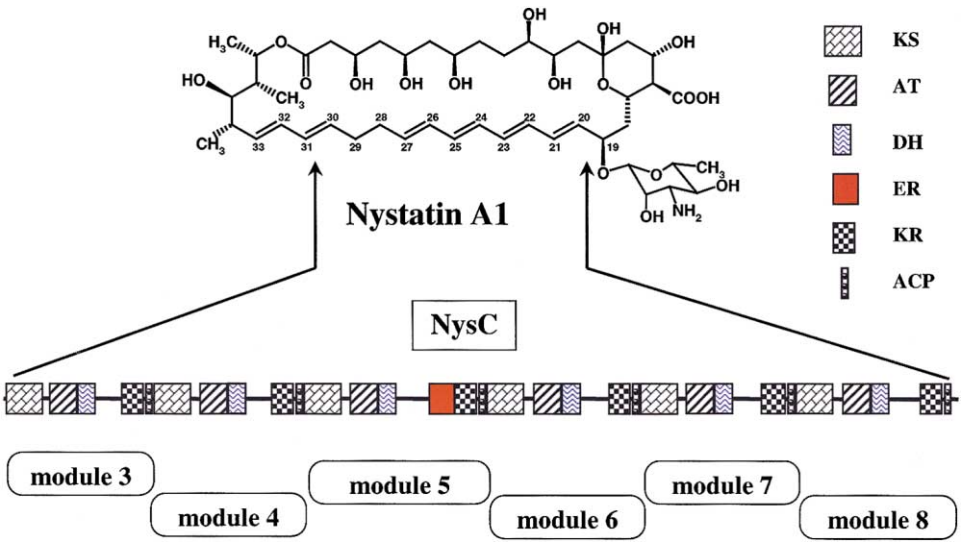


Figure 1. Role of the NysC PKS in the Biosynthesis of the Nystatin Macrolactone Ring

KS: β-ketoacyl synthase, AT: acyl transferase, DH: dehydratase, ER: enoyl reductase, KR: ketoreductase, ACP: acyl carrier protein.

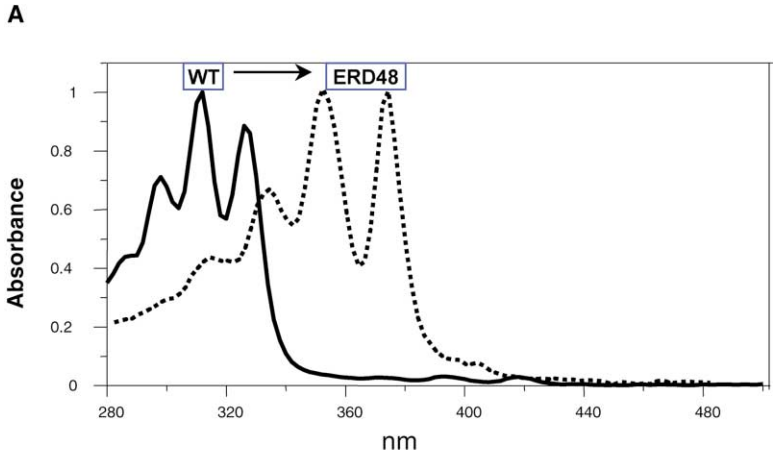
lide compound. To perform such an inactivation, the method of "in-frame" deletion by double homologous recombination using the suicide plasmid pERD4.2 (see the Experimental Procedures) was chosen. A gene replacement procedure resulted in isolation of the mutant ERD44 that, according to Southern blot analysis, contained the desired deletion eliminating a major portion of the ER5 domain-coding region within the *nysC* gene (data not shown). Preliminary analysis of the ERD44 mutant revealed that it did not produce nystatin, although polyene compounds with UV spectra characteristic for heptaens [8] were detected (data not shown). However, we have also detected traces of the heptaene-like compound in the extract of the wild-type (WT) strain in addition to nystatin (P.B., unpublished data). Because of that, and the low yield of the heptaene-like compound produced by the ERD44 mutant, the latter was not considered for further analysis during this study (also see the Discussion).

Deletion of One Complete Module from the NysC PKS Leads to the Production of Hexaene Compounds

During the screening for the ER5 deletion, a mutant ERD48 was isolated, which, according to the Southern blot analysis, contained a larger deletion compared to the ERD44 strain (data not shown). Surprisingly, UV-absorption spectra of DMSO extract of the ERD48 culture revealed an upward shift of about 60 nm compared to that of the wild-type culture extract containing nystatin (Figure 2A). The characteristic absorption spectra of polyenes are shifted according to the number of conjugated double bonds, and the three-peak absorption in the 340–380 nm range of the ERD48 extract is consistent with a hexaenic structure of the conjugated region on the polyene polyketide molecule [8]. For compounds produced by ERD48, this could be explained by a loss of C-28 and C-29 methylene functions in the nystatin molecule, leading to a joining of the two sets of conjugated double bonds.

Theoretically, the fully post-PKS-modified hexaene product of the ERD48 mutant should have a molecular weight of 897.6, corresponding to nystatin (MW 925.6) minus a CH_2-CH_2 moiety (MW 28). This molecular weight difference is referred to below as Δ28. Methanol extracts of the ERD48 and WT cultures were subjected to LC-diode array detector (DAD)-MS analysis, which showed that ERD48 produced a mixture of hexaene compounds (Figure 2B), while no hexaene-like substances were produced by the WT strain (data not shown). The molecular weights for the compounds represented by three major peaks are given in the DAD plot. The molecular weight of the compound eluting at 16 min (MW 897.6) corresponds to a 36-membered macrolactone ring completely decorated by the post-PKS modification enzymes. This novel compound was designated S48HX. Furthermore, the molecular weight 736.6 for the hexaene compound eluting at 7.5 min suggests that it represents a precursor of S48HX, which lacks both the C-10 hydroxyl and the C-19 mycosamine moiety. The third compound with molecular weight 752.6 eluting at 6.5 min is presumably an S48HX precursor lacking only the mycosamine moiety. Thus, it appears that the post-PKS modification of the hexaene aglycone produced by the ERD48 mutant proceeds at a variable extent, reflecting the specificity of the decorating enzymes toward the new substrate.

Extracts of the ERD48 and WT cultures were also subjected to LC-DAD-MS/MS analysis. The iontrap-isolated parent ions were fragmented in an automatic mode with a range of fragmentation amplitudes assuring optimal fragmentation conditions. Representative fragmentation patterns for the S48HX compound and nystatin are given in Figures 3A and 3B, respectively. A total of 11 major S48HX fragments (878.5, 816.5, 810.5, 799.5, 635.3, 617.5, 588.5, 407.3, 394.3, 341.3, and 305.3) could



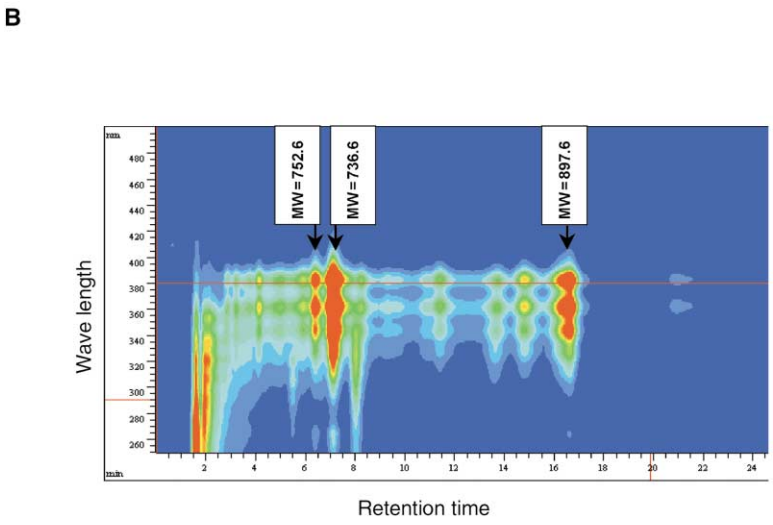


Figure 2. Analysis of Metabolites Produced by the *S. noursei* Wild-Type and ERD48 Strains

(A) A UV scan of DMSO extracts of *S. noursei* ATCC 11455 and ERD48 strains.

(B) Diode-array HPLC data for the ERD48 culture extract. Molecular weights (MW) for three major compounds determined by LC-MS/MS are indicated (see text for details).

be correlated by Δ28 to 11 major nystatin fragments (906.5, 844.5, 838.6, 827.5, 663.5, 645.5, 616.4, 435.1, 422.2, 369.1, and 333.3). These 11 peaks are interpreted as fragments of S48HX including the region with conjugated double bonds, which explains the difference in mass of 28 compared to the nystatin fragments. Therefore, both the UV spectrum and fragmentation analysis of S48HX tentatively suggest the structure of S48HX presented in Figure 3C.

Production of the S48HX compound by the ERD48 mutant would be consistent with an "in-frame" deletion of a DNA fragment encoding one complete module of the NysC PKS. In order to investigate this hypothesis in more detail, a PCR-amplification of the DNA fragment encompassing the suggested deletion was carried out from the genomes of both ERD48 and the WT strains (see the Experimental Procedures). As expected, a 9.0-kb fragment (including the entire module 5) was amplified from the genome of the *S. noursei* WT, while an ~2.7-kb fragment was amplified from the ERD48 genome (data not shown). Cloning and DNA sequencing of the latter fragment confirmed that it encodes part of a hybrid PKS module, consistent with recombination between DNA sequences encoding the highly homologous KR domains in modules 4 and 5 of NysC. This recombination has apparently led to a deletion of the DNA sequences encoding the C-terminal part of the KR domain and the complete ACP domain of module 4, and the KS, AT, DH, ER, and N-terminal part of the KR domain of module 5, thus resulting in the loss of one complete module from the NysC PKS (Figure 4). Based on the above data, we were able to predict the organization of the truncated version of NysC protein (NysCΔ48) in ERD48 (Figure 4), which is in agreement with the production of hexaene nystatin derivatives by this mutant.

Purification and Antifungal Activity of the S48HX Compound

A polyene fraction containing >95% S48HX was purified from the ERD48 culture extract by preparative reverse-phase HPLC and reverse-phase solid phase extraction (data not shown). The extinction coefficient ($E^{1\%}_{1cm}$) of 1499 (379-nm peak) was experimentally determined for this fraction. This value was used to calculate the level of

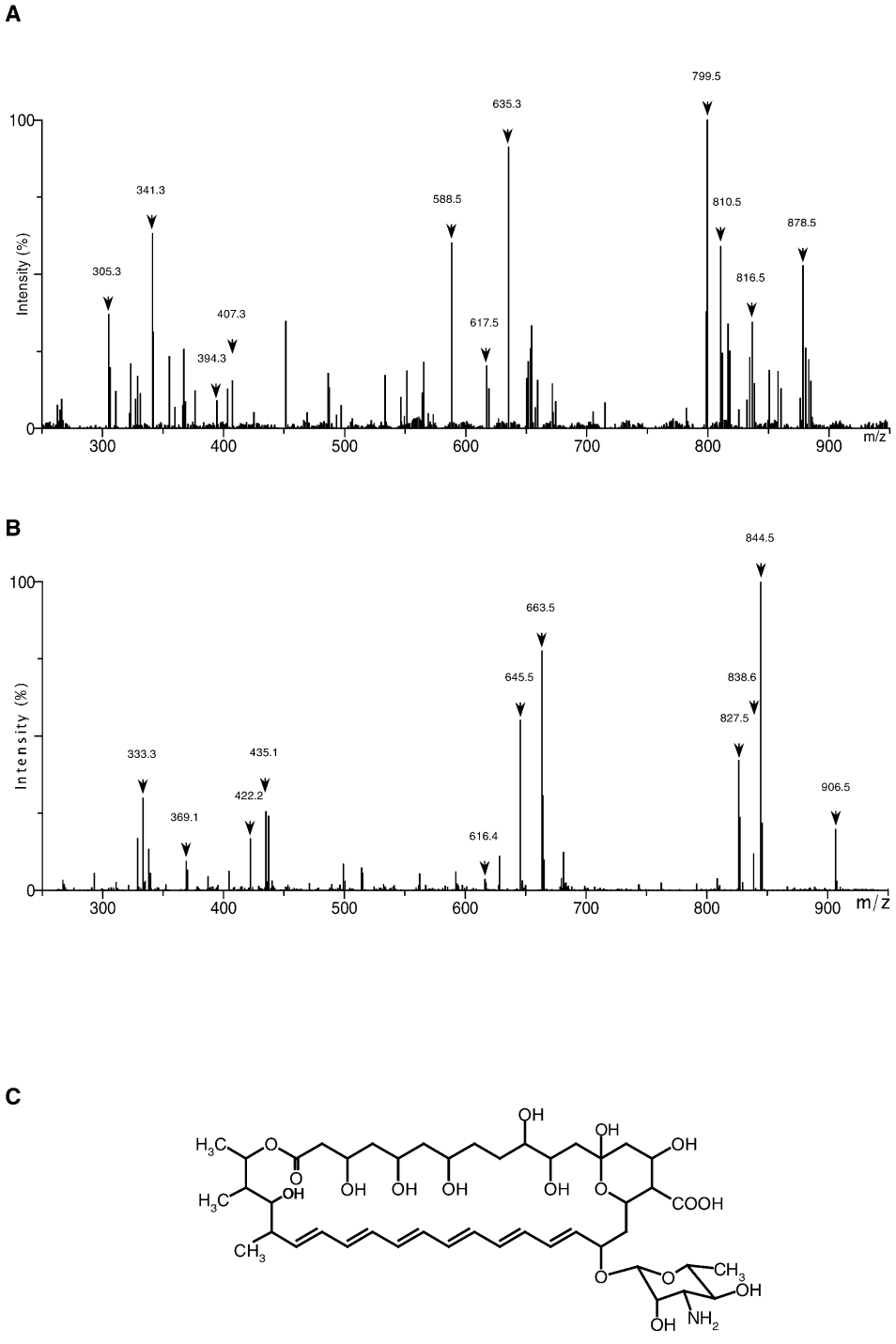


Figure 3. Preliminary Structural Characterization of the Hexaene Nystatin Derivative S48HX

(A and B) Fragmentation patterns for the (A) S48HX compound and (B) nystatin obtained during LC-MS/MS analysis of the culture extracts from the ERD48 mutant and the WT strain, respectively. Fragments correlated by Δ28 and described in the Results are indicated with arrows. (C) Proposed planar chemical structure of the S48HX compound.

S48HX production by the ERD48 mutant, which constituted approximately 1.2 g/l. In the same conditions, the level of nystatin production by the wild-type *Streptomyces noursei* was 4–5 g/l. The antifungal activity of the purified S48HX compound, along with that of nystatin, was assessed in a bioassay using *Candida albicans* ATCC 10231 as a test organism (see the Experimental Procedures). According to our results, the MIC_{50} for S48HX was shown to be 19.9 μg/ml, while the MIC_{50} for nystatin in the same experiment was found to be 0.7

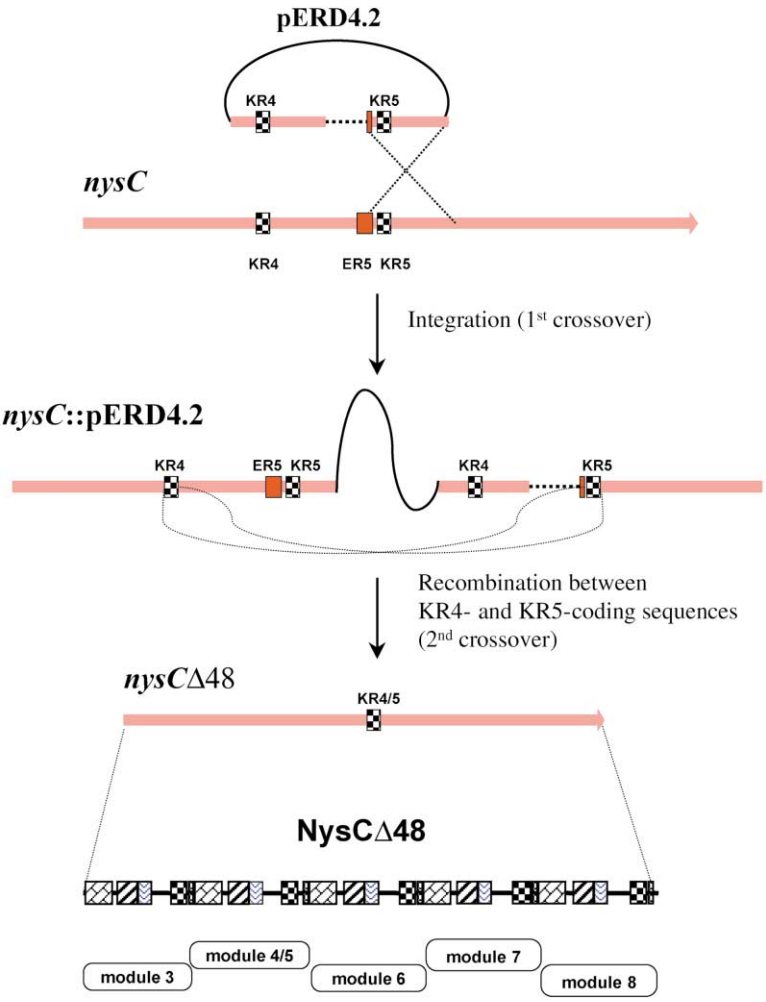


Figure 4. Model Explaining the Generation of the ERD48 Mutant

After integration of the pERD4.2 vector into the *nysC* gene, a second crossover between highly homologous KR4- and KR5-encoding sequences might take place. This event results in the elimination of one complete module from the NysC PKS (NysCΔ48).

μg/ml. The antifungal activity of S48HX is therefore approximately 30 times lower than that of nystatin.

Discussion

Genetic manipulation of polyketide synthases is a promising strategy for extending the diversity of the naturally occurring polyketide compounds [6]. With the aim of creating novel derivatives of the polyene antibiotic nystatin, we have attempted to modify the nystatin PKS NysC in the producing organism *S. noursei*. Originally, our aim was to produce a heptaene nystatin derivative via inactivation of the ER domain in module 5 of NysC that would be responsible for the formation of the saturated bond between C-28 and C-29. The resulting mutant ERD44 did not produce nystatin; although, traces of heptaene compounds were detected in the culture extracts. Upon the detailed analysis of the extracts from the WT *S. noursei*, we have also detected traces of a heptaene compound that disappeared upon insertional inactivation of the nystatin PKS genes (S.B.Z. and H.S., unpublished data). We are currently trying to understand the mechanisms behind this phenomenon with regard to nystatin biosynthesis by studying the heptaene biosynthesis in both WT and ERD44 *S. noursei* strains.

Fortuitously, integration of the plasmid designed for the ER5 inactivation into *S. noursei* and its subsequent elimination via second crossover led to the generation of another mutant, ERD48, producing hexaene nystatin derivatives. From the DNA sequencing data, it became clear that a homologous recombination had occurred between the DNA fragments encoding the KR4 and KR5 domains within the *nysC* gene disrupted by the vector, leading to a perfect "in-frame" deletion of one complete PKS module. These DNA fragments indeed share 99.9% sequence identity over the entire KR-encoding regions (742 nt) and most probably represent a case of a relatively recent duplication in the process of the nystatin gene's evolution. Moreover, the G + C content of KR4- and KR5-encoding DNA is substantially lower than that of the rest of the nystatin gene cluster (65% versus 74%), suggesting that this sequence was acquired from another organism with lower G + C content. It seems likely that the recombination event that yielded the ERD48 mutant was triggered by the integration of the gene replacement vector into this particular region of the *nysC* gene and that an alternative second crossover took place according to the model presented in Figure 4. This notion is supported by the fact that the hexaene-producing *S. noursei* strain was never found during the

previous extensive screening (E. Fjærvik and H.S., unpublished data). The data presented above support the hypothesis on PKS evolution in *Streptomyces* that proposes the acquisition of new modules brought in by mobile genetic elements, their integration into the PKS-encoding genes, and later rearrangement [9]. In this respect, the finding of IS-like elements in several PKS-encoding gene clusters [10, 11] might be of significant value to support this hypothesis.

The molecular weight of the new hexaene nystatin derivative S48HX produced by the ERD48 mutant is consistent with the fully modified 36-membered macrolide, suggesting a substantial tolerance of the post-PKS enzymes toward the new substrate. The LC-MS/MS fragmentation patterns for S48HX also are in agreement with the structural formula suggested for this compound (Figure 3C). The antifungal activity of S48HX was shown to be lower compared to nystatin, implying that not only a number of conjugated double bonds, but also the size of a macrolactone ring, might be important for the biological activity. Nevertheless, the real pharmaceutical value of the S48HX compound can be assessed only upon studying its cytotoxicity and other possible effects on the biological systems.

The fact that post-PKS modification enzymes can recognize and process contracted nystatin macrolactone rings further confirms the relaxed substrate specificity for macrolide aglycone-decorating enzymes. It has been shown previously that certain glycosyltransferases and hydroxylases are quite tolerant of changes in the macrolactone substrate [12, 13]. Data for the ERD48 mutant imply that at least three enzymes in the nystatin biosynthetic pathway, monooxygenases NysL and NysN, and putative mycosamine transferase NysDI, have rather relaxed substrate specificities. The degree of such flexibility for the nystatin aglycone modification enzymes is reflected in the metabolite profile of the ERD48 mutant (Figure 2B). All three major polyene compounds produced by this mutant seem to contain a carboxy group at C-16, suggesting that a monooxygenase responsible for this reaction has relaxed substrate specificity. The NysDI, putative mycosamine transferase, and C-10 hydroxylase, however, do not seem to possess the same tolerance toward the substrate, as more than half of the polyene compounds produced by ERD48 are represented by S48HX aglycone lacking the C-10 hydroxyl. Besides raising interesting issues on the enzyme-substrate interactions in the antibiotic biosynthesis, these data might also be useful in the future design of the experiments aimed at the production of novel nystatin derivatives.

Significance

Polyene antibiotics are the drugs of choice for fighting fungal infections. However, their use for treatment of systemic mycoses is limited due to their relatively high toxicity. Thus, there is a need for new polyene antibiotics with improved pharmacological properties. In the current study, we demonstrate the production of novel derivatives of the polyene antibiotic nystatin as a result of the rearrangement within the PKS gene that specifies its biosynthesis. Characterization of the mutant producing the hexaene nystatin derivative provides insight into the possible routes for the evolution of PKSs and paves the way for rational manipulation of the nystatin PKS with the aim of high-yield production of novel polyene antibiotics.

Experimental Procedures

Strains, Plasmids, and Growth Conditions

S. noursei strains were maintained on solid agar medium ISP2 (Difco) at 30°C. For total DNA isolation from *S. noursei* strains, the liquid medium TSB (3%, Oxoid) was used. The *Escherichia coli* strains DH5α and XL1-Blue were grown and manipulated as described elsewhere [14]. DNA transfer to *S. noursei* was accomplished via conjugation from the *E. coli* ET12567 (pUZ8002) strain according to the procedure established earlier [11]. The recombinant phage N45 [7] served as a template for PCR-assisted construction of a gene replacement plasmid. The vector pGEM3Zf(−) (Promega) was utilized for general cloning, while pSOK201 vector [11] was used for construction of the conjugative gene replacement plasmid. Fermentation of the WT and recombinant *S. noursei* strains as well as the gene replacement procedure were carried out essentially as described earlier [15]. *Candida albicans* ATCC 10231 used as a test organism for measuring antifungal activity was purchased from ATCC and was maintained according to recommendations from the supplier.

DNA Manipulation and Sequencing

DNA manipulation was performed essentially according to Sambrook et al. [14]. The DNA sequencing was carried out at QIAGEN GmbH (Germany).

Construction of the pERD4.2 Vector and the Gene Replacement Procedure

PCR-assisted amplification of a 394-bp DNA fragment representing the coding sequence for the C-terminal part of the ER domain and the N-terminal part of the KR domain in module 5 of NysC was performed. Two primers were used: ERD1 (5′-GTTGGTACCCCACT CCCGGTCCGCAC-3′, sense) and ERD2 (5′-CCAGCCGCATGCAC CACC-3′, antisense), with KpnI and SphI restriction enzyme cleavage sites, respectively (underlined). The resulting PCR fragment was digested with KpnI and SphI and ligated together with the 1828-bp BamHI/KpnI DNA fragment (encompassing the DH4-coding region of *nysC*) and the 1273-bp SphI/EcoRI DNA fragment (encompassing the KR5-, ACP5-, and KS6-encoding segments of *nysC*) into the EcoRI/BamHI-digested pGEM3Zf(−) vector. The recombinant plasmid of 6.7 kb designated pERD4.1, which contained a hybrid DNA fragment with an "in-frame" deletion in the NysC-coding region, was recovered. This deletion eliminated the coding region for the part of the DH-ER interdomain linker and the N-terminal part of the ER domain containing a putative NADP(H) binding site (aa 4829–5200 of NysC). From pERD4.1, the 3.7-kb EcoRI/HindIII insert was excised and ligated with the 3.0-kb EcoRI/HindIII DNA fragment from plasmid pSOK201, resulting in the pERD4.2 construct.

PCR-Assisted Amplification and Cloning of the PKS-Encoding DNA Fragment from the *S. noursei* ERD48 Genome

The PCR reaction aimed at amplification of the part of the rearranged region of the *nysC* gene in *S. noursei* ERD48 was carried out with oligonucleotide primers KR48.1 (5′-CCGCGTCGGATCCGCCGAC-3′) and KR48.2. (5′-AGCCTTCGAATTCGGCGCC-3′), which were derived from the DH4- and KS6-coding sequences of *nysC* and encompassed BamHI and EcoRI restriction sites, respectively (underlined). The 50 μl PCR mixture contained 0.1 μg *S. noursei* ERD48 total DNA, 25 pmol of each primer, dNTPs (350 μm), 1× PCR buffer from Expand High Fidelity PCR System (Roche Molecular Biochemicals), and 1.5 U of the DNA polymerase mixture from the same system. The PCR was performed on the Perkin Elmer GeneAmp PCR System 2400 with the following program: 1 cycle of denaturation at 96°C (4 min), 35 cycles of denaturation at 94°C (45 s) and annealing/synthe-

sis at 70°C (10 min), and 1 cycle of final annealing/synthesis at 72°C (10 min). The 2.7-kb DNA fragment obtained with this procedure was digested with EcoRI and BamHI restriction enzymes and ligated with pGEM3Zf(−) vector DNA digested with the same enzymes. The resulting plasmid, pKR48, was subjected to DNA sequence analysis. As a control, PCR amplification with the same primers was performed using the *S. noursei* ATCC 11455 DNA as a template.

Characterization of the Nystatin Derivatives

Cell pellets from 1-ml cultures were extracted with 10 ml methanol for 1 hr. After centrifugation, the methanol phase was applied directly both to the analytical and preparative LCs. The LC-DAD-MS/MS analysis was carried out using the Agilent 1100 series LC/MSDTrap system. The LC was operated as a gradient system at a flow rate of 0.3 ml min^{-1}. Eluent A contained 30% acetonitrile and 1.4% tetrahydrofuran in MilliQ water, and the pH was adjusted to 4.0 by 1 M acetic acid. Eluent B was pure acetonitrile. The initial conditions were 100% A (20 min), 40% B (25 min), and 100% B for 10 min. A Waters Nova-Pak C_{18} 2 × 150-mm column was used. The Iontrap mass spectrometer was operated with the electrospray ionization source in the negative ion mode. Drying gas flow was 8 l/min, and nebulizer pressure was 30 psi. Drying gas temperature was 350°C; Skim1 = −59.7V, the cap exit offset = −87.2V, and the trap drive setting = 69.1. During auto MS/MS experiments of eluting parent ions, the fragmentation amplitude was varied between 0.6 and 1.5V.

Preparative HPLC and Bioassay

The preparative HPLC system consisted of a manual sampling port, a Waters pump, a Shimadzu UV analyzator set at 370 nm, and a Pharmacia fraction collector. A Waters Nova-Pak C_{18} 3.9 × 300-mm column was used with a flow rate of 8 ml/min. The preparative LC was run isocratically with the mobile phase identical to eluent A (see above) supplemented with 0.1 g/l EDTA (pH was adjusted to 4.0 with 1 M acetic acid). Fractions (8 ml) were collected, and S48HX fractions from run 25 were pooled and diluted in the same volume of MilliQ water. A buffer change and concentration step were performed with solid phase extraction using Waters Nova-Pak C_{18} 3-ml 200-mg matrix columns, and the pure S48HX compound was eluted with methanol. The methanol was further evaporated on a Speed-Vac, and the sample was redissolved in a lower volume of methanol prior to bioactivity testing. The S48HX fraction was analyzed with the analytical LC-DAD system, and the purity was estimated by area comparison of all significant peaks to be >95%.

Bioactivity testing of S48HX was performed in 96-well plates with 120 μl TSB medium (3%, Oxoid) and 500 CFU of *Candida albicans* ATCC 10231 per well. A total of 30 μl of sample was added in appropriate dilutions. The plates were incubated for 16–20 hr at 30°C on a rotary shaker, and the OD_{490} was measured. Nystatin treated in the same manner with solid phase extraction and solvent evaporation was used as a standard.

Acknowledgments

We are grateful to Espen Fjærvik for his help on bioassays and participation in the discussions and to Jens Nielsen for providing the equipment for LC-MS/MS analyses. We wish to thank Randi Aune for running the *S. noursei* fermentations. This work was supported by the Research Council of Norway, SINTEF, and Alharma AS.

Received: October 24, 2001
Revised: December 14, 2001
Accepted: December 20, 2001

References

1. Bolard, J. (1986). How do the polyene macrolide antibiotics affect the cellular membrane properties? Biochim. Biophys. Acta *864*, 257–304.
2. Resat, H., Sungur, F.A., Baginski, M., Borowski, E., and Aviyente, V. (2000). Conformational properties of amphotericin B amide derivatives – impact on selective toxicity. J. Comput. Aided Mol. Des. *14*, 689–703.
3. Donadio, S., Staver, M.J., McAlpine, J.B., Swanson, S.J., and Katz, L. (1991). Modular organization of genes required for complex polyketide biosynthesis. Science *252*, 675–679.
4. Gil, J.A., and Martin, J.F. (1997). Polyene antibiotics. In Biotechnology of Antibiotics, W.R. Strohl, ed. (New York: Marcel Dekker), pp. 551–575.
5. Mendes, M.V., Recio, E., Fouces, R., Luiten, R., Martin, J.F., and Aparicio, J.F. (2001). Engineered biosynthesis of novel polyenes: a pimaricin derivative produced by targeted gene disruption in *Streptomyces natalensis*. Chem. Biol. *8*, 635–644.
6. Katz, L., and McDaniel, R. (1999). Novel macrolides through genetic engineering. Med. Res. Rev. *19*, 543–558.
7. Brautaset, T., Sekurova, O.N., Sletta, H., Ellingsen, T.E., Strøm, A.R., Valla, S., and Zotchev, S.B. (2000). Biosynthesis of the polyene antibiotic nystatin in *Streptomyces noursei* ATCC 11455: analysis of the gene cluster and deduction of the biosynthetic pathway. Chem. Biol. *7*, 395–403.
8. Omura, S., and Tanaka, H. (1984). Production, structure, and antifungal activity of polyene macrolides. In Macrolide Antibiotics: Chemistry, Biology, and Practice, S. Omura, ed. (New York: Academic Press), pp.351–405.
9. Hopwood, D.A. (1997). Genetic contributions to understanding polyketide synthases. Chem. Rev. *97*, 2465–2497.
10. Donadio, S., and Staver, M.J. (1993). IS*1136*, an insertion element in the erythromycin gene cluster of *Saccharopolyspora erythraea.* Gene *126*, 147–151.
11. Zotchev, S.B., Haugan, K., Sekurova, O., Sletta, H., Ellingsen, T.E., and Valla, S. (2000). Identification and partial characterisation of the gene cluster governing biosynthesis of a novel antibacterial polyketide-derived antibiotic in *Streptomyces noursei* ATCC 11455. Microbiology *146*, 611–619.
12. Graziani, E.I., Cane, D.E., Betlach, M.C., Kealey, J.T., and McDaniel, R. (1998). Macrolide biosynthesis: a single cytochrome P450, PicK, is responsible for the hydroxylations that generate methymycin, neomethymycin, and picromycin in *Streptomyces venezuelae*. Bioorg. Med. Chem. Lett. *8*, 3117–3120.
13. Tang, L., and McDaniel, R. (2001). Construction of desosamine containing polyketide libraries using a glycosyltransferase with broad substrate specificity. Chem. Biol. *8*, 547–555.
14. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Second Edition (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).
15. Sekurova, O., Sletta, H., Ellingsen, T., Valla, S., and Zotchev, S.B. (1999). Molecular cloning and analysis of a pleiotropic regulatory gene locus from the nystatin producer *Streptomyces noursei* ATCC 11455. FEMS Microbiol. Lett. *177*, 297–304.

Accession Numbers

The DNA sequence for the PCR fragment amplified from the *S. noursei* ERD48 genome was deposited into the GenBank under the accession number AF420594.