Engineering of a minimal modular polyketide synthase, and targeted alteration of the stereospecificity of polyketide chain extension

Ines Böhm[1], Inès E Holzbaur[2], Ulf Hanefeld[2]*, Jesús Cortés[1], Jim Staunton[2] and Peter F Leadlay[1]

Background: Polyketides are a large and structurally diverse group of natural products that include antibiotics, antifungal agents and immunosuppressant compounds. Polyketides are biosynthesised in filamentous bacteria on modular polyketide synthases (PKSs) in which each cycle of chain extension requires a different 'module' of enzymatic activities. The recently proposed dimeric model for modular PKSs predicts that even a single-module PKS should be catalytically active in the absence of other PKS components. Researchers are also interested in manipulating the stereochemical outcome of polyketide chain extension using genetic engineering of domains within each module.

Results: We have constructed a minimal modular PKS from the erythromycin-producing PKS (DEBS) of *Saccharopolyspora erythraea*. The diketide synthase (DKS1-2) consists of a single chimaeric extension module, derived from the DEBS module 1 ketoacyl-ACP synthase (KS), sandwiched between a loading module and a chain-terminating thioesterase. When DKS1-2 was expressed in *S. erythraea*, the strain preferentially accumulated the diketide (2*R*,3*S*)-2-methyl-3-hydroxy pentanoic acid.

Conclusions: These results demonstrate that, as predicted, even a single-module PKS is catalytically active in the absence of other DEBS proteins. In its normal context, the ketosynthase domain KS1 is thought to generate a (2*S*)-2-methyl-3-hydroxy intermediate by epimerising the initial product of carbon–carbon chain formation, the (2*R*)-2-methyl-3-ketoester. The observed formation of the alternative (2*R*)-2-methyl-3-hydroxy product catalysed by DKS1-2 provides strong support for this proposal, and indicates how targeted alteration of stereospecificity can be achieved on a modular PKS.

Addresses: [1]Cambridge Centre for Molecular Recognition and Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1GA, UK. [2]Cambridge Centre for Molecular Recognition and University Chemical Laboratory, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK.

*Present address: Technische Universiteit Delft, Julianalaan 67, 2628BC Delft, The Netherlands.

Correspondence: Peter F Leadlay
E-mail: pfl10@mole.bio.cam.ac.uk

Key words: minimal polyketide synthase, modular polyketide synthase, module swap, *Saccharopolyspora erythraea, Streptomyces*

Received: **1 May 1998**
Revisions requested: **29 May 1998**
Revisions received: **3 June 1998**
Accepted: **4 June 1998**

Published: **6 July 1998**

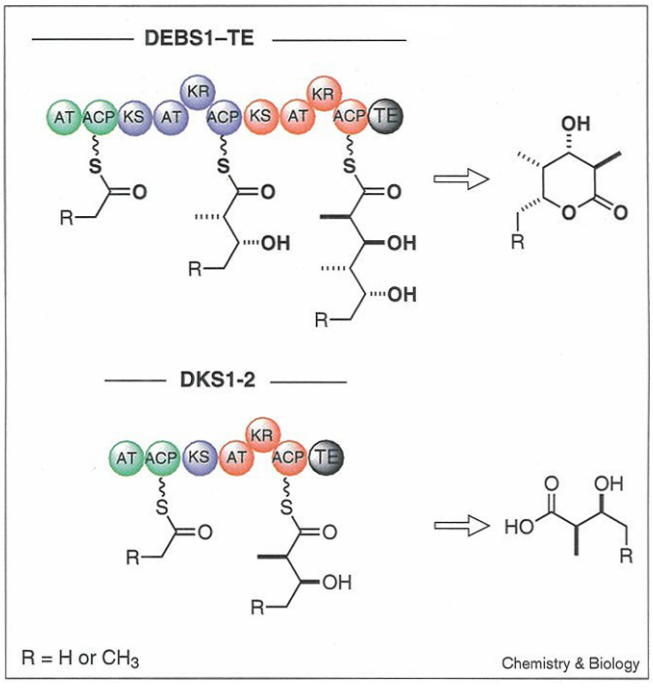
Chemistry & Biology August 1998, **5**:407–412
http://biomednet.com/elecref/1074552100500407

Introduction

Macrocyclic polyketides form a large and structurally diverse class of natural products, synthesized principally by soil microorganisms like *Streptomyces* and related filamentous bacteria, and including numerous clinically important antibiotics, antifungal, antiparasitic and antitumor compounds, as well as immunosuppressants. They are synthesized on modular polyketide synthases (PKSs) that consist of giant multienzymes [1–9] housing sets or 'modules' of enzymic activities, each module containing the enzymes required to catalyze a particular round of polyketide chain extension and (where appropriate) reduction, using as substrates simple carboxylic acids activated as their coenzyme A (CoA) esters. The erythromycin-producing PKS (6-deoxyerythronolide B synthase, DEBS), for example, contains six extension modules distributed between three dimeric multienzymes DEBS1, DEBS2 and DEBS3 [10]. Initiation of polyketide synthesis occurs on a loading module at the amino terminus of DEBS1, and chain termination/cyclisation is catalyzed by a thioesterase (TE)/cyclase at the carboxyl terminus of DEBS3 [1–3].

The unusual size and complexity of modular PKSs (the rapamycin-producing PKS, for example, has more than 65 different active sites that are used in succession [5,6]) has hindered studies of their structure and function, but a convenient model system has been engineered by relocating the DEBS chain-terminating TE to the carboxyl terminus of the DEBS1 multienzyme [11,12]. The resulting bimodular PKS (DEBS1–TE, M_r 780,000 efficiently catalyzes the production of triketide lactones, both *in vivo* [11,12] and *in vitro* [13,14], demonstrating that the DEBS1 multienzyme is catalytically competent in the absence of the other DEBS proteins (Figure 1). Relocation of the TE domain to the carboxyl terminus of module 3 in DEBS2 [15] and of module 5 in DEBS3 [12] also produces the anticipated truncated products. The DEBS1–TE model

Figure 1



Domain organisation of DEBS1–TE and of diketide synthases DKS1-2. Green spheres represent domains originating from the loading domain, blue spheres represent domains from DEBS module 1 and red spheres from DEBS module 2. AT, acyltransferase; ACP, acyl carrier protein; KS, ketosynthase; KR, ketoreductase; TE, thioesterase.

system has been used to demonstrate that interspecific hybrid modular PKSs are functional: transplantation of a malonyl-CoA-specific acyltransferase (AT) domain from extension module 2 of the rapamycin PKS to replace the methylmalonyl-CoA-specific AT domain of extension module 1 of DEBS gave rise to the expected altered triketide lactones [16]. Numerous further examples of such domain swaps have since been reported [8,17–19] in which a polyketide template has been specifically modified, raising the hope that significant numbers of novel bioactive compounds might in future be produced using such methods [20]. However, the efficiency of this engineering would be greatly enhanced by a better understanding of the structure and function of the PKS. In particular, we are far from being able rationally to alter the stereochemical outcome of polyketide chain extension of the PKS to produce all the possible stereoisomers.

The initial purification of individual DEBS proteins to homogeneity [10] showed that, as judged by gel-filtration chromatography, each of the multienzymes DEBS1, DEBS2 and DEBS3 appeared to be a homodimer. This finding was supported by data from analytical ultracentrifugation, limited proteolysis and chemical cross-linking experiments [21]. Consideration of the known, head-to-tail, arrangement of the homodimeric mammalian fatty acid synthase [22] led to the proposal [21] of a dimeric structure common to all modular PKSs. For example, the DEBS1 proteins are intertwined and in the dimer module 1 makes functional contacts with module 1 in the other protein chain and module 2 likewise makes functional contacts with module 2 in the other protein chain. The dimeric arrangement has been confirmed by recent complementation studies [23,24] using elegant methodology developed by Smith and coworkers [25].

The dimeric helical model predicts that even a single module PKS should be catalytically competent. Although several natural PKSs are known to contain proteins housing a single extension module [8,9], and others have been engineered [12,15,18], none of these have been shown to function in the absence of other PKS proteins. We report here the design and construction of a PKS derived from DEBS that contains a single extension module between the DEBS loading module and the DEBS TE. Here we have shown that a recombinant strain housing this enzyme and no other DEBS proteins, does indeed synthesise diketide. Our finding that the diketide produced is substantially the (*2R*,*3S*) isomer strongly supports recent conclusions about the molecular basis of the stereochemical control of polyketide chain extension, as embodied in Celmer's rules [26], and also illustrates how targeted alteration in the configuration of a polyketide product can be achieved.

Results and discussion

Construction of the diketide synthase DKS1-2

A loading module is required for efficient chain initiation on modular PKSs, so the DEBS loading module that consists of a propionyl-CoA-selective AT–ACP didomain was selected. Similarly, efficient chain termination requires catalysis [11], and the DEBS TE, which has been shown to possess the required broad specificity [13,14], was chosen. For the single extension module, the ketosynthase (KS) domain of extension module 1 of DEBS was combined with the AT, ketoreductase (KR) and acyl carrier protein (ACP) of the DEBS extension module 2. The resulting chimaeric diketide synthase (DKS1-2) is illustrated in Figure 1.

For the construction of the diketide synthase DKS1-2 an additional restriction site was engineered into the structural gene for DEBS1–TE using the polymerase chain reaction (PCR) with mutagenic primers, to produce a plasmid in which the DNA regions encoding the KS domains from module 1 (KS1) and module 2 (KS2), were flanked on the 3′ end by a *Hin*dIII site. This was carried out with a pT7-7 based plasmid containing the entire open reading frame of DEBS1–TE (in which the original *Hin*dIII site had previously been removed). Digestion with *Hin*dIII and religation resulted in a plasmid carrying the DKS1-2 gene. The gene for DKS1-2 was then cloned

into the expression plasmid pCJR24 [27], using the *Nde*I site at the start codon and an *Xba*I site six bases downstream from the stop codon, resulting in plasmid pIB015. A strain of *Saccharopolyspora erythraea* from which the resident DEBS genes had been deleted [27] was transformed with pIB015 giving strain JC2/pIB015.

Production of diketide acids by the engineered diketide synthase

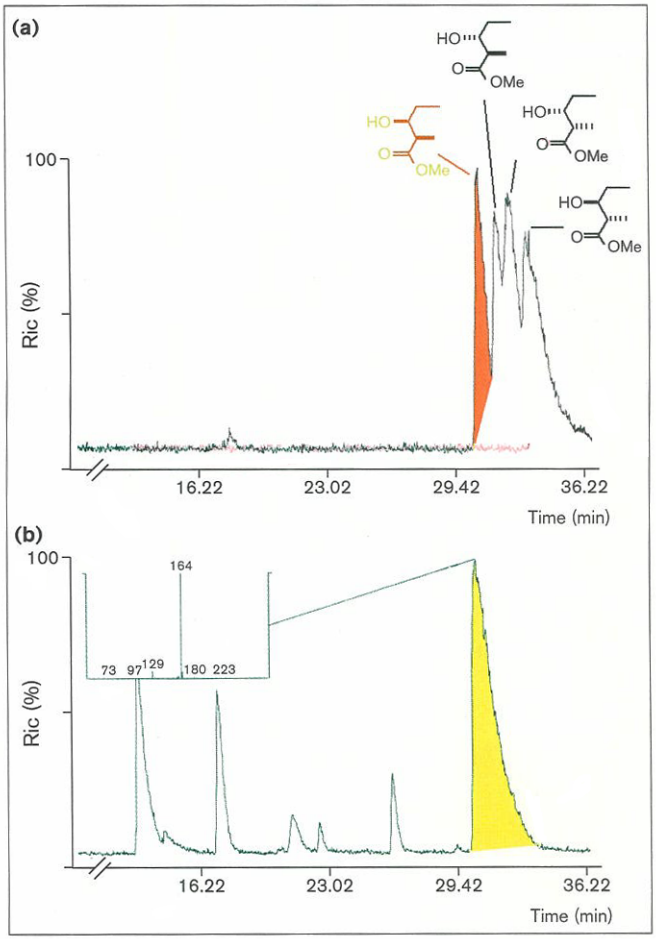
S. erythraea strain JC2/pIB015 was grown for five days. Organic acids were extracted and esterified with diazomethane and the methyl esters were separated and analysed by chiral gas chromatography coupled to a mass spectrometer (GC–MS). The GC column was calibrated using methyl esters of synthetic samples of each of the four possible stereoisomers of 2-methyl-3-hydroxypentanoic acid methyl ester (Figure 2). The extract of the fermentation broth from strain JC2/pIB015 contains substantially (>90%) the specific isomer (2*R*,3*S*)-2-methyl-3-hydroxypentanoic acid, and in reasonable yield (30 mg/l). The exact mass of this product, determined as the monosodium adduct, was in good agreement with the proposed structure (M 169.08606 calculated; M 169.08439 found). No diketide products were detected in extracts of the fermentation broth of the untransformed control strain JC2.

Feeding of diketide acids to polyketide-producing actinomycetes has been reported to result in the degradation of the diketide precursor by β-oxidation inside the cell [28]. Such an effect might have biased our results if the enzymes of β-oxidation in *S. erythraea* were active and highly selective for isomers of 2-methyl-3-hydroxypentanoic acid other than the (2*R*,3*S*)-isomer. Addition of 4-(tetradecylthio) propionic acid (0.1 mg/ml) and 4-pentynoic acid (0.1 mg/ml), known inhibitors of β-oxidation, to the fermentation of the recombinant strain (on day 2) did not significantly increase the amount of diketide acid found and the only isomer of the diketide acid detected under these conditions was again substantially the (2*R*,3*S*)-2-methyl-3-hydroxypentanoic acid.

When cells of the recombinant strain were disrupted and subjected to sodium dodecyl sulfate– polyacrylamide gel electrophoresis (SDS–PAGE), a polypeptide band was visible after Coomassie blue staining that was absent from extracts of the control strain and that had a molecular mass of 240 kDa, in good agreement with the subunit molecular mass expected for the diketide synthase (data not shown). Gel filtration of the cell extracts on a calibrated gel-filtration column confirmed that this protein behaved as a dimeric species, with an apparent M_r of 567 000.

The production of (2*R*,3*S*)-2-methyl-3-hydroxypentanoic acid by the recombinant strain provides direct evidence that the engineered chimaeric diketide synthase is competent in polyketide chain extension in the absence of other DEBS

Figure 2

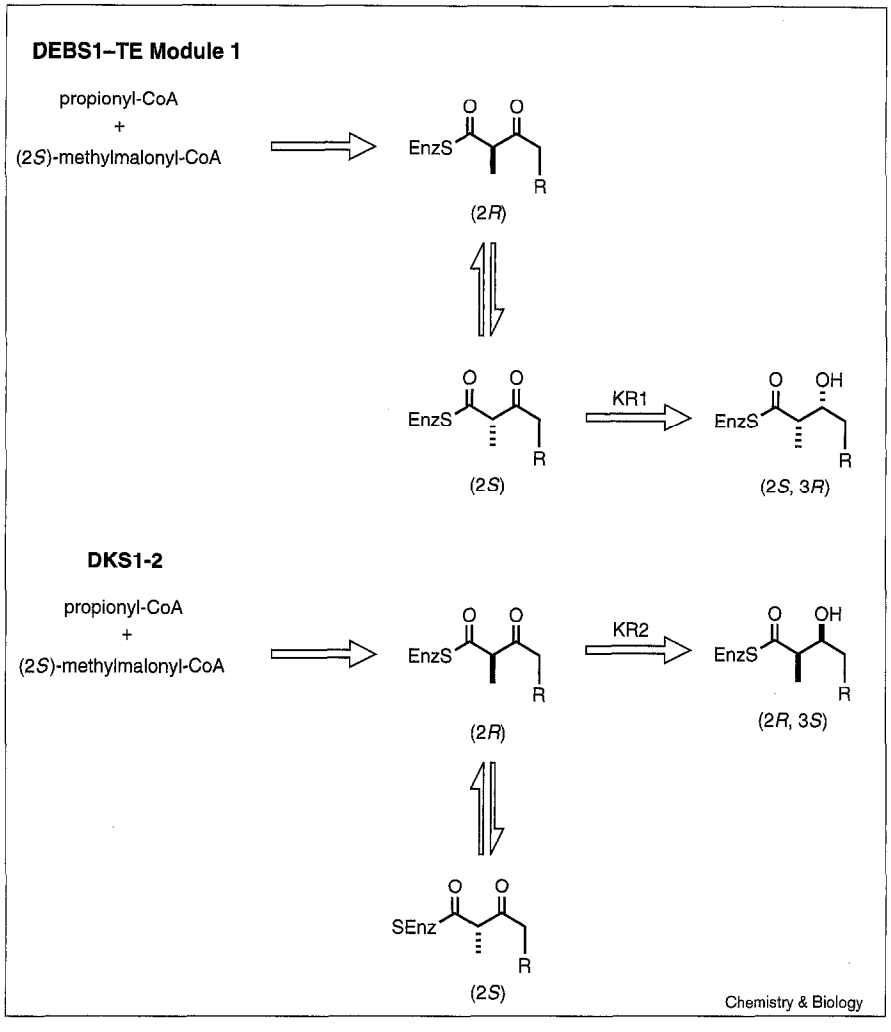


GC–MS traces of **(a)** the four stereoisomers of the methyl esters and **(b)** the methyl ester found in the broth from strain JC2/pIB015, including the MS spectrum of the (2*R*, 3*S*)-3-hydroxy-2-methyl-pentanoic acid methyl ester (ammonia as ionisation gas). Peaks corresponding to the (2*R*, 3*S*)-3-hydroxy-2-methyl-pentanoic acid methyl ester are shaded in red.

proteins. The single module is therefore the minimal functional unit of a modular PKS, as predicted by the dimeric model. The ability to create a single module catalytically competent PKS strengthens still further the functional and structural relationship between the bacterial modular PKSs and the dimeric mammalian fatty acid synthase. Interestingly, a fatty acid synthase deficient in dehydratase activity has been shown to produce the diketide (*R*)-3-hydroxybutyrate as its CoA ester [29]. The diketide synthase is also an attractive target for future structural study because of its favourable size and its catalytic competence.

These results also reinforce the broad utility of the TE domain in catalyzing chain release — diketides normally remain tightly covalently bound to a modular PKS during polyketide synthesis. Previous model studies with purified DEBS ACP–TE and the *N*-acetylcysteamine esters

Figure 3



The predicted stereochemical outcome of polyketide chain extension on truncated PKS multienzymes.

of the four possible stereoisomers of (2*R*,3*S*)-2-methyl-3-hydroxypentanoic acid have shown that hydrolysis of all four isomers occurs with approximately the same efficiency [30]. The observed production (>90%) of the (2*R*,3*S*)-isomer by DKS1-2 cannot therefore be ascribed to a difficulty in off-loading another isomer of the diketide, a situation that would have additionally led to a rapid inhibition of the enzyme.

DKS1-2 was designed to contain a chimaeric module, in which the ketosynthase domain of DEBS module 1 is combined with the AT, KR and ACP domains of DEBS module 2 (Figure 1). As shown in Figure 1, the stereochemical outcome of catalysis by DEBS module 1 is exactly the opposite of the outcome catalyzed by module 2. Such variations are consistently seen in natural macrocyclic polyketides, and have been codified into a set of empirical configurational rules known as Celmer's rules [31]. The activity of DKS1-2 therefore also provided an opportunity to examine the consequences of pairing domains from modules that normally produce different stereochemical outcomes.

Previous work has shown that the AT domains of DEBS, contrary to early speculation, do not contribute to the stereochemical outcome — all six AT domains in DEBS extension modules bind (2*S*)-methylmalonyl-CoA and not the (2*R*)-isomer *in vitro*, and only the (2*S*) isomer is used as substrate by purified DEBS1–TE [32]. This observation conclusively eliminated the hypothesis that both (2*R*)- and (2*S*)-isomers serve as substrates in the DEBS1–TE system and hold the key to the stereochemical outcome. More recent work on DEBS1–TE has also revealed that module 1, but not module 2, possesses an epimerase activity that converts the initially formed (2*R*)-2-methyl-3-oxopentanoic thioester (Figure 3) into the (2*S*)-isomer, which is then reduced by KR1 [26]. For KS2, condensation of (2*S*)-methylmalonyl-CoA also leads to the (2*R*)-ketoacyl thioester, but epimerisation is not necessary to provide the KR2 with its preferred substrate.

The predicted outcomes of diketide synthesis on DKS1-2 (housing the chimaeric module containing KS1 and KR2) are also shown in Figure 3. Initial condensation catalyzed by KS1 leads to the (2*R*)-ketoacyl thioester, which equilibrates with the (2*S*)-isomer on the enzyme. But the (2*R*)-isomer is the preferred substrate for KR2, and so the predicted diketide product is (2*R*,3*S*)-2-methyl-3-hydroxypentanoic acid, rather than the (2*S*,3*R*)-isomer that is produced by module 1 of DEBS. This outcome is exactly what was seen for DKS1-2 (Figure 2). Our observation that (2*R*,3*S*)-2-methyl-3-hydroxypentanoic acid is the predominant product (>90%) of the chimaeric diketide synthase is, therefore, strong evidence that in module 1 of DEBS KS1 is indeed capable of synthesis of the (2*R*)-ketoester [26], that epimerisation to the (2*S*)-ketoester occurs after C–C bond formation and not before and that KR1 is responsible for selection of the correct enantiomer for reduction. The potential synthetic utility of these observations is clear — the judicious choice of components in a chimaeric extension module can bring about a targeted alteration of the stereochemistry at both of the methyl- and hydroxy-bearing centres in appropriate polyketide products.

Significance

Modular polyketide synthases (PKSs) are promising tools for the combinatorial biosynthesis of complex polyketides, including both analogues of known polyketide drugs and compounds with wholly novel bioactivity. The demonstration that a minimal PKS, consisting of a single chimaeric extension module sandwiched between a loading module and a chain-terminating thioesterase, is catalytically competent in the absence of other PKS components both supports a recently proposed dimeric model for these enzymes and also provides a more accessible target for structural studies, in which all the key protein–protein interactions of a modular PKS are retained. The recombinant strain containing the minimal PKS, derived from the erythromycin-producing PKS (DEBS), produces predominantly one stereoisomer (>90%) of the diketide product, whose configuration differs from that produced by module 1 of DEBS. This outcome reinforces the idea that the epimerisation found in certain modules such as DEBS module 1 occurs after C–C bond formation and not before, and also that the stereochemical outcome of polyketide chain extension depends upon interplay between both the ketosynthase domain and the ketoreductase domain within each module. It also suggests how targeted alterations in polyketide configuration can be achieved.

Materials and methods

Strains and plasmids

All DNA manipulations were performed in *Escherichia coli* DH10B (Gibco) using standard culture conditions [33]. *S. erythraea* strains containing plasmids with SCP2* were grown at 30°C in TSB (Gibco) containing thiostrepton (5 mg/l; Sigma).

Manipulation of DNA

Routine cloning and transformation procedures were as previously described for *Escherichia coli* [33]. PCR was performed using *Pwo* polymerase (Boehringer) according to the manufacturer's instructions. Transformation of *S. erythraea* was carried out using the method of Yamamoto *et al.* [34]; plasmid-containing *S. erythraea* were selected on solid medium containing thiostrepton (25 mg/l).

Construction of the expression plasmid pIB015

The *Hin*dIII site from plasmid pNTEP2, a pT7-7 based plasmid containing the entire open reading frame for DEBS1–TE [16], was removed to give pNTEPH. Using PCR with pNTEPH as a template two new *Hin*dIII restriction sites were created at positions 2895 and 7316 (numbered starting with the ATG of the open reading frame), at the end of the conserved KS1 and KS2 domains, respectively. The resulting plasmid was called pIB007. Plasmid pIB007 was digested with *Hin*dIII and religated to give pIB011. The resulting diketide synthase DKS1-2 contains the loading module comprising AT and ACP domains, KS1, AT2, KR2 domains, a chimaeric ACP from DEBS modules 2 and 6, and the TE domain. The diketide synthase was subsequently cloned into pCJR24, an expression plasmid for *S. erythraea*, as an *Nde*I/*Xba*I fragment to give plasmid pIB015.

Analysis of the (2R,3R)-3-hydroxy-2-methyl-pentanoic acid product

Fermentation broths containing diketide acid were acidified to pH 2.5, solid NaCl was added to near saturation, and the aqueous broth was extracted three times with an equal volume of ethyl acetate. The combined extracts were esterified with diazomethane and analysed by GC–MS on a GCQ instrument (Finnigan MAT). A HydrodexβPM column (25 m × 0.25 mm ID) (Machery-Nagel, Germany) was used, with helium as the carrier gas. The constant gas velocity was 50 cm/s and the injector temperature was 250°C. The temperature program used to separate the four possible diketide methyl esters was as follows: 40°C for 2 min, then a gradient of 3°C/min to 70°C, and then 70°C for 25 min.

The four diketide esters [35] had the following retention times on the chiral GC column: (2*R*, 3*S*)-3-hydroxy-2-methyl-pentanoic acid methyl ester: 30:50 min; (2*R*,3*R*)-3-hydroxy-2-methyl-pentanoic acid methyl ester: 31:51 min; (2*S*, 3*R*)-3-hydroxy-2-methyl-pentanoic acid methyl ester: 32:27 min ; (2*S*, 3*S*)-3-hydroxy-2-methyl pentanoic acid methyl ester: 33:30 min. The fragmentation pattern for an authentic standard was as follows (GC–MS, CI mode with methane as reagent gas): $(M)H^+$ m/z = 147; $(M\text{-}H_2O)H^+$ m/z = 129; $(M\text{-}CH_3OH)H^+$ m/z = 115; $(M\text{-}CH_3OH\text{-}H_2O)H^+$ m/z = 97. With NH_3 as reagent gas: $(M)NH_4^+$ m/z = 164. The product from strain JC2/pIB015 showed a retention time and fragmentation patterns identical to those of the authentic sample of (2*R*, 3*S*)-3-hydroxy-2-methyl-pentanoic acid methyl ester (Figure 2). When identical quantities were injected the peak width at half height for this isomer was the same, irrespective of the origin of the sample.

Acknowledgements

We thank Laurenz Kellenberger and Marko Oliynyk for helpful discussions, John Lester and Kate Pennock for DNA sequence analysis, and Gordon Kearney for assistance with GC–MS analysis. This research was supported by grants from Glaxo-Wellcome and from the Biotechnology and Biological Sciences Research Council to P.F.L. and J.S., and by grants from the Fonds pour la Formation de Chercheurs et l'aide à la Recherche and from the Cambridge Commonwealth Trust to I.E.H.

References

1. Cortés, J., Haydock, S.F., Roberts, G.A., Bevitt, D.J. & Leadlay, P.F. (1990). An unusually large multifunctional polypeptide in the erythromycin-producing polyketide synthase of *Saccharopolyspora erythraea*. *Nature* **348**, 176-178.
2. Donadio, S., Staver,M.J., McAlpine, J.B., Swanson, S.J. & Katz, L. (1991). Modular organisation of genes required for complex polyketide biosynthesis. *Science* **252**, 675-679.

3. Bevitt, D.J., Cortés, J., Haydock, S.F. & Leadlay, P.F. (1992). 6-Deoxyerythronolide-B synthase 2 from *Saccharopolyspora erythraea*. *Eur. J. Biochem.* **204**, 39-49.
4. Swan, D. G., Rodriguez, A. M. , Vilches, C., Mendez, C. & Salas, J.A. (1994). Characterisation of a *Streptomyces antibioticus* gene encoding a type I polyketide synthase which has an unusual coding sequence. *Mol. Gen. Genet.* **242**, 358-362.
5. Schwecke, T. *et al.*, & Leadlay, P.F. (1995). The biosynthetic gene cluster for the polyketide immunosuppressant rapamycin. *Proc. Natl Acad. Sci. USA* **92**, 7839-7843.
6. Aparicio, J.F., *et al.*, & Leadlay, P.F. (1996). Organisation of the biosynthetic gene cluster for rapamycin in *Streptomyces hygrocopicus*: analysis of the enzymatic domains in the modular polyketide synthase. *Gene* **169**, 9-16.
7. MacNeil, D.J. *et al.*, & Danis, S.J. (1992). Complex organisation of the *Streptomyces avermitilis* genes encoding the avermectin polyketide synthase. *Gene* **115**, 119-125
8. Kuhstoss, S., Huber, M., Turner, J.R., Paschal, J.W. & Rao, R.N. (1997). Production of a novel polyketide through the construction of a hybrid polyketide synthase. *Gene*, **183**, 231-236.
9. Kakavas, S.J., Katz, L. & Stassi, D. (1997). Identification and characterisation of the niddamycin polyketide synthase genes from *Streptomyces caelestis*. *J. Bacteriol.* **179**, 7515-7522.
10. Caffrey, P., Bevitt, D.J., Staunton, J. & Leadlay, P.F. (1992). Identification of DEBS1, DEBS2 and DEBS3, the multienzyme polypeptides of the erythromycin-producing polyketide synthase from *Saccharopolyspora erythraea*. *FEBS Lett.* **304**, 225-228.
11. Cortés, J., Wiesmann, K.E.H., Roberts, G.A., Brown, M.J., Staunton, J. & Leadlay, P.F. (1995). Repositioning of a domain in a modular polyketide synthase to promote specific chain cleavage. *Science* **268**, 1487-1489.
12. Kao, C.M., Luo, G., Katz, L., Cane, D.E. & Khosla, C. (1995). Manipulation of macrolide ring size by directed mutagenesis of a modular polyketide synthase. *J. Am. Chem. Soc.* **117**, 9105-9106.
13. Wiesmann, K.E.H., Cortés, J., Brown, M.J.B., Cutter, A.L., Staunton, J. & Leadlay, P.F. (1995). Polyketide synthesis *in vitro* on a modular polyketide synthase. *Chem. Biol.* **2**, 583-589.
14. Pieper, R., Luo, G., Cane, D.E. & Khosla, C. (1995). Cell-free synthesis of polyketides by recombinant erythromycin polyketide synthase. *Nature* **378**, 263-266.
15. Kao, C.M., Luo, G., Katz, L., Cane, D.E. & Khosla, C. (1996). Engineered biosynthesis of structurally diverse tetraketides by a trimodular polyketide synthase. *J. Am. Chem. Soc.* **118**, 9184-9185.
16. Oliynyk, M., Brown, M.J., Cortés, J., Staunton, J. & Leadlay, P.F. (1996). A hybrid modular polyketide synthase obtained by domain swapping. *Chem. Biol.* **3**, 833-839.
17. Marsden, A.F.A., Wilkinson, B., Cortés, J., Dunster, N.J., Staunton, J & Leadlay, P.F. (1998). Engineering broader specificity into an antibiotic-producing polyketide synthase. *Science* **279**, 199-202.
18. McDaniel, R., Kao, C.M., Hwang, S.J. & Khosla, C. (1997). Engineered intermodular and intramodular polyketide synthase fusions. *Chem. Biol.* **4**, 667-674.
19. McDaniel, R., Kao, Fu, H., Hevezi, P., Gustaffson, C., Betlach, M., Cane, D.E. & Khosla, C. (1997). Gain-of-function mutagenesis of a modular polyketide synthase. *J. Am. Chem. Soc.* **119**, 4309-4310.
20. Leadlay, P.F. (1997) Combinatorial approaches to polyketide biosynthesis. *Curr. Opin. Chem. Biol.* **1**, 162-168.
21. Staunton, J., Caffrey, P., Aparicio, J.F., Roberts, G.A., Bethell, S.S. & Leadlay, P.F. (1996). Evidence for a double-helical structure for modular polyketide synthases. *Nat. Struct. Biol.* **3**, 188-192.
22. Wakil, S.J. (1989) Fatty acid synthase, a proficient multifunctional enzyme. *Biochemistry* **28**, 4523- 4530
23. Kao, C.M., Pieper, R., Cane, D.E. & Khosla, C. (1996). Evidence for two catalytically independent clusters of active sites in a functional modular polyketide synthase. *Biochemistry* **35**, 12363-12638.
24. Gokhale, R.S., Lau, J., Cane, D.E. & Khosla, C. (1998). Functional orientation of the acyltransferase domain in a module of the erythromycin polyketide synthase. *Biochemistry* **37**, 2524-2528.
25. Joshi, A.K., Witkowski, A. & Smith, S. (1997). Mapping functional interactions between domains of the animal fatty acid by mutant complementation *in vitro*. *Biochemistry* **36**, 2316-2322
26. Weissman, K.J., *et al.*, & Leadlay, P.F. (1997). The molecular basis of Celmer's rules: the stereochemistry of the condensation step in chain extension on the erythromycin polyketide synthase. *Biochemistry* **36**, 13849-13855.
27. Rowe, C.J., Cortés, J., Gaisser, S., Staunton, J. & Leadlay P.F. (1998). Construction of new vectors for regulated high-level expression on actinomycetes. *Gene*, in press.
28. Li, Z., Martin, F.M. & Vederas, J.C. (1992). Biosynthetic incorporation of labelled tetraketide intermediates into dehydrocurvularin, a phytotoxin from *Alternaria cineraiae*, with assistance of β-oxidation inhibitors. *J. Am. Chem. Soc.* **114**, 1531-1533.
29. Williams, M.D., Rahn, J.A., Smith, S., Joshi, A.K. & Sherman D.H. (1998). Production of a polyhydroxyalkanoate biopolymer in insect cell with a modified eucaryotic fatty acid synthase (vol 62, pg 2540, 1996). *Appl. Env. Microbiol.* **64**, 383.
30. Weissman, K.J., *et al.*, & Leadlay, P.F. (1998).The thioesterase of the erythromycin-producing polyketide synthase: influence of acyl chain structure on the mode of release of substrate analogues from the acyl enzyme intermediates. *Angew. Chem. Int. Ed.*, **37**, 1437-1440.
31. Celmer, W.D. (1965). Macrolide Stereochemistry. III A configurational model for macrolide antibiotics. *J. Am. Chem. Soc.*, **87**, 1801-1802.
32. Marsden, A.F.A., Caffrey, P., Aparicio, J.F., Loughran, M.S., Staunton, J., & Leadlay, P.F. (1994). Stereospecific acyl transfers on the erythromycin-producing polyketide synthase. *Science*, **363**, 378-380.
33. Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). *A Laboratory Manual* (2nd edn), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
34. Yamamoto, H., Maurer, K.H. & Hutchinson, C.R. (1986). Transformation of *Streptomyces erythraeus*. *J. Antibiot.* **34**, 1306-1313.
35. Harris, R., Cutter, A.L., Weissman, K.J., Hanefeld, U., Timoney, M.C. & Staunton, J. (1998). Enantiospecific synthesis of analogues of the diketide intermediate of the erythromycin polyketide synthase (PKS). *J. Chem. Res.*, in press.

Because *Chemistry & Biology* operates a 'Continuous Publication System' for Research Papers, this paper has been published via the internet before being printed. The paper can be accessed from http://biomednet.com/cbiology/cmb – for further information, see the explanation on the contents pages.