Cyclosporin Synthetase
THE MOST COMPLEX PEPTIDE SYNTHESIZING MULTIENZYME POLYPEPTIDE SO FAR DESCRIBED*

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Cyclosporin A and its homologues are synthesized by a single multifunctional enzyme from their precursor amino acids. Cyclosporin synthetase is a polypeptide chain with a molecular mass of approximately 800 kDa. In 3% polyacrylamide-sodium dodecyl sulfate gels it shows a single band of approximately 650 kDa, which appears to not be glycosylated. The enzyme could be purified to near-homogeneity in five steps. A 72-fold purification was obtained. All constitutive amino acids of cyclosporins are activated as thioesters via aminoadenylation by the same enzyme. Then N-methylation of the thioester-bound amino acids which are present in methylated form in the cyclosporin molecule takes place, whereby S-adenosyl-l-methionine serves as the methyl group donor. Methyltransferase activity is an integral entity of the enzyme; this could be shown by a photoaffinity labeling method. 4'-Phosphopantetheine is a prosthetic group of cyclosporin synthetase similar to other peptide and depsipeptide synthetases. Cyclosporin synthetase shows cross-reactions with monoclonal antibodies directed against enniatin synthetase.

Cyclosporin A (Fig. 1) is a cyclic undecapeptide with anti-inflammatory, immunosuppressive, antifungal, and antiparasitic properties (1). It is used in transplantation surgery and in the treatment of autoimmune diseases (2, 3). Cyclosporin A shares with the depsipeptides enniatins and beauvericin which have been shown to be synthesized by large multienzyme complexes from their primary precursors (amino and hydroxy acids) under ATP and AdoMet consumption (7–9). Synthesis of depsipeptides involves amino-adenylation of precursors, binding of the activated precursors as thioesters, N-methylation of the corresponding enzyme-bound amino acids, elongation, and cyclization reactions (10, 11).

Previous attempts to characterize the enzyme system responsible for synthesis of cyclosporine first led to the enrichment of an enzyme fraction catalyzing the synthesis of the diketopiperazine cyclo-(D-Ala-MeLeu), representing a partial sequence (positions 8 and 9) of cyclosporin A (12). Although this preparation was able to activate all constitutive amino acids of cyclosporin A as thioesters via aminoadenylation, total synthesis of cyclosporin A was not observed. Further efforts guided to total in vitro synthesis of several cyclosporins by partially purified cyclosporin synthetase fractions (13) and led recently to the in vitro biosynthesis of cyclosporins not obtainable by fermentation (14).

This paper describes further purification and characterization of cyclosporin synthetase and confirms that cyclosporin synthetase follows a thiotemplate mechanism (15), which has been shown previously for the biosynthesis of various other peptides and depsipeptides (16).

MATERIALS AND METHODS

Growth of Organisms—B. nivea, strain 7399/45, was donated by Sandoz Ltd. (Basel, Switzerland) and cultured as described in Ref. 14. Lactobacillus plantarum, DSM 20 205, was maintained on pancreatic agar (Difco) plates. Pre- and maincuture were incubated in pantothenate assay medium (Difco) at 37 °C. Preculture was har vested by centrifugation, washed several times with 0.9% NaCl, and used as inoculum.

Radioisotopes and Chemicals—All radiochemicals were purchased from Amersham Corp. Bmt and cyclosporin A were donated by Dr. R. Traber, Sandoz Ltd. (Basel, Switzerland), MeBmt was donated by Dr. R. M. Wenger, Sandoz Ltd. (Basel, Switzerland). ATP was from Boehringer Mannheim. Partially purified tyrocidine synthetase III from B. nivea and beauvericin which have been shown to be synthesized by large multienzyme complexes from their primary precursors (amino and hydroxy acids) under ATP and AdoMet consumption (7–9). Synthesis of depsipeptides involves amino-adenylation of precursors, binding of the activated precursors as thioesters, N-methylation of the corresponding enzyme-bound amino acids, elongation, and cyclization reactions (10, 11).

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formed was extracted as described in Ref. 13. TLC analysis was done as described (14).

Thioester Formation—This was measured by incubating 100 ml of enzyme in a total volume of 160 ml with 31 mM MgCl₂, 28 mM ATP, and 0.5 μCi of [³H]-labeled amino acids (specific activities: threonine, 288 mCi/mmol; glycine, 108 mCi/mmol; leucine, 348 mCi/mmol; valine, 285 mCi/mmol; alanine, 171 mCi/mmol; d-alanine, 40 mCi/mmol) or (in the presence of 156 μM Bmt) 0.35 μCi of [methyl-³H]AdoMet (56 mCi/mmol) for 10 min at 25 °C. The reaction was stopped by addition of 2 ml of 7% trichloroacetic acid. After 30 min on ice the precipitate was collected on membrane filters (ME 25, Schleicher & Schull, Dassel, West Germany). Filters were washed twice with 7% trichloroacetic acid and water each; after drying, the radioactivity was determined.

Enzyme Purification—Extraction of lyophilized mycelium and precipitations with 30-50% (NH₄)₂SO₄ were achieved as described earlier (14). 10 ml of the resuspended (NH₄)₂SO₄ precipitation material were loaded onto a Fractogel HW-55 (F) column (4 x 63 cm) and eluted with buffer B. Due to the high molecular weight of the enzyme, further purification could be achieved by glycerol gradient ultracentrifugation (Fig. 4A). Examination of the different purification steps by SDS-polyacrylamide gradient gel electrophoresis shows that cyclosporin synthetase is the major protein after the ultracentrifugation step (Fig. 4A).

Purification of the cyclosporin synthetase activity was followed by measuring the cyclosporin A synthesis rate as described under "Materials and Methods." Subsequently TLC and autoradiography were performed to confirm the cyclosporin A production (Fig. 4B).

| Step | Volume | Protein | Units | Specific activity |
|------|--------|---------|-------|------------------|
| 1. Crude extract | 500 | 2,226 | 470 | 211 |
| 2. Polyethyleneimine precipitation | 500 | 1,722 | 828 | 481 |
| 3. 30-50% (NH₄)₂SO₄ precipitation | 9 | 299 | 505 | 1,689 |
| 4. Fractogel HW-55 column | 103 | 59 | 988 | 16,746 |
| 5. Glycerol gradients (25-50%) | 108 | 31 | 345 | 11,129 |

Results

Purification of Cyclosporin Synthetase—Cyclosporin synthetase from B. nivae, strain 79349/45, was purified 72-fold. The purification protocol is presented in Table I. At any step of the purification procedure, the enzyme could be stored at −80 °C for over 12 months without loss of activity. Preparation of the crude extract and precipitation with polyethyleneimine and with (NH₄)₂SO₄ were achieved as described in Ref. 14. The redissolved ammonium sulfate precipitation material was separated by gel filtration on a Fractogel HW-55 (F) column; the activity resided in a single peak (Fig. 2).

Fig. 2. Elution profile of Fractogel HW-55 (F) column. 8 ml of resuspended ammonium sulfate precipitate were loaded onto a Fractogel HW-55 (F) column (4 x 63 cm) and eluted with buffer B. 8-ml fractions were collected, protein content (●) and cyclosporin synthetase activity (▲) of each fraction were measured.
Interestingly cyclosporin synthetase activity could also be isolated from spores of *B. nivea* using the extraction procedure described above.

**Molecular Mass Determinations**—Measurements of the molecular weight of the native cyclosporin synthetase were performed by ultracentrifugation in glycerol gradients along with standard proteins. A molecular mass of about 800 kDa was obtained. The apparent molecular weight of denatured enzyme was determined by SDS-polyacrylamide gel electrophoresis (3% gels); extrapolation of molecular masses of calibration proteins results in a molecular mass between 600 and 700 kDa for cyclosporin synthetase (Fig. 5). When protein fragments yielded from trypsin digestion of cyclosporin synthetase were separated in SDS-gradient gels, addition of their molecular masses gave a value of about 750 kDa, which is in good agreement with the findings described above.

**Carbohydrate Stain**—To check whether cyclosporin synthetase is a glycoprotein we used a staining procedure which is sensitive for carbohydrate portions of proteins. However no staining with Schiff’s reagent could be observed. This finding together with the fact that cyclosporin synthetase does not bind to concanavalin A-agarose led us to the assumption that cyclosporin synthetase is not a glycoprotein.

**Biosynthesis of Cyclosporins**—To synthesize cyclosporins *in vitro* cyclosporin synthetase needs the appropriate amino acids in unmethylated form (including D-alanine or a homologue D-amino acid (14); the nonchiral amino acid glycine can also substitute D-alanine), ATP, Mg$^{2+}$, and AdoMet as the methyl donor (13, 14). Mg$^{2+}$ ions can be substituted by Mn$^{2+}$ ions. However, the rate of cyclosporin A formation decreases to about 50% compared to the reaction with Mg$^{2+}$. As shown in Ref. 14, the optimal temperature for *in vitro* cyclosporin A synthesis is 24 °C, the reaction proceeds linearly for at least 15 min under substrate saturating conditions. Cyclosporin A synthesis is inhibited by the reaction products AMP, PPi, (not by P$_i$) and S-adenosyl-L-homocysteine, but not by cyclosporin A itself. The reaction proceeds optimally at pH 7.5, measured in Hepes buffer, which is the best buffer for *in vitro* cyclosporin A synthesis, followed by MOPS, Tris, TES, and, at a
Denatured for gel electrophoresis as described under "Materials and Methods" and separated in a 3% polyacrylamide SDS gel. Extrapolation of molecular masses of the calibration proteins enniatin synthetase (250 kDa), linear gramicidin synthetase (350 kDa), and tyrocidin synthetase III (450 kDa) results in a molecular mass of 650 kDa for cyclosporin synthetase (CySyn).

A great distance (50% of the synthesis rate measured in Hepes), by phosphate buffer.

Activation of Amino Acids and N-Methylation—As it has been reported in Ref. 12 for the cyclo-(d-Ala-MeLeu) synthesizing enzyme, the cyclosporin synthetase described here catalyzes ATP-pyrophosphate exchange reactions dependent on all constitutive amino acids of cyclosporin A in their unmethylated form, whereas the N-methyl amino acids are not activated by the enzyme. Furthermore all amino acids required for cyclosporin C synthesis could be shown to be bound covalently as thioesters to the enzyme (Fig. 6). Cyclosporin C, in which 2-aminobutyric acid in position 2 is replaced by threonine (= [Thr]cyclosporin A) was selected for these experiments because 2-aminobutyric acid was not commercially available in a 14C-labeled form. The same holds true for Bmt; covalent binding of this compound to the enzyme was measured indirectly by formation of [N-methyl-14C]MeBmt using S-adenosyl-[methyl-14C]methionine and unlabeled Bmt. With all 14C-labeled amino acids used in Fig. 6, it was also possible to label cyclosporin synthetase specifically as analyzed in polyacrylamide gradient gels (not shown).

Photoaffinity Labeling of Cyclosporin Synthetase—We were interested to clarify, whether the methyltransferase activity of the enzyme is an integral part of the cyclosporin synthetase molecule or whether it is an associated but different enzyme. For this purpose we used a method for site-specific affinity labeling of methyltransferases (21), which has been previously helpful to demonstrate that the methyltransferase activity of enniatin synthetase is an integral part of the enzyme (22). By irradiation with short-wave UV light in the presence of AdoMet labeled in the methyl group various methyltransferases could be labeled covalently. Like these enzymes cyclosporin synthetase, too, was labeled when irradiated in the presence of [methyl-14C]AdoMet or [methyl-3H]AdoMet (Fig. 7). Irradiation of the enzyme in the presence of [carboxyl-14C]AdoMet or [U-14C]ATP did not give any labeling (not shown).

When the affinity-labeled enzyme was digested either with trypsin or with S. aureus V8 protease, three radiolabeled protein bands arose (Fig. 7), suggesting the presence of more than one methyltransferase activity per cyclosporin synthetase molecule, probably three; work is in progress to determine the exact stoichiometry.

Immunological Examinations—At the ultracentrifugation stage of purification only the major protein band and some minor bands of the preparation running just a little faster in the gel show a positive reaction with a polyclonal rabbit antiserum,4 suggesting the presence of one methyltransferase activity per cyclosporin synthetase molecule, probably three; work is in progress to determine the exact stoichiometry.

The cyclosporin synthetase band cross-reacts in immunoblots with a polyclonal antiserum directed against enniatin synthetase as well as with the monoclonal antibodies against enniatin synthetase described in Ref. 24. With the latter antibodies, the strongest reactions could be detected with monoclonal antibodies 21.1 and 25.91, which inhibit the thioester formation with valine and recognize the denatured form of enniatin synthetase.

Furthermore, cyclosporin synthetase preparations show a very significant cross-reaction with a polyclonal antibody preparation directed specifically against pentetene in enzyme-linked immunosorbent assay,5 suggesting the presence of phosphopantetheine as a prosthetic group similar to a 6-

5. Molecular mass estimation of denatured cyclosporin synthetase. 25 μl of a glycerol gradient enzyme preparation were denatured for gel electrophoresis as described under "Materials and Methods" and separated in a 3% polyacrylamide SDS gel. Extrapolation of molecular masses of the calibration proteins enniatin synthetase (250 kDa), tyrocidin synthetase III (450 kDa) results in a molecular mass of 650 kDa for cyclosporin synthetase (CySyn).

FIG. 6. Thioester-bound amino acids of cyclosporin C. Individual fractions of a glycerol gradient ultracentrifugation were tested for their capacity to bind the constitutive amino acids of cyclosporin C as thioesters. The 14C-labeled amino acids were incubated together with ATP, MgCl2, and cyclosporin synthetase as described under "Materials and Methods." The protein was precipitated with 7% trichloroacetic acid, and the protein-bound radioactivity was measured. Values were corrected with results from incubations without ATP resp. Bmt. The peak fraction (when measured for in vitro synthesis of cyclosporin A) was fraction 8.

FIG. 5. Molecular mass estimation of denatured cyclosporin synthetase. 25 μl of a glycerol gradient enzyme preparation were denatured for gel electrophoresis as described under "Materials and Methods." The protein was precipitated with 7% trichloroacetic acid, and the protein-bound radioactivity was measured. Values were corrected with results from incubations without ATP resp. Bmt. The peak fraction (when measured for in vitro synthesis of cyclosporin A) was fraction 8.
proteins are indicated after fluorography (R). The molecular masses of standard preparations from an ultracentrifugation were irradiated at 254 nm with [merhy-"C"]AdoMet as described under “Materials and Methods.” The protein was separated by polyacrylamide gel electrophoresis in a 15-2% Laemmli gel and stained with Coomassie blue (A) or autoradiographed after fluorography (B). The molecular masses of standard proteins are indicated (A).

number of other peptide and depsipeptide synthetases.

Presence of 4'-Phosphopantetheine in Cyclosporin Synthetase—To confirm the assumption that 4'-phosphopantetheine forms part of cyclosporin synthetase, we performed a microbiological assay with Lactobacillus as a test organism. Fractions from the glycerol gradient ultracentrifugation step were analyzed in order to determine their 4'-phosphopantetheine content. As shown in Fig. 8 the synthetic activity of cyclosporin synthetase comigrates with pantothenate in the gradient. The fact, that most of the panthotenate was released after alkaline phosphatase treatment proves that it is present as 4'-phosphopantetheine in the enzyme. In addition the typical band of cyclosporin synthetase comigrates with panthotenate release and synthetic activity (Fig. 8). Further evidence for the presence of 4'-phosphopantetheine in cyclosporin synthetase was obtained from specific labeling of the enzyme by in vivo feeding of tritiated β-alanine, which was analyzed by polyacrylamide gel electrophoresis and adjacent autoradiography (not shown).

DISCUSSION

The first attempts to establish the cell-free synthesis of cyclosporin were not successful, but led to an enzyme enrichment actively synthesizing the diketopiperazone cyclo-(D-Ala-MeLeu) (12), which represents a partial sequence of cyclosporin A. Change of the cyclosporin producer strain and the buffer for enzyme preparation (Tris buffer instead of phosphate, glycerol content) resulted in successful in vitro synthesis of cyclosporin (13). From our results Hepes and Tris are appropriate buffer systems for the enzyme in contrast to the previously used phosphate buffer; the presence of glycerol in the buffer is necessary as a stabilizer. We think that in the absence of glycerol some conformational changes of the enzyme take place which lead to the loss of its ability to produce cyclosporins. The main reaction product of such “inactive” enzyme preparations is the diketopiperazine cyclo-(D-Ala-MeLeu). Therefore it seems obvious that our previous preparations described in Ref. 12 contained intact but “inactive,” probably conformationally changed, cyclosporin synthetase polypeptide chains.

Like enniatin synthetase (8, 22) which can be considered as a model system for other N-methylating peptide synthetases cyclosporin synthetase accepts only the unmethylated precursor amino acids of cyclosporins which are methylated while bound to the enzyme as thioesters as previously shown (Ref. 12). The methyltransferase(s) responsible for these N-methylations is integral part of the enzyme as could be shown by the affinity-labeling experiments with [methyl-14C]AdoMet.

It is interesting that all peptide and depsipeptide synthetases from fungi (e.g. enniatin (8), beauvericin (11), δ-(L-α-aminoacidyl)-L-cysteinyl-D-valine (26), ergot peptide lactam, and cyclosporin synthetase) do not exhibit subunit structure. They consist of single polypeptide chains of molecular masses between 250 and 800 kDa, which harbor all catalytic activities necessary for peptide formation. Such enzymes are designated as “multienzyme polypeptides” in the nomenclature according to NC-IUB (27), in contrast to the “multienzyme complexes” from prokaryotes which consist of subunits (e.g. gramicidin, tyrocidin, bacitracin synthetase, for review see Ref. 16).

5 J. Dittmann, R. Zocher, and A. Lawen, unpublished results.

6 N. Quandt and U. Keller, personal communication.
Experiments to determine the exact number of N-methyltransferase(s) and 4'-phosphopantetheine residues per mole of cyclosporin synthetase have been hampered by two difficulties. First, to measure exactly the absolute protein content of our preparations, because the dye-binding method we used is related to the calibration protein (bovine serum albumin in our case). Attempts to determine the protein amount gravimetrically were not successful, as we believe, due to different glycerol quantities remaining associated to the enzyme. Second, we never know the exact quantity of inactivated enzyme in our preparations. As can be seen from Table I during the last purification step, a considerable loss of specific activity is observed.

The molecular mass of cyclosporin synthetase has been determined to be between 650 and 800 kDa. In spite of this high value, we were not able to dissociate the enzyme into subunits; neither with urea nor with detergents like SDS nor with β-mercaptoethanol.

This high molecular mass is not astonishing if one realizes that there are (in the case of cyclosporin A) 7 amino acids which have to be N-methylated and in total 11 amino acids which have to be activated and combined. The overall reaction of cyclosporin synthesis can be divided in at least 40 partial reaction steps: 11 aminoadenylation reactions, 11 transthiolation reactions, 7 N-methylation reactions, 10 elongation reactions, and the final cyclization reaction; possible other transthiolation reactions from one thiol group to another are not included in this calculation. The measured molecular mass is in good agreement with a theory of Lipmann and co-workers (28), which requires a protein domain of 70 kDa for each activation site in a peptide synthetase; so, in the light of this assumption one would expect a molecular mass of 770 kDa for cyclosporin synthetase.

In summary, cyclosporin synthetase appears to be the largest and most complex enzymatically active multienzyme polypeptide chain so far described and is a further example of a N-methylating peptide synthetase from eukaryotes.

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REFERENCES

1. Borel, J. F. (1986) Prog. Allergy 38, 9-10
2. Kahan, B. D. (ed) (1984) Cyclosporin: Biological Activity and Clinical Applications, Grune & Straton Inc., Orlando, FL
3. Schindler, R. (ed) (1985) Cyclosporin in Autoimmune Diseases, Springer Verlag, Berlin
4. Traber, R., Hofmann, H., Looss, H. R., Ponele, M., and von Warburg, A. (1987) Helv. Chim. Acta 70, 13-36
5. Traber, R., Hofmann, H., and Kobel, H. (1989) J. Antibiot. 42, 591-597
6. Zocher, R., Madry, N., Peeters, H., and Kleinkauf, H. (1984) Phytochemistry 23, 549-551
7. Zocher, R., and Kleinkauf, H. (1978) Biochem. Biophys. Res. Commun. 81, 1162-1167
8. Zocher, R., Keller, U., and Kleinkauf, H. (1982) Biochemistry 21, 43-48
9. Peeters, H., Zocher, R., Madry, N., Oelrich, P. B., Kleinkauf, H., and Kraepelin, G. (1983) J. Antibiot. 36, 1762-1766
10. Zocher, R., Keller, U., and Kleinkauf, H. (1983) Biochem. Biophys. Res. Commun. 110, 292-299
11. Peeters, H., Zocher, R., and Kleinkauf, H. (1988) J. Antibiot. 41, 352-359
12. Zocher, R., Nihira, T., Paul, E., Madry, N., Peeters, H., Kleinkauf, H., and Keller, U. (1986) Biochemistry 25, 550-553
13. Billich, A., and Zocher, R. (1987) J. Biol. Chem. 262, 17258-17269
14. Lawrence, A., Traber, R., Geyl, D., Zocher, R., and Kleinkauf, H. (1989) J. Antibiot. 42, 1283-1289
15. Lipmann, F. (1971) Science 173, 875-884
16. Kleinkauf, H., and van Dohren, H. (1987) Annu. Rev. Microbiol. 41, 259-289
17. Blobel, G., and Sabatini, D. (1971) Proc. Natl. Acad. Sci. U. S. A. 68, 390-394
18. Bradford, M. M. (1976) Anal. Biochem. 72, 248-254
19. Laemmli, U. K. (1970) Nature 227, 680-685
20. Fairbanks, G., Steck, T. L., and Wallach, D. F. H. (1971) Biochemistry 10, 2606-2617
21. Hurst, J. H., Billingsley, M. L., and Lovering, W. (1984) Biochem. Biophys. Res. Commun. 122, 499-508
22. Billich, A., and Zocher, R. (1987) Biochemistry 26, 5417-5423
23. Pugh, E. L., and Wallach, D. (1965) J. Biol. Chem. 240, 4727-4733
24. Billich, A, Zocher, R., Kleinkauf, H., Braun, D. G., Lavanchy, D. and Hochkoppel, H.-K. (1987) Biol. Chem Hoppe-Seyler 368, 521-529
25. Deleted in proof
26. van Liesveld, H., von Döhren, H., and Kleinkauf, H. (1989) J. Biol. Chem. 264, 3680-3684
27. Nomenclature Committee of the International Union of Biochemistry (NC-IUB) (1989) Eur. J. Biochem. 185, 486-496
28. Lee, S. G., Roskoski, R., Jr., Bauer, K., and Lipmann, F. (1973) Biochemistry 12, 398-406
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