Structure of the Complex between the Antibiotic Cerulenin and Its Target, β-Ketoacyl-Acyl Carrier Protein Synthase*

(Received for publication, November 30, 1998)

Martin Moche‡, Gunter Schneider‡, Patricia Edwards‡, Katayoon Dehesh‡, and Ylva Lindqvist‡‡

From the ‡Department of Medical Biochemistry and Biophysics, Doktorsringen 9A1, Karolinska Institutet, S-171 77 Stockholm, Sweden and the §Oils Division, Calgene, Inc., Davis, California 95616

In the biosynthesis of fatty acids, the β-ketoacyl-acyl carrier protein (ACP) synthases catalyze chain elongation by the addition of two-carbon units derived from malonyl-ACP to an acyl group bound to either ACP or CoA. The enzyme is a possible drug target for treatment of certain cancers and for tuberculosis. The crystal structure of the complex of the enzyme from Escherichia coli, and the fungal mycotoxin cerulenin reveals that the inhibitor is bound in a hydrophobic pocket formed at the dimer interface. Cerulenin is covalently attached to the active site cysteine through its C2 carbon atom. The fit of the inhibitor to the active site is not optimal, and there is thus room for improvement through structure based design.

β-Ketoacyl synthases, condensing enzymes, comprise a structurally and functionally related family that play critical roles in the biosynthesis of a variety of natural products, including fatty acids (1, 2) and the polyketide precursors leading to antibiotics, toxins, and other secondary metabolites (3, 4). They catalyze carbon-carbon bond forming reactions by condensing a variety of acyl chain precursors with an elongating carbon source, usually malonyl or methylmalonyl moieties, that are covalently attached through a thioester linkage to an acyl carrier protein (ACP). Condensing enzymes can be part of multienzyme complexes, domains of large, multifunctional polypeptide chains as the mammalian fatty acid synthase, or single enzymes as the β-ketoacyl synthases in plants and most bacteria (1–5).

In fatty acid biosynthesis, the chain elongation step consists of the condensation of acyl groups, derived from acyl-ACP or acyl-CoA with malonyl-ACP. These reactions are catalyzed by a group of enzymes, the β-keto-ACP synthases (KAS, EC 2.3.1.41). Several species of β-keto-ACP synthases in plants and bacteria have been identified, distinct in amino acid sequence, chain length specificity for their substrates, and sensitivity to cerulenin, an inhibitor of condensing enzymes (6).

We recently determined the crystal structure of KASII from Escherichia coli at 2.4-Å resolution (7). The subunit consists of two mixed five-stranded β-sheets surrounded by α-helices. The two sheets are packed to each other in such a way that the fold can be described as consisting of five layers, α-β-α-β-α. The enzyme is a homodimer, and the subunits are related by a crystallographic 2-fold axis. The proposed nucleophile in the reaction, Cys-163 is located at the bottom of a mainly hydrophobic pocket at the dimer interface. The structure allowed us to suggest the specific function of conserved residues in the catalytic reaction. The conservation of key residues in the whole family of condensing enzymes suggests that they all exhibit a similar three-dimensional molecular architecture.

Condensing enzymes have been identified with properties subject to exploitation in the areas of plant oil modification (8), polyketide engineering, and ultimately design of anti-cancer (9, 10) and anti-tuberculosis (11, 12) agents. One of the molecular targets of isoniazid, which is widely used in the treatment of tuberculosis, is KAS (12). Cerulenin (Fig. 1A), a mycotoxin produced by the fungus Cephalosporium caerulens, acts as a potent inhibitor of KAS by covalent modification of the active cysteine thiol (13, 14). Condensing enzymes from many pathways and sources have all been shown to be inactivated by this antibiotic (6, 13–16) with the exception of the synthase from C. caerulens (17) and KASIII (16), the isozyme responsible for the initial condensation of malonyl-ACP with acetyl-CoA in plant and bacterial fatty acid biosynthesis. Inhibition of the KAS domain of fatty acid synthase by cerulenin is selectively cytotoxic to certain cancer cells (9, 10).

We have now determined the structure of E. coli KASII complexed with cerulenin with the objectives of defining the active site pocket, providing a basis for understanding differences in substrate specificities and sensitivity toward cerulenin between different condensing enzymes and laying a foundation for efforts at structure based drug design with this target.

EXPERIMENTAL PROCEDURES

The KASII-cerulenin complex was prepared as described previously (18). Crystals of the complex were grown by the hanging drop method. Droplets consisting of equal amounts of protein solution (6 mg ml−1 protein, 0.3 M NaCl, 25 mM Tris, pH 8.0, 5 mM imidazole, and 10% v/v glycerol) and reservoir solution were equilibrated against 26% w/v polyethylene glycol 8000 and 0.1% v/v 2-mercaptoethanol in water. Data from two crystals were collected at 298 K at the synchrotron in MAX-lab, beamline I711, in Lund. The data was processed with DENGIO (19) and programs from the Collaborative Computing Project 4 Suite (20) and the two data sets were scaled together in SCALA (21) (Table I). The crystals are very radiation-sensitive, but cannot be frozen in a cryostream. Due to non-isomorphism, data of only two crystals could be merged. The crystals of the complex have space group P321 with similar cell dimensions as the native enzyme (Table I). The coordinates of the native enzyme (7) were used to calculate initial electron density maps with SIGMAA (22). All data were used in the refinement; no sigma cutoff was applied. After an initial cycle of positional refinement, the model was rebuilt and a model of cerulenin was included. Further cycles of refinement of the complex were carried out using the program
have moved substantially. These conformational changes provide access for cerulenin to the active site cysteine and open a hydrophobic pocket for the hydrophobic tail of the inhibitor.

From the initial $F_\text{obs}$ – $F_\text{o}$ electron density map these structural changes could be readily seen as well as the binding site for the inhibitor (Fig. 1B). Cerulenin is bound covalently through its C2 carbon atom to the Cys-163-Sy atom. Its hydrocarbon tail fits in a hydrophobic pocket formed at the dimer interface (Figs. 2 and 3). The structure of the adduct of cerulenin and cysteine, isolated by tryptic digestion of the cerulenin-fatty acid synthase complex, has been determined by NMR and mass spectroscopy (14). This study revealed that the inhibitor reacts at its C2-epoxide carbon with the SH group of cysteine and that cerulenin formed a hydroxylactam ring (Fig. 1A). The electron density observed in the KASII-cerulenin complex is not consistent with this structure. It was not possible to model bound cerulenin in the closed ring form but the open form of the inhibitor could readily be fitted to the electron density map (Fig. 1, B and C). We thus conclude, that the hydroxylactam ring, which is formed preferentially in proptic solvents (14), is not present in the hydrophobic environment of the protein.

In the KASII-cerulenin complex, the inhibitoramide carbon-oxycarbon is within hydrogen bond distance to the Ne atoms of the side chains of His-340 and His-303, while the amide NH$_2$ group does not make any close interactions (Fig. 3). It is, however, not possible from the structure to exclude the opposite conformation and interactions for the amide group. The hydroxyl group at C3 forms a hydrogen bond to the main chain NH of Phe-400. The carbonyl oxygen at C4 does not form any polar interactions, in fact, it is located in a very hydrophobic pocket formed by side chains Phe-400, Phe-202, and Val-134 from the other subunit in the dimer. The binding site for the hydrophobic part of the inhibitor is also lined with hydrophobic residues: Ala-162, Gly-107, Leu-342, Phe-202, Val-134, Ala-193, Gly-198; and from the second subunit in the dimer, Ile-138, Val-134, and Phe-133. The two double bonds with trans configuration give the hydrophobic tail a shape that fits to the hydrophobic groove once residue Ile-108 has changed rotamer.

In comparison, binding of tetrabutylercerulenin would cost entropy, and as expected it shows more than 2 orders of magnitude less inhibitory activity (27). The influence of the length of the hydrocarbon chain, maintaining the double bond positions, has been studied using fatty acid synthase from Saccharomyces cerevisiae (28). Cerulenin (12 carbons) had the highest inhibitory activity, with slightly decreasing binding strength upon increase in chain length. However, when increasing the length from 16 to 18 carbon atoms, the inhibition decreased by 2 orders of magnitude. The size of the hydrophobic pocket in KASII, which binds the hydrocarbon tail of cerulenin, suggests that there is space for a longer hydrophobic tail only if the side chains of Leu-111 and of Phe-133 in the second subunit change conformation and interactions for the amide group. The hydroxy group at C3 forms a hydrogen bond to the main chain NH of Phe-400. The carbonyl oxygen at C4 does not form any polar interactions, in fact, it is located in a very hydrophobic pocket formed by side chains Phe-400, Phe-202, and Val-134 from the other subunit in the dimer. The binding site for the hydrophobic part of the inhibitor is also lined with hydrophobic residues: Ala-162, Gly-107, Leu-342, Phe-202, Val-134, Ala-193, Gly-198; and from the second subunit in the dimer, Ile-138, Val-134, and Phe-133. The two double bonds with trans configuration give the hydrophobic tail a shape that fits to the hydrophobic groove once residue Ile-108 has changed rotamer.

In comparison, binding of tetrabutylercerulenin would cost entropy, and as expected it shows more than 2 orders of magnitude less inhibitory activity (27). The influence of the length of the hydrocarbon chain, maintaining the double bond positions, has been studied using fatty acid synthase from Saccharomyces cerevisiae (28). Cerulenin (12 carbons) had the highest inhibitory activity, with slightly decreasing binding strength upon increase in chain length. However, when increasing the length from 16 to 18 carbon atoms, the inhibition decreased by 2 orders of magnitude. The size of the hydrophobic pocket in KASII, which binds the hydrocarbon tail of cerulenin, suggests that there is space for a longer hydrophobic tail only if the side chains of Leu-111 and of Phe-133 in the second subunit change their conformation. Thus, possible differences in the sensitivity of condensing enzymes toward cerulenin might be controlled by the size of this cavity.

The structure of the cerulenin complex can be considered to mimic the intermediate formed upon reaction of KAS with the acyl-ACP. In such a complex the hydrophobic cavity would harbor the hydrocarbon tail of the acyl intermediate. The acyl hydrophobic tails will not be restricted by two double bonds (as in the case of cerulenin), and this will allow longer acyl chains to be buried in this pocket. Inspection of the active site cavity suggests that it would not be possible to harbor a linear acyl chain longer than 14 carbon atoms without structural changes. Such conformational changes must occur since KASII is able to elongate 16:1 to 18:1 (29).
Cerulenin Resistance—There are few exceptions to the inhibition of KAS by cerulenin, one is the fatty acid synthase of the fungus synthesizing this antibiotic, *C. caerulens* (17), another is KASIII (16), the variant of KAS that catalyzes the initial condensation of malonyl-acyl carrier protein with acetyl-CoA in plant and bacterial fatty acid biosynthesis. Unfortunately there is no amino acid sequence available of KAS from *C. caerulens*, therefore the structural basis for the lack of inhibition can not yet be analyzed. The sequence of a 12-residue peptide supposed to span the active site cysteine (30) showed no homology to other KAS enzymes, suggesting that the enzyme from *C. caerulens* might be very different.

Amino acid sequences for KASIII show very low degrees of sequence identities to KASI, KASII, and to the fungal and animal fatty acid synthases, which makes an alignment of the sequences very unreliable and prevents a proper analysis. However, considering the size of its substrates, a reasonable explanation for the lack of inhibition of KASIII is that it has a smaller active site cavity available that can not accommodate the hydrophobic tail of cerulenin.

Mutations in the gene coding for fatty acid synthase that result in cerulenin resistance in *S. cerevisiae* have been observed (31). One of these mutations is a Gly→Ser replacement. This glycine residue corresponds to Gly-107 in KASII, adjacent to Ile-108, which changes rotamer upon binding of cerulenin. Exchange of this glycine for serine introduces steric hindrance to binding of cerulenin and influences the polarity in the hydrophobic cavity.
Acknowledgment—We thank the staff at MAX-lab, Lund University for help at the beamline.

REFERENCES

1. Magnuson, K., Jackowski, S., Rock, C. O., and Cronan, J. E. (1994) Microbiol. Rev. 57, 522–542
2. Somerville, C., and Browse, J. (1991) Science 252, 80–87
3. Hopwood, D. A., and Sherman, D. H. (1990) Annu. Rev. Genet. 24, 37–66
4. Katz, L., and Donadio, S. (1993) Annu. Rev. Microbiol. 47, 875–912
5. Wakil, S. J. (1989) Biochemistry 28, 4523–4530
6. Vance, D. E., Goldberg, I., Mitsuhashi, O., Bloch, K., Omura, S., and Nomura, S. (1972) Biochem. Biophys. Res. Commun. 48, 649–656
7. Huang, W., Jia, J., Edwards, P., Dehesh, K., Schneider, G., and Lindqvist, Y. (1998) EMBO J. 17, 1183–1191
8. Dehesh, K., Edwards, P., Fillatti, J., Slabaugh, M., and Byrne, J. (1998) Plant J. 15, 383–391
9. Pizer, E. S., Wood, F. D., Pasternack, R., and Kuhajda, F. P. (1996) Cancer Res. 56, 745–751
10. Pizer, E. S., Jackisch, C., Wood, F. D., Pasternack, R., Davidson, N. E., and Kuhajda, F. P. (1996) Cancer Res. 56, 2745–2747
11. Rastogi, N., Goh, K. S., Horgen, L., and Barrow, W. W. (1998) FEMS Immunol. Med. Microbiol. 21, 149–157
12. Mdluli, K., Slayden, R. A., Zhu, Y., Ramaswamy, S., Pan, X., Mead, D., Crane, D. D., Musser, J. M., and Barry, C. E., III (1998) Science 280, 1607–1610
13. Kleywegt, G. J., and Jones, T. A. (1994) Acta Crystallogr. Sect. D Biol. Crystallogr. 50, 760–763
14. Evans, P. R. (1993) in Proceedings of the Collaborative Computing Project 4 (Sawyer, L., Isaacs, N., and Bailey, S. S., eds) pp. 114–122, SERC Daresbury Laboratory, Warrington, UK
15. Child, C. J., and Shoolingin-Jordan, P. M. (1998) Biochem. J. 330, 933–937
16. Jaworski, J. G., Clough, R. C., and Barnum, S. R. (1989) Plant Physiol. 90, 41–44
17. Tomoda, H., Okuda, S., and Iwasaki, S. (1989) Biochem. (Tokyo) 105, 751–755
18. Tomoda, H., Hashimoto, A., Takeshima, H., and Okuda, S. (1994) Mol. Gen. Genet. 244, 90–96
19. Kraulis, P. (1991) J. Appl. Crystallogr. 24, 946–950
20. Mendel, E. A., and Murphy, M. E. P. (1994) Acta Crystallogr. Sect. D Biol. Crystallogr. 50, 869–873
21. Kleywegt, G. J., and Jones, T. A. (1994) Acta Crystallogr. Sect. D Biol. Crystallogr. 50, 178–185
22. Esnouf, R. M. (1997) J. Mol. Graph. 15, 133–138

FIG. 2. Schematic diagram of the dimer of KASII with cerulenin bound at the interface as a CPK model. The figure was generated using the programs Molscript (32) and Raster3D (33).

FIG. 3. The cerulenin binding groove of KASII. The figure was prepared using Voodoo (34), Bobscript (35), and Raster3D (33).