Chemistry & Biology, Vol. 10, 101–106, February, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S1074-5521(03)00029-2

Previews

Mammalian Fatty Acid Synthase: Closure on a Textbook Mechanism?

Mammalian fatty acid synthase is a classic example of a chain-building multienzyme. A cornerstone of its mechanism has been the obligatory collaboration of two identical subunits, with fatty acyl intermediates transferring between them. Now, fresh evidence has upset this view.

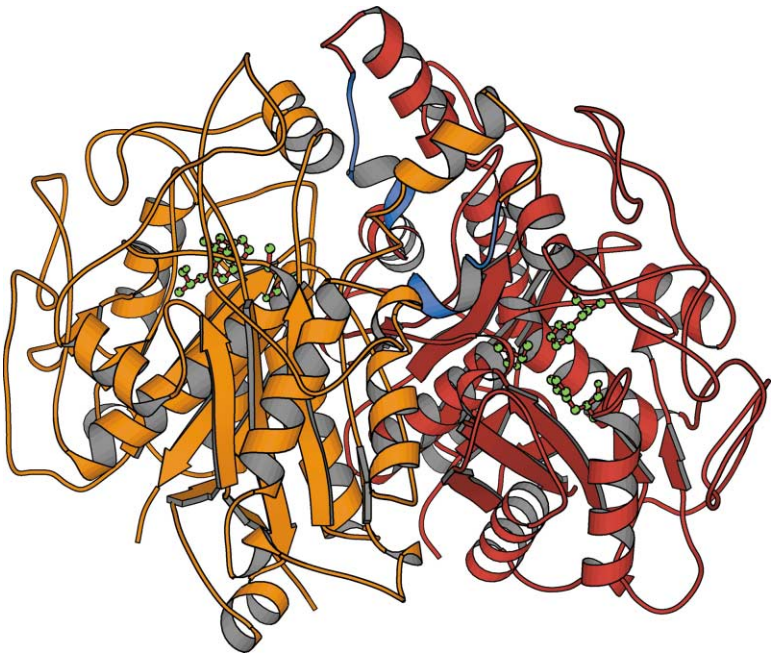
Classic biochemical studies on the enzymes of fatty acid biosynthesis by the research groups of Lynen, Bloch, Wakil, and others have given us an enduring and satisfying picture of the individual enzyme activities involved and of the way in which they are orchestrated to produce fatty acids. The growing chain, attached to the active site of a ketosynthase (KS) enzyme, is condensed with a malonyl unit (covalently attached to an acyl carrier protein [ACP]) through the action of an acyltransferase (AT) to produce a β-ketoacyl product on the ACP with release of carbon dioxide; the ketoacyl moiety is then reduced to saturated acyl by the successive action of ketoreductase, dehydratase, and enoylreductase enzymes, and the lengthened chain hops back to the KS to initiate the next cycle. For the mammalian and yeast fatty acid synthases, the analysis was particularly challenging because (in contrast to bacterial fatty acid synthase) the individual enzymes were found to be covalently associated as domains of a large multifunctional protein, but by the late 1980s it was possible to write a coherent account of the currently accepted mechanism [1, 2]. One of the key features of the mechanism had been revealed by analysis of the rapid stochiometric inhibition of the mammalian fatty acid synthase by the bifunctional chemical 1,3-dibromopropan-2-one [3]. In the inhibited multienzyme, it appeared that the active-site cysteine of each KS enzyme was crosslinked specifically to the ACP on the *opposite* subunit of a homodimer. Together with evidence from electron microscopy and neutron scattering [4], this immediately suggested why the multienzyme polypeptide needed to be dimeric: the ACP and KS domains on the same polypeptide were simply too far apart to interact. Indeed, when monomeric versions of the mammalian FAS were made, they were confirmed to be wholly inactive in overall synthesis, although certain individual activities were still detectable using model substrates. Also, limited proteolysis of purified FAS protein, which severs the flexible linkers between the enzymatic domains, was shown to give patterns of smaller fragments, which could be readily interpreted in terms of a model in which two identical polypeptides, each containing seven active sites, lie head-to-tail in an extended planar arrangement [5]. This suggests that the catalytic domains in the N-terminal region of one subunit work together with the catalytic domains from the C-terminal region of the other subunit.

This widely accepted mechanism and the associated structural model only started to be seriously questioned after Smith and his collaborators succeeded in expressing recombinant FAS in insect cells and began to use site-directed mutagenesis to knock out individual active sites [6]. More importantly, they developed a tedious but reproducible methodology for scrambling together the subunits of two different purified FAS mutants and then allowing them to redimerize [7, 8]. Now, they could interrogate the heterodimers for activity, and in a meticulous series of complementation experiments they built a detailed map of the functional relationships between individual domains of the FAS dimer. Later, affinity tags were introduced so that the heterodimers could be separated away from the homodimeric parents before the kinetic analysis was done [9]. The results of this complementation analysis soon unveiled a major surprise: the dehydratase domain makes functional contact with the ACP in its own polypeptide chain, although separated from it by some 1200 amino acids [8]. This finding is incompatible with the classical structural model.

Meanwhile, advances in our understanding of the related process of polyketide biosynthesis were shedding additional and unexpected light on FAS mechanism and structure. Production of the complex polyketide antibiotic erythromycin A by the bacterium *Saccharopolyspora erythraea* had been shown as early as 1990 [10] to involve giant multienzymes akin to mammalian FAS, in which each cycle of chain extension is catalyzed by a different set or module of FAS-related activities. Strikingly, the order and identity of the active sites along the polypeptide chain was identical in each module to comparable active sites of the mammalian FAS [10–12]. In 1996, a "helical" model was proposed for modular PKSs in which the multienzymes were homodimeric, and within each module the subunits interacted both head-to-head (KS in contact with KS, ACP in contact with ACP) *and* head-to-tail (KS in contact with ACP of the opposite subunit) [13]. This model, based on the results of hydrodynamic experiments, on limited proteolysis and chemical crosslinking, was supported by the results of qualitative mutant complementation studies based on the Smith approach [14]. In particular, since ACP-thioesterase didomain fragments were also homodimeric, the modular PKS structure had to be coiled and compact compared to the classic FAS model.

More recent mutant complementation studies in Smith's laboratory showed that a single KS or AT domain could "service" [15] the ACP domains on both subunits of FAS [16]. This in turn prompted a reinvestigation of the classical crosslinking experiment with 1,3-dibromopropan-2-one, and a previously overlooked minor product could be characterized as representing chemical crosslinking of KS and ACP active sites within the same subunit [17]. Clearly, the FAS dimer is also coiled and compact [18].

The most recent experiments, reported by Smith and



Homology Model of KS Domain Dimer

Homology model, created by Nuria Campillo, Mark Williams, Bojana Popovic, and Tom L. Blundell, of a KS domain dimer from a modular PKS, based on FabF of *E. coli* [20]. Active-site residues are shown in green; the active-site loop contributed by the other subunit is highlighted in blue.

his colleagues in this issue of *Chemistry & Biology* [19], have set the seal on these insights: when a subunit of the FAS mutated in each and every one of its seven active sites was combined in a heterodimer with a wild-type FAS subunit, the resulting enzyme was found, remarkably, to be capable of synthesizing long chain fatty acids, and at a very efficient rate—roughly one-third of each of the active sites in a fully functional wild-type dimer. This result could have been produced if the heterodimers of active and inactive subunits reassorted, but several lines of evidence were used to eliminate the possibility that this had occurred. Taken together, these results provide compelling evidence for Smith's view that the textbook model for FAS mechanism and structure needs revision. Clearly, a fatty acid can be synthesized even when covalent attachment of intermediates and extension units is only possible to one of the subunits.

Smith now proposes [19] that the dimerization of FAS subunits merely serves to stabilize an optimal conformation for the monomer within the context of the dimer, allowing productive interactions between domains, both inter- and intra-subunit. This is a brave assertion because it amounts to saying that none of the domains of the FAS contributes in any *direct* way to the structure and activity of an active site in the other subunit. In the absence of X-ray crystal structural information for the FAS, it is hard to be sure that this is true. Moreover, there is evidence to the contrary from X-ray crystal structures of the homodimeric KS enzymes of the *Escherichia coli* FAS [20–22]. For example, the loop between Ile108 and Ile129 of one subunit of FabF forms part of the wall of the active site of the other subunit. Homology modeling of the KS domain of a typical modular PKS onto this structure (see Figure) (Williams, M.G., Campillo, N., Popovic, B., and Blundell, T.L., personal communication) suggests that the same functional interaction may be present in the KS domains of both PKS and FAS multienzymes. X-ray crystal structures of (at least appropriate portions of) the FAS itself are eagerly awaited to allow this question to be finally settled. Smith's findings also have implications for our understanding of the structure and function of the modular PKS systems: the convergence of the FAS structural model toward that originally proposed for the modular PKS further underlines the profound similarity between the two multienzyme systems.

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Selected Reading

1. Wakil, S.J. (1989). Biochemistry *28*, 4523–4530.
2. Chang, S.I., and Hammes, G.G. (1990). Acc. Chem. Res. *23*, 363–369.
3. Stoops, J.K., and Wakil, S.J. (1981). J. Biol. Chem. *256*, 5128–5133.
4. Stoops, J.K., Wakil, S.J., Uberbacher, E.C., and Bunick, G.J. (1987). J. Biol. Chem. *262*, 10246–10251.
5. Witkowski, A., Rangan, V.S., Randhawa, Z.I., Amy, C.M., and Smith, S. (1991). Eur. J. Biochem. *198*, 571–579.
6. Joshi, A.K., and Smith, S. (1993). J. Biol. Chem. *268*, 22508–22513.
7. Witkowski, A., Joshi, A., and Smith, S. (1996). Biochemistry *35*, 10569–10575.
8. Joshi, A.K., Witkowski, A., and Smith, S. (1997). Biochemistry *36*, 2316–2322.
9. Joshi, A.K., Rangan, V.S., and Smith, S. (1998). J. Biol. Chem. *273*, 4937–4943.
10. Cortés, J., Haydock, S.F., Roberts, G.A., Bevitt, D.J., and Leadlay, P.F. (1990). Nature *348*, 176–178.
11. Donadio, S., Staver, M.J., McAlpine, J.B., Swanson, S.J., and Katz, L. (1991). Science *252*, 675–679.

12. Bevitt, D.J., Cortés, J., Haydock, S.F., and Leadlay, P.F. (1992). Eur. J. Biochem. *204*, 39–49.
13. Staunton, J., Caffrey, P., Aparicio, J.F., Roberts, G.A., Bethell, S.S., and Leadlay, P.F. (1996). Nat. Struct. Biol. *3*, 188–192.
14. Kao, C.M., Pieper, R., Cane, D.E., and Khosla, C. (1996). Biochemistry *35*, 12363–12368.
15. Perham, R.N. (1991). Biochemistry *30*, 8501–8512.
16. Joshi, A.K., Witkowski, A., and Smith, S. (1998). Biochemistry *37*, 2515–2523.
17. Witkowski, A., Joshi, A.K., Rangan, V.S., Falick, A.M., Witkowska, H.E., and Smith, S. (1999). J. Biol. Chem. *274*, 11557–11563.
18. Rangan, V.S., Joshi, A.K., and Smith, S. (2001). Biochemistry *40*, 10792–10799.
19. Joshi, A.K., Rangan, V.S., Witkowski, A., and Smith, S. (2003). Chem. Biol., this issue, 169–173.
20. Huang, W., Jia, J., Edwards, P., Dehesh, K., Schneider, G., and Lindqvist, Y. (1998). EMBO J. *17*, 1183–1191.
21. Olsen, J.G., Kadziola, A., von Wettstein-Knowles, P., Siggaard-Andersen, M., Lindquist, Y., and Larsen, S. (1999). FEBS Lett. *460*, 46–52.
22. Davies, C., Heath, R.J., White, S.W., and Rock, C.O. (2000). Structure *8*, 185–195.

Chemistry & Biology, Vol. 10, February, 2003, DOI 10.1016/S1074-5521(03)00028-0

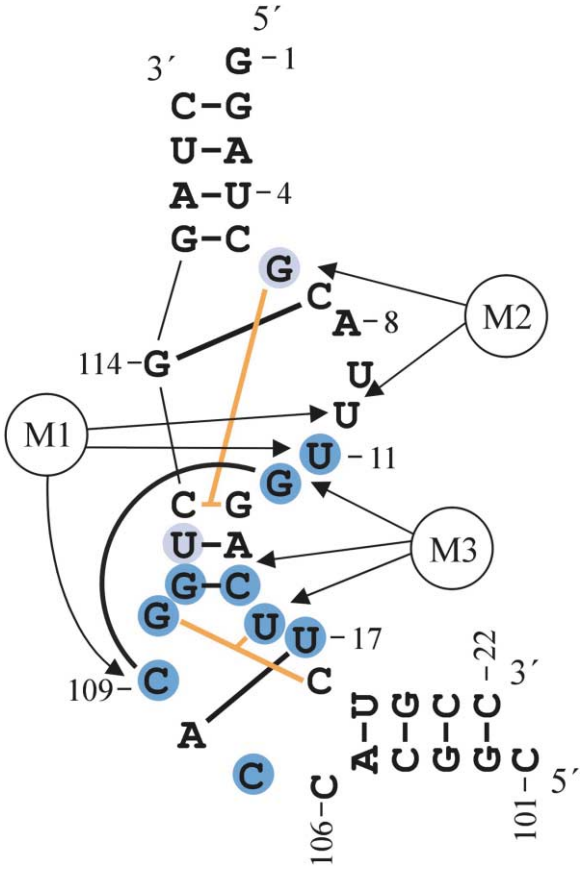
Aptamer Structures: A Preview into Regulatory Pathways?

The crystal structure of a streptomycin binding RNA aptamer displays a novel bipartite fold able to clamp the antibiotic. In view of the recent findings that metabolites directly control mRNA translation, we might expect that similar structures exist in natural RNAs.

The notion that RNA molecules are able to fold and build binding pockets for small molecules first emerged when it was discovered that self-splicing group I introns have a cofactor [1]. Guanosine was the first of a list of metabolites that interact with high affinity and specificity with RNA. The same binding site located in the group I intron core can accommodate the amino acid arginine and many antibiotics, among them streptomycin, neomycin, and viomycin [2]. Today it is clear that RNA is a potent target for therapeutic drugs. In the past year, a plethora of high-resolution structures of antibiotic-ribosome complexes shed light into the binding mode and recognition principles of RNA-antibiotic interactions [3].

With the development of in vitro selection procedures, it became possible to isolate RNA aptamers for probably every water-soluble ligand, and the small size of these aptamers made them perfect tools to explore the rules that govern recognition of small molecules by RNA. High-resolution structures of several ligand-aptamer complexes have been determined, demonstrating the diversity of structural motifs RNA can fold into [4]. Both simple noncomposite folds that form tight binding pockets as well as complex composite modular shapes can be found. One important outcome of these studies will be a database with an extensive repertoire of RNA structural modules. The streptomycin binding aptamer presented by Tereshko et al. in this issue of *Chemistry & Biology* represents a novel RNA fold with a distinct way to encapsulate a small molecule [5].

To enhance the crystallization procedure, the original aptamer was split into two strands with dangling 5′ ends, a procedure that allowed crystals to develop a few minutes [6]. The streptomycin-aptamer complex adopts an unusually sophisticated structure characterized by a 90° kink between residues C106 and C107 at the bottom of the lower asymmetrical loop, giving the complex its L



Secondary Structure of the Streptomycin Aptamer

The secondary structure of the streptomycin aptamer with the 90° kink between bases C106 and C107 is shown. Solid black lines represent base pairing. Base triples are indicated with orange lines. Bases highlighted in dark blue interact directly with streptomycin, whereas the interaction of G6 and U112 (highlighted in light blue) with the antibiotic is mediated by a water molecule. Arrows indicate binding sites of the three metal ions (labeled M1 to M3).