Structure-Function Relationships of the Saccharomyces cerevisiae Fatty Acid Synthase

THREE-DIMENSIONAL STRUCTURE

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The three-dimensional structure of the Saccharomyces cerevisiae fatty acid synthase was computed from electron microscopy of stain images. The barrel-shaped structure (point group symmetry 32) has major and minor axes of ~245 × 220 Å, respectively, and consists of two different subunits organized in an α6β6 complex (Mr = 2.5 × 10⁶). Two sets of three β subunits form triangle-shaped caps that enclose the ends of the barrel. The wall of the barrel appears to consist of three N-shaped α subunit pairs each with an over and underlying arch-shaped β subunit. Inside the molecule there are three major interconnected cavities that are tilted ~20° with respect to its major axis. An axle-shaped structure extends the length of the cavity on the 3-fold axis and is connected to the two ends of the barrel. The cavities are partially divided on the equator of the molecule by three spokes that extend from the axle on the 2-fold axis to the exterior wall. We propose that these six cavities constitute the six equivalent sites of fatty acid synthesis resulting in an extraordinary structure-function relationship with the 42 catalytic sites involved in fatty acid synthesis inside the molecule. The six cavities each have two funnel-shaped openings (~20 Å in diameter) which may serve to permit the diffusion of substrates and products in and out of these functional units. The subunits appear to be arranged in a manner that affords extensive intermolecular interactions contributing to the stability of this macromolecular complex.

The Saccharomyces cerevisiae fatty acid synthase is a multienzyme complex (Mr = 2.5 × 10⁶) consisting of six copies each of α (Mr = 207,863) and β (Mr = 220,077) subunits (α6β6) (1, 2). The subunits are multifunctional enzymes since they consist of multiple catalytic domains that comprise the seven catalytic activities associated with fatty acid synthesis. Even though the distribution and the order of their activities from amino to carboxyl terminus in the α and β subunits are known (1, 3–5), there is little structural information regarding the organization of the subunits and the architecture of this macromolecular complex.

Single particle images from stain (2, 6–9) and cryoelectron (9) microscopy indicate that the molecule has the shape of a prolate ellipsoid with minor and major axes of ~220 and 250 Å, respectively. The molecule has a high protein density band at its equator and protein density on its major axis that appears in the center of the ring-shaped end views of the structure. Immunoelectron microscopy showed that the α subunits are located in a central region of the oval-shaped particles and, the β subunits are distributed on its two ends (6). The β subunits appear to extend from a central plate in a finger-like (6) or arch-like (9) fashion. Average images of the end views derived from stain and frozen-hydrated particles revealed a hexagonal ring consisting of three each of alternating kidney and egg-shaped components with a Y-shaped feature in its center (9). These studies indicated that the structure has point group symmetry 32 (6, 9).

The finding that there are 6 mol of fatty acids synthesized per mol of enzyme (10) and the kinetics and stoichiometry of its reaction with the substrate analog p-nitrophenyl thioacetate support the proposal that the molecule comprises six equivalent centers of fatty acid synthesis each with its seven catalytic domains (11). Thus, it is not an exaggeration to describe the molecule as a proficient factory for fatty acid synthesis. These remarkable functional characteristics serve to heighten interest in the molecule’s structural organization which the above electron microscopy studies cannot address. Moreover, three-dimensional reconstructions of the fatty acid synthase which consisted of one (12) and eight particles (8) have failed to give significant new information regarding its structure because of their low resolution. These efforts were thwarted by the inability to obtain appropriate alignment of a significant number of side projections of the molecules, which have their major axis parallel to the support film and, consequently, present a continuum of views of the structure. We have developed methods to obtain random orientations of the molecules, and it was determined that the end views of the structure could be readily aligned to obtain the average images described above (9). Accordingly, we report the 25 Å resolution structure of the yeast fatty acid synthase which reveals an elaborate structural organization of the subunits and a most unusual structure-function relationship in that apparently all of the catalytic domains reside inside this barrel-like structure.

EXPERIMENTAL PROCEDURES

Enzyme Purification—S. cerevisiae fatty acid synthase was assayed as described previously (11). A yeast cake (Red Star, 670 g) was added to phosphate buffer (0.2 M KP₂O₇, 1 mM EDTA, 1 mM cysteine, 2 mM benzanilide, 1.7 mM phenylmethanesulfonyl fluoride, pH 6.5) to give a final volume of 1.5 liters. The mixture was blended for 30 s at low speed to disperse the clumps, and the cells were disrupted using the Bead Beater with its container in an ice bath. It was determined that five 30-s pulses with 30-s intervals to permit cooling resulted in the maximum release of the fatty acid synthase activity in the supernatant. Centrifuga- tion at 20,000 × g at 4 °C (for 30 min) yielded the supernatant with a lipid cake on top, which was removed in a cold room by filtering

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through glass wool. All of the following procedures were performed in an atmosphere of dry nitrogen. Polyethylene glycol 8000 (Sigma Chemical Co.) was added to the supernatant with an aim to give a 5.5% (v/v) solution. After centrifugation as above, the polyethylene glycol concentration of the supernatant was increased to 10% (v/v) and, after centrifugation, the resulting pellet contained approximately 70% of the activity. The pellet was dissolved in 250 ml of 0.1 M KPi, 1 mM EDTA, 1 mM cysteine, and 0.5 mM phenylmethanesulfonyl fluoride, pH 7.5, and calcium phosphate gel in H2O was added until the fatty acid synthase activity remaining in the supernatant was <10%. During the addition, the pH was maintained at 6.5 by the addition of acetic acid. The gel was centrifuged as above and suspended in 0.1 M NaPi, 1 mM EDTA, 1 mM cysteine, and 1 mM benzamidine, pH 7.5. After adjusting the pH to 7.5 with 1 M NaOH, the gel was centrifuged at 17,000 × g for 30 min. The enzyme in the supernatant was precipitated with 10% polyethylene glycol and centrifuged. The pellet was dissolved in 60 ml of 0.1 M NaPi, 1 mM EDTA, 1 mM benzamidine, pH 7.2 by gentle stirring overnight. The enzyme was fractionated by gel filtration chromatography on a Sepharose 4B (Pharmacia Biotech Inc.) column (5 × 55 cm) using the same buffer. Fractions containing specific activity greater than 300 nmol of NADPH oxidized min⁻¹ mg⁻¹ were pooled to give a specific activity of ~1000 nmol of NADPH oxidized min⁻¹ mg⁻¹. The pooled fractions were precipitated by slowly adding powdered ammonium sulfate (ICN, ultrapure) to 50% of saturation while maintaining the pH at 7.0 by the addition of 1 M NaOH. The enzyme, which had a specific activity of ~2000, was stored as a suspension containing 10% glycerol at −10 °C or purified further by Superose 6 column chromatography (0.8 × 30 cm) using a fast protein liquid chromatography system (Pharmacia). The enzyme containing specific activity greater than 5000 nmol of NADPH oxidized min⁻¹ mg⁻¹ was used in these studies and was obtained within 48 h after the start of the isolation. This procedure not only reduced the time of isolation from ~6 to 2 days but also consistently increased the yield from ~10 to ~60 mg compared with our previously developed procedure (12, 11).

**Isolation of the α and β Subunits—**The fatty acid synthase (5–10 mg) was dissociated as described previously (14). After reacting with di- methy溜aleic anhydride for 30 min at 0 °C, the inactive enzyme was equilibrated rapidly with 0.1 M Tris-HCl, 1 mM EDTA, 1 mM diithiothreitol, pH 8.1, by gel filtration using a Sephadex G-75 column (1.1 × 17 cm) to promote complete dissociation of the complex and to stabilize the anhydride derivative of the protein. The resulting protein was characterized by ultracentrifugation, gel filtration chromatography, and electron microscopy. Its s20, w of 3.7 S compares with a value of 9.1 S (15) for the monomeric rat liver fatty acid synthase (Mw = 240,000), whereas its elution volume on a Superose 6 column was similar to that for the native yeast fatty acid synthase. The sedimentation coefficient was determined with a Beckman model E analytical ultracentrifuge as described previously (15). These results indicate that the introduction of the negative charge by the maleyl groups results in not only complete dissociation of the complex but also extensive unfolding of the subunits to assume an extended configuration. Stain electron microscopy showed that the protein had a string-like appearance (~300 Å in length). The α subunit was separated from β using a Mono Q column (Pharmacia) using a fast protein liquid chromatography system (Pharmacia) and a linear NaCl gradient (0–0.5 M NaCl in 0.1 M Tris-HCl, 1 mM EDTA, 1 mM diithiothreitol, pH 8.0). The α subunit eluted at ~0.1–0.2 M NaCl, and its purity was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The β subunit was separated from α as described previously (16). Immediately after the isolation of the subunits, the maleyl groups were removed, and the enzyme was activated as described previously (15). Solid ammonium sulfate was added to 70% of saturation, then saturated ammonium sulfate (pH lowered to 2 with acetic acid) was added to lower the pH of the protein to 6.0. After 2.5 h, the protein was centrifuged at 36,000 × g and the pellet resuspended in 0.05 M NaPi, 1 mM EDTA, 1 mM cysteine, 1 mM diisopropyl phosphosphate, or 10 μg/ml human α2, macroglobulin, 5% glycerol, pH 7.0 (reaction buffer) so that its final concentration was ~0.1 mg/ml. Approximately half of the α subunit was degraded within 48 h at 0 °C unless one of the proteinase inhibitors was included in the reaction buffer. The often used phenylmethanesulfonyl fluoride was not effective in preventing proteinase. Removal of the maleyl groups from the enzyme before separation of the subunits and reactivation in the presence of 10 μM FMN as described above resulted in recovery of 90% of its specific activity, and electron microscopy indicated that the αβ2 structure had reformed.

**Electron Microscopy—**A 240-μl sample of yeast fatty acid synthase (10 μg/ml) in 0.25% methylamine tungstate was applied to Butvar 76 film by the spray method (17). To minimize shrinkage of the molecules during beam irradiation (18), the film was carbon coated on one side before application of the protein. A random distribution of the orientations of the particles was achieved by applying the Butvar side of the carbon-coated Butvar film. Images were recorded at 100 kV using conventional irradiation procedures. An underfocus of ~1 μm at the center of the stage was used to record the 50° tilted image and 0.5 μm underfocus for the corresponding nontilted image. The images of the α subunit preparation were prepared for electron microscopy and reactivated β subunits did not have a consistent shape and were judged unsuitable for image analysis.

**Three-dimensional Reconstruction—**Digitization of the micrographs was performed as described previously (18). The micrographs were checked for underfocus, astigmatism, and drift using a digitally generated power spectrum. Initial image analyses were performed using Silicon Graphics 4D25 and Indigo workstations using our SUPRIM (19) image processing software. The individual images that had a ring shape (end views) were collected from the 0° tilt micrograph together with their corresponding 50° tilted images. Correspondence analysis and hierarchical ascendant classification of the average end view image obtained using a model-based alignment (data set n = 2,035) revealed a small group of images (n = 46) which were significantly different from the grand average; they were discarded. The remaining cluster averages were similar to the grand average, indicating a homogeneous data set. After three passes of alignment a spectral signal to noise resolution (20) of 22 Å was obtained. A three-dimensional reconstruction of the tilted end views was performed by the weighted back projection of a random conical tilt series as described previously (21). Side view images were also picked (oval shape with a high density band bisecting its major axis (9), for subsequent refinement of the three-dimensional structure.

**Refinement of the Three-dimensional Reconstruction—**The refinement of the SUPRIM reconstruction was carried out using the three-dimensional projection alignment method (22) implemented in the SPI-DER system (23). A set of projections was generated by tilting the model 0–90° (θ) and rotating it 0–360° (φ) about its major axis in 2° increments (22). The candidate images consisted of 50° tilted end views (n = 1,989) used in the original reconstruction. Two passes of refinement were usually sufficient to achieve a stable resolution value. Orthogonal orientations of the molecules (side views) were incorporated in the reconstruction in two steps. (i) A data set of 50° tilted images (n = 137) which were judged to have their major axis approximately perpendicular to the tilt axis in the corresponding nontilted field were incorporated in the candidate list above. The refinement was repeated as described, and (ii) the new model was used to incorporate 554 nontilted side views in the reconstruction. Two successive refinements of the collected data sets (2,676 images) achieved a stable resolution value.

The reconstruction of the complex was estimated by randomly dividing the data sets into halves, and two iterative refinements were generated using the same number of iterations and parameters of the reconstruction as those for the complete data set. The resolution was estimated (based on the radius of the spherical shell) by differential phase residual (DFR) (24) and the Fourier ring correlation methods (25) with a ring correlation coefficient of 0.67 (20) after thresholding the reconstructions to a volume that corresponds to the molecular weight of the fatty acid synthase (Mw = 2.5 × 106).

**Display—**The three-dimensional displays were displayed as shaded solid surface models using SUPRIM rendering. Selected stereo views of the symmetrized, shaded solid surface and wire frame renderings were generated using SYNU (26). A solid representation of the central cavity was created by excising the exterior and inverting the pixel values. The resulting cavity image was filtered and thresholded at values corresponding to those for the reconstruction. The images were produced using a Mitsubishi S3600-30U dye sublimation color printer.

**RESULTS**

The initial three-dimensional reconstruction of the yeast fatty acid synthase was computed by weighted back projection from a conical tilt series employing the 50° tilted end view images (Fig. 1A). Even though the end view projection of the structure (Fig. 1A) shows a good concordance with the corresponding average image (Fig. 1F), its side view projection lacks the prominent band of protein density that bisects the major axis of the molecule (Fig. 1F). This band of high protein density

1 The abbreviation used is: DFR, differential phase residual.
is also a prominent feature of the individual stain (2, 6–8) and frozen-hydrated molecular images (9). Instead, a vertical high density band is prominent (Fig. 1A), which may be attributed to the missing cone artifact (27). Furthermore, it is apparent that significant structural information is lacking in the equator of the reconstruction where the six α subunits are located (see below). Therefore, we have pursued methods to incorporate the orthogonal side views of the molecules into the reconstruction.

Refinement (22) of the reconstruction reduced the surrounding noise but, not surprisingly, did not improve the concordance between the side view projection (Fig. 1B) and the average image (Fig. 1F). The resolution of the refined reconstruction (25 Å, DPR) represented a modest improvement in the initial reconstruction (27 Å), indicating that the end view images utilized consisted primarily of close to on axis projections of the molecules.

An effort to incorporate nontilted side views in the reconstruction using the refined reconstruction (Fig. 1B) as a model failed to align these images. However, it was possible to incorporate 137 tilted 50° side view orientations of the molecules. The resulting reconstruction (Fig. 1C) shows no significant improvement in the concordance between the projection and the average image, but the resulting model had sufficient information in the region of the missing cone to align the nontilted side views appropriately. The entire data set consisting of tilted end views, tilted side views, and untitled side views was combined to give the refined reconstruction in Fig. 1D (resolution = 24 Å). Its end view and side view projections show good concordance with the corresponding average images, indicating that the missing cone artifact has been minimized. Further refinement of the reconstruction shown in Fig. 1D resulted in a slight improvement in the resolution, 22 Å DPR and 20 Å Fourier ring (a similar value of the resolution was also obtained by employing a soft mask and no threshold (28), 26 Å DPR). Moreover, some of the minor features such as the three protrusions on the two ends of the structure are better defined (compare Figs. 1D and 2A). The reconstruction (Fig. 2) was low pass Fermi filtered (29) to 25 Å to minimize contrast transfer function effects.

A reconstruction was also performed consisting of only 50° tilted images: 1,989 and 682 end and side views, respectively. The resulting structure (24 Å resolution) was very similar to the structure in Fig. 1D in which the corresponding 545 nontilted side views were utilized (data not shown). The inclusion of the orthogonal side views, either tilted or nontilted, in the reconstruction improves its resolution and the concordance between its projections and the average images. Consequently, we conclude that the structural information of the molecule is reproduced faithfully by the methods employed to image it. The average image of the side view (Fig. 1F, right) appears slightly longer (~8%) than the side view projection of the structure (Fig. 1E, right). This difference may result from the larger number of end views (1,989) than side views (682) used in the refinement or may simply reflect differences in the thresholding.

The excellent concordance between the 32 symmetrized (Fig. 2) and nonsymmetrized (Fig. 1D) reconstructions indicates that the molecule has point group symmetry 32. It is also interesting to note that the projection of the end view of the symmetrized reconstruction (Fig. 1E) shows additional features not seen in the average image (Fig. 1F) probably because of its higher resolution.

Stereo views of the structure viewed on the 3-fold axis (Fig. 2A) and two presentations on the 2-fold axis, front (Fig. 2B) and back (Fig. 2C) show that the structure is barrel-shaped. The molecule has dimensions of ~245 × 220 Å. It is capped at both ends by triangle-shaped bodies (~70 Å in a side) in approximately the same orientation (see below) (Fig. 2A). Three narrow extensions are near the base of the cap and are positioned near the vertices of the triangle. Two larger triangle-shaped structures (200 Å on a side) lie underneath the caps in approximately the same orientation. The middle section of the structure (Fig. 2, B and C) is ~170 Å in length and has 12 openings, six on either side of the equator. Three prominent protrusions on the 2-fold axis extend ~25 Å from the side of the barrel and are oriented 120° with respect to each other normal to the major axis. These features are responsible for the protrusions on the ring seen in the end view average image (Fig. 1F). The outside wall of the barrel has a zig-zag appearance with the prominent protrusion in the center and four surrounding openings (Fig. 2B). Three of these shapes comprise the side wall of the barrel.

Serial sections cut normal to the barrel’s major axis show the
protein density distribution of the structure and reveal an elaborate internal cavity (Fig. 3). An initial inspection of the slices shows that most of the protein and the highest protein density of the molecule are associated with the middle portion of the structure (compare rows 3–5 with 1, 2, 6, and 7). It is expected that this portion of the structure comprises the activity domains associated with the α and β subunits since the two subunits are most proximal to each other in this region of the molecule (see below).

The central slices (rows 3–5) have three approximately round high density patterns that are associated with the prominent triangular protein density in the center slice (4C). Alternating
between these strands are three protein density patterns of variable shape which are associated with the kidney-shaped protein density in the central slice (4C). These pairs of strands separate in sections 3B–E and 5A–D to form the openings to the internal cavity. The triangle-shaped caps (1A–D and 7B–E) at both ends of the structure form the roof over the three cavities that traverse through the middle portion of the molecule (2A–6E). The middle sections (4B–D) show a prominent triangle-shaped rod on the 3-fold axis which is attached by low density connections to the three triangular components in the outer ring. The six protein strands rotate ~20° clockwise through the middle portion of the molecule.

The cut-away view of the end of the structure (Fig. 4A) reveals its roof and shows that the cavity is divided in three sections. However, the partitions are absent in the body of the molecule (Fig. 4B). This section of the structure has a cartwheel appearance: a triangular axle on the 3-fold axis is connected by three spokes to the outer ring of protein on the 2-fold axis. The axle extends to both ends of the molecule, and its three spokes divide the three major cavities into six segments with interconnecting openings (Fig. 4D). Pairs of diagonally opposite round and oval openings lead into the cavity through funnel-shaped grooves in the outer wall of the structure (Fig. 4C). The slanted appearance of the cavity in the cut-away structure (Fig. 4, C and D) is related to the twist in the protein strands (Fig. 3).

A wire frame rendering of the structure with its solid shaded cavity in red (Fig. 5, A and B) shows that the cavity is buried deep in the molecule and further illustrates that the three major interconnected cavities are tilted with respect to the major axis of the structure.

**DISCUSSION**

**Electron Microscopy and Image Processing**—In conventional stain and cryoelectron microscopy the yeast fatty acid synthase molecule assumes an orientation so that its major axis is approximately parallel to the support or the air-water interface (9). Even though the molecule appears to interact with the surfaces, the variant appearance of both stain and frozen-hydrated images indicates that it is free to rotate about its major axis. The inability to obtain side views of the structure with a preferred orientation has made their alignment problematic.

Molecules interact little or not at all with the Butvar support film (17) making it possible to obtain multiple orientations of the fatty acid synthase (9). The end view images were aligned successfully to give the average image (Fig. 1F) with spectral signal-to-noise resolution of 22 Å, and the three-dimensional structure was computed from the corresponding 50° tilted images with a DPR resolution of 27 Å. We have utilized a novel approach to refine this reconstruction by using its model to align 50° tilted side view images. Consequently, a major improvement in the reconstruction was achieved after orthogonal side view images were incorporated as shown by the good concordance between the projections of the reconstruction and the corresponding average images (Fig. 1, E and F) and the betterment of the resolution to 22 Å. Thus, the Butvar support film and the methylamine tungstate stain obviate the need to employ the double carbon film technique (30), which may flatten the molecule (28). The statement “The important lesson for data merging is that [three-dimensional] reconstructions from negatively stained molecules cannot be merged unless they are based on the same view of the molecule, i.e. on images showing the molecule facing the support grid in the same orientation” (28) does not apply to our methods of preparing the molecules for imaging (17). In addition to the present study, successful three-dimensional reconstructions of the *S. cerevisiae* truncated dihydrolipoamide acetyltransferase (31) and native human α2-macroglobulin (18) from particle images in multiple orientations (views) are further support for the utility of this stain and support film in the imaging of macromolecules. The ~20% isotropic shrinkage of the molecules in the former reconstruction (31) has been avoided by employing the carbon-backed Butvar film (see “Experimental Procedures”).

**Structure-Function Relationships**—Most enzymes have their catalytic sites disposed in crevices or pits on the outside surface of the structure, whereas on the inside there is little or no water and an abundance of hydrophobic residues (32). *S. cerevisiae* fatty acid synthase is unusual: it has a solvent filled cavity (Fig. 4) which is lined with 42 catalytic sites organized in groups of seven to constitute the six centers of fatty acid synthesis (10). The evidence for the internal disposition of the catalytic domains results from antibody binding studies. It was shown that polyclonal antibodies (anti-α) resulted in only a slight decrease (~10%) of fatty acid synthase activity and no inhibition of the partial reactions. Similarly, anti-β inhibited only the malonyl-palmitoyl transferase activity and fatty acid synthase activity by 50%. The other two activities (enoyl reductase and dehydratase) were unaffected (6). A 50-kDa Fab fragment isolated from the IgG against the acyl carrier protein domain did not inhibit fatty acid synthase activity but did cross-react with the α subunit in a Western transfer experiment.3 Our three-dimensional structure affords an explanation for the inaccessibility of the catalytic sites. The six 23-Å-diameter oval and six 17-Å-diameter round openings leading into the cavity are too small to accommodate the IgG molecule or the Fab fragment (Fig. 4). Furthermore, the internal cavity with its catalytic sites is sequestered deep inside the structure (Fig. 5, A and B).

The cut-away structures (Fig. 4) and the structural organization of the molecule (see below) afford a plausible proposal for

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1. U. Klueppelberg, R. H. Cheng, M. A. Yazdí, C. Y. Maeng, T. S. Baker, L. J. Reed, and J. K. Stoops, unpublished results.
2. A. H. Mohamed, J. K. Stoops, and S. J. Wakil, unpublished results.
Fig. 4. **Stereo views of the cut-away structure.** Approximately one-third of the structure on its 3-fold axis was cut away, giving the cap (A) and the remaining two-thirds of the molecule (B). Similarly, the front one-third (C) and back two-thirds (D) were cut away with the structure on its 2-fold axis. After the top and front sections were cut away, they were rotated 180° about the vertical axis to expose their interior. The cap (A) contains three cavities, the body a central axle with three spokes (B and D), and the wall of the barrel has 12 funnel-shaped openings that permit the diffusion of substrates and products in and out of the cavities containing the six centers of fatty acid synthesis.
the functional organization of the molecule. A center for fatty acid synthesis comprises a wedge that includes the interior wall formed by the axle (Fig. 4D), a roof (Fig. 4A), and the exterior wall with its two funnel-shaped openings and the spoke that forms the floor (Fig. 4C). Thus, two centers of fatty acid synthesis are displayed in the cut-away structures, one above and below the spoke on the 2-fold axis (Fig. 4, C and D). There are six of these structures inside the barrel, which is consistent with functional studies that indicate that the enzyme has six equivalent centers of fatty acid synthesis (10).

Each compartment contains the seven catalytic domains and the acyl carrier protein component with its 4'-phosphopantetheine prosthetic group. This moiety serves to transfer the covalently bound intermediates of fatty acid synthesis (“swinging arm”) to the catalytic domains (7). Complementation studies of fatty acid synthase mutants have shown that a defect in a catalytic site in one mutant can complement a defect in a different site in another mutant (34). The openings between the cavities located on its wall and floor (Fig. 4, B and D) may permit the transfer of intermediates between centers of fatty

Fig. 5. Stereo views of the wire frame structure and a solid shaded representation of its central cavity in red on its 3-fold (A) and 2-fold axis (B). Shown is an interpretive model of the organization of the α subunits (yellow) and the β subunits (gray) in the structure viewed on the 2-fold axis (front half and back half, left to right). The zig-zag shape of the α subunits forms the wall of the barrel, and two arch-like β subunits over- and underlie pairs of the α subunits and form the caps.
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acid synthesis.

Structural Organization of the Molecule—Immunoelectron microscopy studies (6) and our reconstruction indicate that the six α subunits are disposed near the equator of the structure, whereas the six β subunits are arranged three on either side of the equator and form the ends of the barrel. Inactivation studies of the enzyme with the bifunctional reagent 1,3-dibromo-2-propanone showed that cysteine and pantetheine residues were modified, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated that reaction resulted in formation of primarily α dimers (13). Consequently, it was proposed that pairs of α subunits were cross-linked through their neighboring pantetheine and cysteine residues, and the subunits are juxtaposed to head to tail (13, 33). Consequently, the six α subunits are related to each other by a 180° rotation about an axis normal to equator of the barrel, and as a result, there are two sets of interacting sites between the adjacent subunits. We propose that one of the sites of interaction lies on the 2-fold axis (Fig. 2B) and the other on the 2-fold axis seen on the back half of the structure (Fig. 2C). There are three each of these two sites of interaction around the wall of the barrel.

We have investigated the structure of the α and β subunits by electron microscopy to gain further insight into their disposition in the complex. The β subunits do not exhibit a consistent shape possibly because they have an extended flexible structure. The α subunits have an H-like shape, and multiple views of the structure indicated that they form dimers and tetramers (Fig. 6). There was no indication that the α subunits associated to form an αβ ring-like structure that could be representative of their disposition in the equator of the barrel (Fig. 6A). In this regard, we and others (14) have found that it is not possible to regain fatty acid synthase activity or reconstitute the structure upon mixing the subunits after treating them separately with the reactivation buffer. However, nearly full activity and the structure were reformed if the subunits were reactivated together. These results suggest that the α and β subunits must be together during the renaturation process to form the active structure.

The shape and size of the average image of the isolated α subunits (Fig. 6B) suggest how they are arranged in the structure. We propose that a pair of α subunits has an N-shape, they interface on the 2-fold axis, and that two arch-shaped β subunits overlie the pair of α subunits (Fig. 5C, left) to form the caps. This arrangement represents the αβ2 protomer unit. The structural arrangement in the right image of Fig. 5C could equally represent the protomer unit since both views are on the structure’s 2-fold axis; however, the average image (Fig. 6B) appears to show greater similarity to the left image (Fig. 5C)). A portion of the two α subunits is juxtaposed within the two β arches and, consequently, may afford extensive overlap between the catalytic sites associated with the α and β subunits. In this regard, the α and β subunits together form the funnel-shaped openings leading into the cavities. Pairs of α and β subunits are related by a 180° rotation about axes normal and parallel, respectively, to the major axis of the structure. In other words, the β subunit in the top half of the structure is in the opposite orientation to one directly below it, and the α subunits are oriented in a flip-flop arrangement around the central ring. This organization is consistent with the molecule encompassing six equivalent centers of fatty acid synthesis (8). The three spokes on the 2-fold axis of the molecule (Fig. 4B) consist of pairs of α subunits with their subunit-subunit interface. A portion of the six α subunits joins in the center of molecule to form the large triangular feature (~82 Å on a side) (Fig. 4, B and D) on the axe. The two sets of three α subunits which are disposed in an opposite orientation extend in opposite directions to the caps where they interact with the β subunits. The three blobs seen in the slices (Fig. 3, 1D and 7B) may represent strands of the three α subunits. This structural arrangement of the subunits affords extensive intermolecular contacts. Even though the exact shape and arrangement of the subunits are not known, it is apparent that the architecture of the structure is designed to form a stable complex with its centers of fatty acid synthesis well protected from the cellular milieu.

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