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ARTICLE

^{1}H, ^{15}N, ^{13}C resonance assignment of the acyl carrier protein subunit of the *Saccharomyces cerevisiae* fatty acid synthase

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Abstract Acyl carrier proteins participate in the synthesis of fatty acids. Here we report the NMR resonances assignment of the acyl carrier protein domain of the *Saccharomyces cerevisiae* fatty acid synthase which corresponds to the fragment 138A-302L in the primary structure. The assignment will allow performing NMR studies with the aim to investigate the intrinsic dynamics of this protein, and to study the structural changes upon apo-holo transformation in order to unveil the mechanism of binding of the growing acyl chain.

Keywords Acyl carrier protein (ACP) · *Saccharomyces cerevisiae* · Resonance assignments · Fatty acid synthase (FAS)

Biological context

Acyl carrier proteins (ACPs) are part of a family of structurally conserved carrier proteins that participate in the synthesis of fatty acids. In bacteria and fungi they are also involved in the synthesis of polyketides (PK) and non ribosomal peptides (NRPs). Polyketides and NRPs are secondary metabolites for which an extraordinary variety of pharmacological properties has been discovered (Cane et al. 1998). In all the biosynthetic processes in which ACPs are involved, the metabolites are assembled by elongation of carboxylic acid building blocks that are activated as acyl/aminoacyl thioesters to drive catalyzed condensation steps that result in production of a peptide or ketide chain (Cane et al. 1998; Hopwood 1997; White et al. 2005).

In the fatty acid synthesis as well as in most of the biosynthetic processes involving the carrier proteins, the building blocks are tethered to the terminal thiol of a phosphopantetheine prosthetic group that is covalently attached to the ACP (Lai et al. 2006). Acyl carrier protein shuttles the acyl intermediates in fatty acid synthase (FAS) between the functional enzyme domains involved in the cyclic condensation of 2-carbon acyl units from malonyl-CoA that typically yields (16:0) palmitate as a product. In bacteria the ACP and the catalytic domains of FAS are separate proteins (type II FAS) (Byers and Gong 2007), whereas in yeast and mammals ACP is one domain integrated along with the biosynthetic enzymes into large multidomain megasynthase (type I FAS) (Leibundgut et al. 2008). The ACP structure is highly conserved in the family of the carrier proteins and consists of a minimal fold with three major alpha helices (I, II, and IV) and a short alpha helical segment (III), with a conserved serine residue that serves as an anchor site for the covalent attachment of the phosphopantetheine cofactor (Byers and Gong 2007).

Acyl carrier proteins of type II FAS as present in bacteria and plants shuttle the substrates and elongating chains to the different catalytic domains of the FAS system while diffusing free in the cytoplasm. Acyl carrier protein establishes low affinity but highly specific interactions with the enzyme partners mediated by a recognition site located in its α-helix II [for a review see (Byers and Gong 2007)]. As an interesting characteristic feature this type of ACPs exhibit a highly dynamic scaffold which was revealed by NMR studies; e.g. the *E. coli* ACP structure is an average between conformations in fast exchange (Kim and Prestegard 1989), and the spinach ACP appears to exist in a conformational equilibrium in slow exchange (Kim and

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Prestegard 1990). *E. coli* ACP has been shown to be only marginally stable at physiological conditions, and it sequesters the growing acyl chain in a flexible hydrophobic binding pocket (Roujeinikova et al. 2007) increasing the protein stability (Kim and Prestegard 1990). The binding of an enzyme partner increases the stability of the ACPs and in some cases ACPs of type II FAS fold only upon binding with the enzyme partners (Gong et al. 2008). The strong reduction in entropy upon binding of this naturally unfolded proteins has been proposed to enable highly specific binding with low affinity, allowing the kind of reversible interactions often seen for proteins that have multiple partners (Tompa 2002).

Interestingly, the mammalian ACP (type I FAS) does not sequester the growing acyl chain suggesting a different mechanism of recognition in the multidomain type I FAS. The crystal structure of *Saccharomyces cerevisiae* FAS (Leibundgut et al. 2007) was solved with the ACP domain stalled at the active site of the ketoacyl synthase (KS), the first catalytic enzyme in the reaction cycle of elongation of the fatty acid chain. The structure showed the arm of the phosphopantetheine cofactor reaching deep into the cleft of the active site of KS. In this case the ACP not only establishes interactions with the enzyme partner (KS) but also with regions of the structural scaffold of the fatty acid megasynthase. This mechanism of recognition is different than the one observed in the bacterial FAS suggesting that the ACP of type I FAS possibly does not need to have the dynamic nature observed in ACPs in type II FAS. The importance of unveiling the nature of ACP-enzyme recognition and the stabilization of the growing acyl chain by ACP reaches far beyond the research of fatty acid biosynthesis; it would also help to understand the recognition mechanisms of PKs and NRPs, opening avenues to manipulate those processes for the production of new natural product variants with high efficiency (Lai et al. 2006). Here we present the NMR assignment of the *S. cerevisiae* ACP (part of a type I FAS) that enables the NMR study of dynamic aspects and the binding of the growing acyl chains to the protein.

Methods and experiments

Protein preparation

Fragment A138-L302 of the alpha subunit of the *S. cerevisiae* fatty acid synthase comprising the acyl carrier protein globular domain cloned into the expression vector pET28a with N-terminal His-tag (M120–N137) was kindly provided by Prof. Nenad Ban (ETH Zuirch). For isotope labeling, a freshly transformed overnight culture of *E. coli* BL21(DE3) cells (Stratagene, La Jolla, CA, USA), containing the plasmid for expression of *S. cerevisiae* ACP (*sc*ACP) was added at 37 °C to 2 l of minimal medium (M9) containing 1 g/l [^{15}N]-ammonium chloride, 4 g/l [^{13}C-6]glucose, a vitamin cocktail (5 mg/l thiamine, 1 mg/l D-biotin, 1 mg/l choline chloride, 1 mg/l folic acid, 1 mg/l niacinamide, 1 mg/l D-pantothenic acid, 1 mg/l pyridoxal hydrochloride, 0.1 mg/l riboflavin), trace elements and kanamycin (40 µg/ml). At $OD_{600} = 0.7$, expression was induced with isopropyl L-D-galactopyranoside (IPTG) added to a final concentration of 1 mM. Cells were harvested 4 h after induction, centrifuged, and resuspended in 100 ml buffer A (100 mM potassium phosphate, pH 8.0). After lysis by french press, sonication and centrifugation, the soluble protein fraction was added to 25 ml of nickel-nitrilotriacetic acid (NTA) agarose resin (Qiagen, Valencia, CA, USA) and was stirred for 1 h at room temperature. The resin was poured into a glass column and washed with 250 ml buffer B (100 mM potassium phosphate, 20 mM imidazole, pH 8.0).

The immobilized *sc*ACP was eluted with buffer C (100 mM potassium phosphate, 150 mM imidazole, pH 8.0). The purified protein (incl. His-tag) was analyzed by SDS–polyacrylamide gel electrophoresis. Protein elution was monitored by UV at 280 nm. The collected fractions were analyzed by SDS–polyacrylamide gel electrophoresis and the fractions containing ACP were concentrated to 5 ml by filtration–centrifugation using Centriprep YM-3. The protein solution was dialyzed three times against buffer D (10 mM K_2PO_4, pH6.5) 1:100. The protein concentration was determined using the calculated molar extinction coefficient, $\varepsilon_{280} = 9{,}970\ M^{-1}\ cm^{-1}$ for *sc*ACP. The protein was further concentrated to obtain samples for NMR spectroscopy by filtration–centrifugation. The NMR sample was obtained with a final dialysis overnight against NMR buffer; 90% H_2O, 10% D_2O, 10 mM potassium phosphate at pH 6.5.

NMR measurements and resonance assignments

Two NMR samples of *sc*ACP were prepared at 1 mM protein concentration, one uniformly ^{15}N-labeled, and the other uniformly ^{13}C/^{15}N-labeled. All NMR measurements were performed at 298°K on a Bruker AVIII 600, or a Bruker AVIII 750 spectrometer, both equipped with room temperature triple-resonance probes with z-gradient coils. All spectra were processed with the software Topspin (Bruker, Karlsruhe, Germany). For the spectral analysis the program CARA (Keller 2004) was used. The following spectra were recorded: a [^{15}N,^{1}H]-COSY and a [^{13}C,^{1}H]-COSY spectrum (Bodenhausen and Ruben 1980); a 3D HNCA-spectrum (Kay et al. 1990); a 3D HNCACB (Wittekind and Mueller 1993); a 3D HNCO (Grzesiek and Bax 1992); a 3D HNCACO (Clubb et al. 1992); a 3D

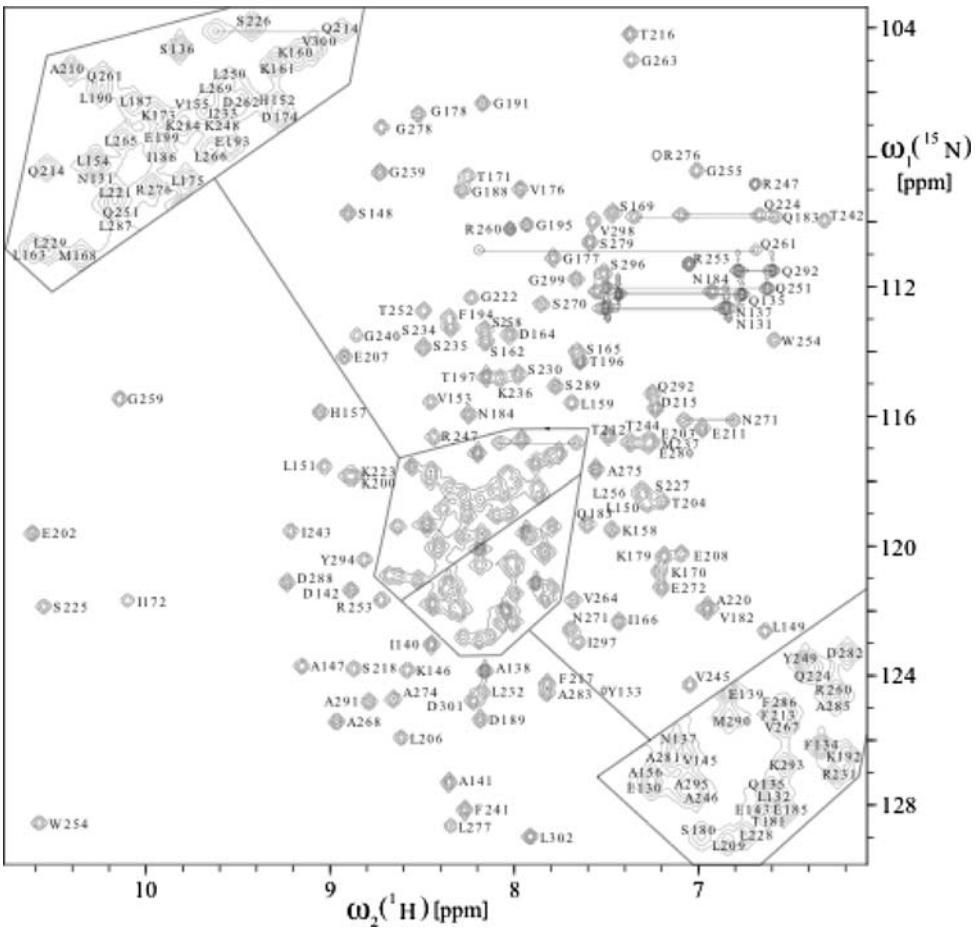

Fig. 1 [^{15}N,^{1}H]-COSY spectrum of 1 mM ^{13}C,^{15}N-labeled acyl carrier protein (ACP) domain of *Saccharomyces cerevisiae* fatty acid synthase (FAS), 10 mM potassium phosphate (pH 6.5), 90% (v/v) H_2O/10% (v/v) $^{2}H_2O$, 298°K, recorded at 750 MHz ^{1}H resonance frequency. Two expansions of the crowded region of the spectrum are shown in the *upper left* and *bottom right* corners. The resonance assignments for the backbone amide ^{15}N–^{1}H signals of all the residues of the globular domain ACP (138A-302L) except 219G are indicated in addition to some of the resonances assignment of the N-terminal flexible His-tag used for purification (residues M120–N137); all side chain NH_2 resonances of Asn and Gln residues could be assigned, the pair of resonances are connected by *horizontal lines*

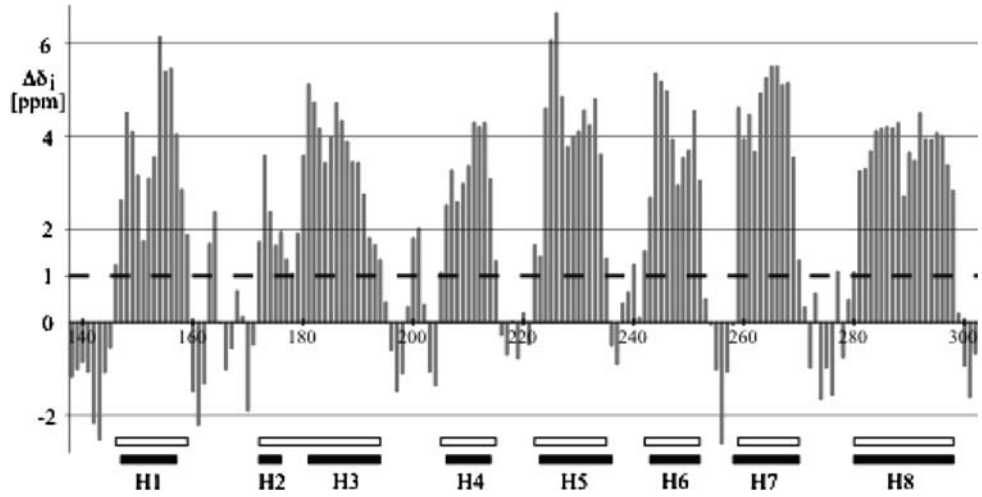

Fig. 2 Deviations, $\Delta\delta_i$, of C^{α} and C^{β} chemical shift differences from corresponding random coil values. Regular secondary structures were identified using established empirical relationships (Spera et al. 1991). $\Delta\delta_i$ was calculated from one-third of the differences $\Delta\delta C^{\alpha} - \Delta\delta C^{\beta}$ summed over three consecutive residues (Metzler et al. 1993); *open rectangles* at the *bottom* indicate helical secondary structure determined by $\Delta\delta_i \geq 1$ for three or more consecutive residues; *solid black rectangles* indicate α-helixes in the crystal structure which contains no *β*-sheet (Leibundgut et al. 2007)

HCcH-TOCSY with 23 ms mixing time (Bax et al. 1990; Muhandiram et al. 1993); a 2D (Hb)Cb(CgCd)Hd spectrum (Yamazaki et al. 1993); a 3D ^{15}N-resolved [^{1}H,^{1}H]-NOESY (Fesik and Zuiderweg 1988) and two 3D ^{13}C-resolved [^{1}H,^{1}H]-NOESYs with carriers centered in the aliphatic region and in the aromatic region, respectively (Fesik and

Zuiderweg 1988; Muhandiram et al. 1993), for all three NOESY experiments a mixing time of 60 ms was used.

Extent of the assignment and data deposition

We report the chemical shift assignment for the *S. cerevisiae* acyl carrier protein. As shown in the [^{15}N–^{1}H]-COSY spectrum of the Fig. 1, 156 out of 157 expected backbone amide resonances were assigned. More than 96% of the resonances of the backbone ^{15}N and $^{13}C^{\alpha}$ atoms were assigned. The missing backbone atom resonances consist of nine nitrogen atoms, corresponding to the N atom of eight prolines and of G219, the latter does not appear in a [^{15}N,^{1}H]-COSY (Fig. 1). In addition to the backbone assignments, nearly complete sequence specific assignment of the side chain resonances was achieved. The missing side chain resonances are the Nε amine of R131 that become broad and disappear below a temperature of 303°K, the Hδ protons of lysines (due to overlap) and the exchangeable side chain amines of 5 arginines, 14 lysines, and 2 histidines. The side chain ^{15}N amine chemical shift of the residues N271 and Q214 present unusual ^{15}N chemical shifts of about 116 and 117 ppm, respectively.

The deviations of C^{α} and C^{β} chemical shifts from corresponding random coil chemical shifts were used to identify regular secondary structure elements (Fig. 2). The locations of the secondary structure elements in solution agree well with those observed in the crystal structure [(Leibundgut et al. 2007) PDB entry 2UV8] suggesting only small structural deviations between the solution and crystalline states. The assigned chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 16085.

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