Structure and mechanism of the glycyl radical enzyme pyruvate formate-lyase[6]

Andreas Becker[1], Karin Fritz-Wolf[1], Wolfgang Kabsch[1],
Joachim Knappe[2], Sabine Schultz[2], A. F. Volker Wagner[2]

[1] Max-Planck-Institut für medizinische Forschung, Abteilung Biophysik
Jahnstraße 29, 69120 Heidelberg, Germany
[2] Biochemie-Zentrum Heidelberg, Ruprecht-Karls Universität,
Im Neuenheimer Feld 501, 69120 Heidelberg, Germany

The enzyme pyruvate formate-lyase (PFL) catalyzes the reversible conversion of pyruvate and CoA into acetyl-CoA and formate, which has a central role in anaerobic glucose fermentation by *E. coli* cells and other bacteria [1]. PFL - a 2×85 kDa homodimer - is the first example of a radical enzyme where the spin was found to be located on the polypeptide backbone Cα-atom of a glycyl residue (Gly 734) [2,3]. Biochemical studies revealed that the substrate conversion by PFL proceeds via a homolytic mechanism and occurs through two half-reactions involving an acetyl-enzyme intermediate (E + pyruvate $\rightleftarrows$ acetyl-E + formate; acetyl-E + CoA $\rightleftarrows$ E + acetyl-CoA) [4]. In the active site two adjacent cysteinyl residues (Cys 418, Cys 419) play a central role in carrying the intermediary acetyl. Moreover, it has been proposed that a thiyl radical from one of these two cysteine residues would be generated by the glycyl radical and used to promote the homolytic substrate cleavage.

Crystals of native PFL (nonradical form; $P4_32_12$, a = 158 Å, c = 159 Å) proved problematic with respect to obtaining isomorphous heavy atom derivatives due to the pair of adjacent cysteine residues (418, 419). For initial structure determination we have therefore chosen to work with the C418,419A double mutant of PFL which yielded crystals diffracting to 2.3 Å resolution (DESY/BW7B, 100K) and gave an isomorphous Hg-derivative. Based on the C418,419A mutant, additional isomorphous Hg-derivatives were obtained under identical soaking conditions from mutant protein crystals (Tab. 1). The structure of the C418,419A double mutant was used as vehicle to solve the wild type structure and the wild type structure in complex with oxamate, which is an isosteric and chemically inert analogue of the pyruvate substrate.

The monomers of the PFL-dimer are related by a nearly perfect twofold rotation whereby this redundancy was used for phase extension. The monomer is assembled in an antiparallel manner from two parallel five-stranded β-sheets, forming a 10-stranded β/α barrel flanked on the outside by α-helices. The active site residues Cys 419 and Gly 734 are found at the tips of short loops, each connecting a two-stranded antiparallel β-sheet. These finger loops protrude from the top and bottom surfaces into the centre of the barrel where the Cys and Gly residues meet at a Cα-distance of 4.8 Å. Our atomic model of the enzyme, with oxamate located in the active site, suggests a new view of the catalytic mechanism, in which the central Cys residues (Cys 418, Cys 419) both participate as thiyl radicals. Among the catalytic amino acid residues, Cys 418 is found in the active site at a strategic position, where its Sγ is inserted between the oxamate/pyruvate plane and Trp 333 at a distance of

3.3 Å to C2 atom with an optimal angle for nucleophilic attack on sp^2 carbonyl by Cys 418 thiyl. The short distance of 3.7 Å estimated between Gly 734Cα and 419Sγ as well as between 419Sγ and 418Sγ can readily account for the direct H transfers between these residues that we propose for the initiation and termination phase of catalysis.

Table 1 Data collection

	wild type	wild type-oxamate	C418,419A
Outer shell (Å)	3.0-2.9	2.7-2.6	2.4-2.3
$\langle I/\sigma \rangle$ in outer shell	3.14	3.09	2.95
Completeness (%)	98.5	96.1	95.6
R_{meas}[1](%)	8.8	8.8	7.0

PFL mutants used for phase determination

	C418,419A	C418,419A,Y735C	C418,419A,C122S
Resolution range (Å)	25.0-3.0	25.0-3.2	25.0-4.5
Completeness (%)	93.9	88.5	97.5
Outer shell (Å)	3.0-2.9	3.2-3.3	3.0-2.9[5]
$\langle I/\sigma \rangle$ in outer shell	3.13	2.60	2.94
R_{meas}[1](%)	15.6	16.2	18.0
R_{cullis}[2] (%)	60	62.0	59.3
Phasing power[3]	1.56	1.47	1.53
ρ_{ano}[4]	0.50	0.35	0.45

[1]R_{meas} as defined by Diederichs and Karplus [5] are quality measures of the individual intensity observations. [2]Cullis R factor for centric reflections. [3]Phasing power is the mean value of the heavy atom structure factor amplitude divided by the residual lack-of-closure error. [4]Correlation factor between observed and calculated intensity differences of Bijvoet paires. [5] For C418, 419A, C122S only data of resolution lower than 4.5 Å were used for phasing. Data were collected at BW7B/DESY.

References

[1] Kessler, D. & Knappe, J. Anaerobic Dissimilation of Pyruvate. In *Escherichia coli* and *Salmonella*, cellular and molecular Biology (Neidhardt, F. C. et al., eds) 199-205, American Society for Microbiology, Washington, DC. (1996).

[2] Knappe, J., Neugebauer, F. A., Blaschkowski, H. P. & Gänzler, M. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 1332-1335 (1984).

[3] Wagner, A.F.V., Frey, M., Neugebauer, F. A., Schäfer, W. & Knappe, J. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 996-1000 (1992).

[4] Knappe, J., Blaschkowski, H. P., Gröbner, P. & Schmitt, T. *Eur. J. Biochem.* **50**, 253-263 (1974).

[5] Diederichs, K.& Karplus,P.A., *Nature Struct. Biol.* **4**, 269-275 (1997).

[6] Becker, A., Fritz-Wolf, K., Kabsch, W., Knappe, J., Schultz, S.& Wagner, A.F.V. *Nature Struct. Biol.* **6**, 969-975 (1999).