Activity versus Peroxisomal Targeting of PerCR

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DOI 10.1016/j.str.2008.02.006

Peroxisomal carbonyl reductase (PerCR), a tetrameric enzyme, enters peroxisomes when expressed in human cells, but not when PerCR tetramers are introduced into these cells. The PerCR crystal structure (Tanaka et al., 2008) yields insights that explain these data.

Pig heart peroxisomal carbonyl reductase (PerCR) belongs to the short chain dehydrogenases/reductases (SDRs) family, a large and diverse family of enzymes found in bacteria, yeast, and multicellular animals (Kallberg et al., 2002). The discovery that enzymes that catalyzed the synthesis or inactivation of steroid hormones, such as estradiol, testosterone, and cortisol were SDRs, stimulated initial interest in this protein family (Baker, 2001; Wu et al., 2007). Currently, the PDB contains over 200 crystal structures of wild-type and mutant SDRs cocrystallized with physiological and synthetic substrates, and much is known about the mechanism of action of SDRs (Benach et al., 1998; Tanaka et al., 1996; Wu et al., 2007). SDRs have a catalytically active tyrosine, which forms a triad with a highly conserved lysine and serine at the catalytic site. Of structural and functional importance is the presence of the catalytically active tyrosine in α helix F, which has a hydrophobic exterior surface, and along with α helix E, forms the stabilizing intersubunit interface (Benach et al., 1998; Tanaka et al., 1996; Tsigelny and Baker, 1995) in SDR dimers and tetramers. With one exception (Ghosh et al., 2001; Tsigelny and Baker, 1995), all SDRs are active as either dimers or tetramers. Moreover, the exception contains an extra segment with a hydrophobic α helix that forms an ''internal dimer interface'' with α helix F (Ghosh et al., 2001). It makes sense that SDR monomers would be catalytically inactive because exposure of the hydrophobic surface of α helix F to water would disrupt its structure and the configuration of the essential tyrosine in the catalytic site (Tsigelny and Baker, 1995).

It would appear that with such an extensive body of information about SDR structure and function that there is not much more to learn from these enzymes. In this issue, however, studies by Tanaka et al. (Tanaka et al., 2008) on pig heart PerCR demonstrate that SDRs have more to teach us about the relationship between structure and biological mechanisms, such as protein transport to organelles. Moreover, Tanaka et al.'s report is an excellent demonstration of the value and, in this case, the necessity of having the crystal structure of pig heart PerCR to explain puzzling biochemical data, and in the process uncover a novel mechanism for regulating the trafficking of oligomeric proteins to peroxisomes.

Pig heart PerCR localizes to peroxisomes, as expected in view of the SRL sequence at the carboxyl terminus (Tanaka et al., 2008). SRL is a type 1 peroxisomal targeting sequence (PTS1) and is a variant of SKL, the canonical PTS1 (Leon et al., 2006). SHL, another PTS1 variant, is at the carboxyl terminus of dog liver PerCR. Tanaka et al. (2008) undertook straightforward biochemical and molecular studies to confirm that SRL functioned as a PTS1 in pig PerCR by constructing SLL and SL mutants, which are known to lack PTS1 function (Maynard and Berg, 2007; Swinkels et al., 1992). Transfection of HeLa cells with cDNA for pig PerCR, or mutants with SLL and SL and with SKL and SHL as controls, gave the expected results. The SLL and SL mutants were not targeted to peroxisomes and were enzymatically inactive. Only the SKL and SHL mutants were targeted to peroxisomes. Both mutants were enzymatically active, but the SKL variant was less stable.

A distinguishing feature of protein transport across the peroxisomal membrane is that folded and oligomeric proteins are transported across this membrane, with or without noncovalently-bound cofactors (Subramani, 2002). In fact, many peroxisomal proteins are multimeric and several are known to contain bound cofactors. If oligomeric proteins are imported into peroxisomes, then it follows that their PTSs must be available for binding to the PTS receptors in the oligomeric state. This is borne out by binding studies showing that dimeric dihydroxyacetone synthase DHAS (which has the C-terminal sequence, NKL) can indeed interact with Pex5 (PTS1 receptor) from *Hansenula polymorpha* (Faber et al., 2002).

Unexpectedly, introduction of the wild-type pig PerCR tetramer directly into HeLa cells did not lead to peroxisomal localization (Tanaka et al., 2008). What prevented the transport of pig PerCR to the peroxisome? To answer this question, Tanaka et al. (2008) crystallized pig PerCR with NADPH. It is a homotetramer, with a structure that resembles that of other SDRs including a dimer interface consisting of α helices E and F from each subunit. Pig PerCR contains the triad of tyrosine, lysine, and serine at the catalytic site. Binding of the coenzyme NADPH to pig PerCR resembles that of other SDRs. It is the structure of the C-terminal SRL that explains the perplexing observation that introduction of pig PerCR protein into HeLa cells does not lead to peroxisomal localization. The 3D structure shows that SRL is at the tetramer interface, where it is extensively structured via hydrogen bonding, presumably shielding the PTS1 from interactions with Pex5 (Tanaka et al., 2008). Tanaka et al. (2008) propose that SRL is exposed to solvent in the PerCR monomer, which would allow binding to Pex5 and transport into the

peroxisome, where PerCR would be assembled into a tetramer.

Tanaka et al. (2008) also found that while PerCR ending in SRL, SKL, or SHL (all known from previous work to be functional PTSs; Swinkels et al., 1992) were imported into peroxisomes, PerCR ending in SKL is unstable. Their 3D model of this mutant suggests that replacement of an R with a K in the intersubunit interface reduces the stability of the tetramer. These results suggest that in PerCR a balance has been reached between oligomer stability, which is necessary for enzymatic activity in the SDR family (Tsigelny and Baker, 1995), and efficient peroxisomal targeting, and that monomeric subunit import is the solution that has evolved without compromising protein stability and activity.

The studies of Tanaka et al. (2008) may be relevant to other multimeric proteins that are not imported into peroxisomes, but instead their monomeric subunits are. The best-studied exemplar is alcohol oxidase (AO) of methylotrophic yeasts, which is an octameric protein. Monomers of AO are imported into peroxisomes, presumably because the PTS is indeed available for Pex5 binding in these monomers (Waterham et al., 1997). In contrast, octameric AO is not imported into peroxisomes of *H. polymorpha* when the protein is delivered into these cells by liposome fusion (Douma et al., 2004). Binding studies confirmed that the octameric protein did not bind to Pex5, whereas another control protein, GFP-SKL, did (Faber et al., 2002). However, in contrast to the studies in the paper by Tanaka et al., the structural basis for this presumed lack of availability of the PTS in octameric AO is unknown. Perhaps the crystal structure of AO will yield another novel mechanism for monomer import into peroxisomes.

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A Trigger Squeezed

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DOI 10.1016/j.str.2008.02.002

Qiu et al. (2008) add new weight to the very highly detailed understanding of how extracellular ligand binding-induced dimerization of a receptor tyrosine kinase is first manifested inside the cell.

The ErbB family of receptor tyrosine kinases has four members, all of which are required for normal development. The ErbB nomenclature arose from homology to an avian erythroblastosis gene— an alternate nomenclature derives from similarity of the second identified human homolog, human epidermal growth factor receptor-2 (HER2), to the first identified member, epidermal growth factor receptor (EGFR, also HER1). Dysregulation of one or more of these receptors is associated with many cancers and therapeutic intervention has found wide application. For instance, the anti-HER2 antibody trastuzumab (Herceptin®) is the standard of care for the subset of breast cancers with HER2 overexpression, and the anti-EGFR antibody cetuximab (Erbitux®) is used for metastatic colorectal cancers. The therapeutic potential of ATP-competitive inhibitors directed at ErbB intracellular catalytic domains has also been realized in erlotinib (Tarceva®), gefitinib (Iressa®), and lapatinib (Tykerb®).

Most of these therapies were approved during an ongoing eruption of structural results that have helped rationalize their mechanisms of action (Burgess et al., 2003). The eruption started when X-ray structures of the extracellular domain (ECD) of EGFR with bound ligands, reported by two groups in 2002, became available (Garrett et al., 2002; Ogiso et al., 2002). When these results were combined with the contemporaneous structure of the HER3 ECD with no bound ligand (Cho and Leahy, 2002), it became