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Review

Mechanism of StAR's regulation of mitochondrial cholesterol import

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Abstract

The steroidogenic acute regulatory protein (StAR) mediates the acute steroidogenic response by moving cholesterol from the outer to inner mitochondrial membrane, but the mechanism of StAR's action has remained mysterious. We showed that StAR acts on the outer membrane, requires cholesterol binding, and requires the structural change previously described as a pH-dependent molten globule. The current model is that StAR's interaction with protonated phospholipid head groups on the outer mitochondrial membrane induces a molten globule transition needed for StAR to take up cholesterol. Recent data suggest a functional interaction between StAR and the peripheral benzodiazepine receptor (PBR). Whereas many models have suggested that StAR delivers cholesterol to PBR, we suggest that StAR removes cholesterol from the cholesterol-binding domain of PBR and delivers it to the inner mitochondrial membrane.

Keywords: Acute response; Adrenal; Molecular dynamics; Molten globule; Steroid; Steroidogenesis

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1. Introduction

The steroidogenic acute regulatory protein, StAR, mediates the rapid flow of cholesterol from the outer mitochondrial membrane (OMM) to the inner membrane (IMM), permitting steroidogenic cells to make large amounts of steroid in a short time (Stocco and Clark, 1996). The regulation of StAR expression (Stocco et al., 2005) and the role of StAR in causing congenital lipoid adrenal hyperplasia (Lin et al., 1995; Bose et al., 1996; Miller, 1997) are well-known, but progress on the key biochemical question – the mechanism of StAR's action – has been modest until recently. The structure of StAR has been modeled from the X-ray structures of two closely related proteins—N-216 MLN64 (Tsujishita and Hurley, 2000) and StarD4 (Romanowski et al., 2002), yielding a very similar structure (Mathieu et al., 2002; Yaworsky et al., 2005). These structures feature a helix-grip fold and a hollow hydrophobic pocket of the appropriate size and shape to bind one molecule of cholesterol. This sterol-binding pocket (SBP) is defined primarily by StAR's β-barrel and carboxyl-terminal α-helix (C-helix). This C-helix forms the 'floor' of the pocket, as it is the principal part of the StAR molecule that interacts with the OMM (Yaworsky et al., 2005). Both N-218 MLN64 and N-62 StAR can bind cholesterol with 1:1 stoichiometry (Tsujishita and Hurley, 2000).

Four distinct models of StAR's action have been proposed:

Contact sites. StAR is synthesized as a short-lived cytoplasmic 37 kDa protein with a mitochondrial leader peptide that is cleaved upon mitochondrial import to yield the long-lived

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intramitochondrial 30 kDa form. This suggested that 37 kDa StAR is a precursor and that 30 kDa StAR is the mature active form; mitochondrial import of the 37 kDa 'precursor' was proposed to form contact sites between the OMM and IMM, permitting cholesterol to flow down a chemical concentration gradient from OMM to IMM (Stocco and Clark, 1996). This contact site model was supported by ultrastructural observations of increased numbers of contact sites in adrenal mitochondria stimulated with ACTH (Jefcoate et al., 1986), and by studies suggesting increased cholesterol trafficking at semi-purified contact sites (Cherradi et al., 1997).

Desorption. Roles for contact sites and for intramitochondrial 30 kDa StAR were questioned by observations that StAR remains active when confined to the cytoplasm: 62 N-terminal residues of StAR, including the entire mitochondrial leader, could be removed without affecting activity (Arakane et al., 1996). Other studies showed that N-62 StAR can insert cholesterol into other, non-mitochondrial membranes (Kallen et al., 1998) suggesting that the principal role of StAR's leader peptide is to confine its action to the mitochondrion and to keep it away from other organelles (Miller et al., 1999). It is difficult to envision how StAR moves cholesterol from the OMM to the IMM without leaving the cytoplasm. Strauss proposed that StAR 'desorbs' cholesterol from the OMM, permitting it to traverse the mitochondrial intramembranous space (IMS), presumably as micro-droplets, before being taken up by the IMM (Christenson et al., 2001) but IMS micro-droplets of cholesterol have not been reported, and this model makes few other testable predictions. Nevertheless this suggestion remains consistent with the molten globule model.

IMS shuttle. The structure of N-216 MLN64 and the demonstration that StAR could bind cholesterol suggested that StAR must act in the IMS, where it would shuttle cholesterol from the OMM to the IMM (Tsujishita and Hurley, 2000). Because this proposal was inconsistent with the observations that N-62 StAR is active on the OMM, those data were dismissed as an artifact of in vitro overexpression; and because the structure indicated StAR was a cholesterol-binding protein, the proposal that StAR requires a molten globule conformational change (see below) was dismissed as illogical (Tsujishita and Hurley, 2000). The IMS shuttle model was initially accepted because of its logic, simplicity, and structural basis, but is now clear that this model is incorrect.

Molten globule. Biophysical studies of bacterially expressed human N-62 StAR indicated that the carboxyl-half of StAR was loosely folded and protease-sensitive, whereas the amino-terminal half was tightly folded and protease-resistant; and spectroscopic studies showed that StAR underwent a molten globule conformational change at ∼pH 3.5 (Bose et al., 1999). Molten globules are partially unfolded proteins that have lost some tertiary structure but retain their secondary structure. While pH 3.5 is not achieved within a cell, pH 3.5 in vitro might model StAR's interaction with the protonated phospholipid head groups of the OMM. Thus the model proposed that StAR acts on the OMM, and that activity requires a conformational change elicited by protonated phospholipids (Bose et al., 1999). This view is supported by observations that an intact mitochondrial proton pump is required for StAR activity (King et al., 1999; Allen et al., 2004).

1.1. StAR acts exclusively on the OMM

To determine StAR's site of action and hence discriminate among these models, we affixed StAR on the cytoplasmic side of the OMM, in the IMS, or on the matrix side of the IMM by fusing N-62 StAR to mitochondrial proteins having known topologies (Bose et al., 2002). Cells expressing StAR immobilized on the outside of the OMM by fusion to the C-terminus of Tom20 achieved maximal constitutive steroidogenesis, but StAR bound to Tim9 in the IMS was inactive. The Tim9/StAR protein was active with steroidogenic mitochondria in vitro, showing that this protein was inactive in transfected cells because of its IMS location, not because of its structure. Similarly, StAR was also inactive when localized to the mitochondrial matrix (Bose et al., 2002). Mitochondrial protein-import assays of StAR with a modified leader peptide confirmed that StAR acts exclusively on the OMM: slowing StAR's mitochondrial entry increased activity whereas speeding its entry decreased activity; thus StAR activity was determined by its OMM occupancy time. Finally, immunoquantitation showed cellular transfection achieved physiological, not pharmacological levels of N-62 StAR (Bose et al., 2002). These studies established that StAR acts on the OMM.

1.2. Only the C-helix of StAR interacts with the OMM

To visualize which residues of StAR associate with the OMM, we prepared small unilamellar vesicles (SUV) having the same lipid/cholesterol composition as an OMM, and mixed these with bacterially expressed, biologically active human N-62 StAR. The StAR/SUV mixture was proteolyzed and the peptides were analyzed by mass spectrometry. Comparing the peptides obtained under different pH conditions in the presence or absence of SUV permitted identification of the residues that associate with the lipid membrane, thus inhibiting proteolysis (Yaworsky et al., 2005). These 'liposome protection experiments' show that only the outside aspect of the carboxyl-terminal helix (C-helix) consistently associates with the membrane (Fig. 1A). This helix is amphipathic; most of its residues are charged and interact with the OMM, but its hydrophobic F267, L271, and L275 residues are oriented toward the SBP (Fig. 1B). Proteolysis of N-62 StAR and of a synthetic peptide model of the C-helix confirm that this association is more avid at pH 3.5–4.0 than at pH 7, providing independent evidence for the pH-dependent molten globule model (Yaworsky et al., 2005). Thus these data indicate that StAR does not become deeply buried in the OMM, as one might envision as necessary to pick up a cholesterol molecule, but instead treads lightly on the OMM.

1.3. Visualizing the molten globule by molecular dynamics

Although the SBP can accommodate one cholesterol molecule, the structural data show insufficient room for a choles-