Binding of Steroidogenic Acute Regulatory Protein to Synthetic Membranes Suggests an Active Molten Globule*

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Steroidogenic acute regulatory protein (StAR) mediates cholesterol transport from the outer to the inner mitochondrial membrane during steroid biosynthesis. The mechanism of StAR’s action is not established. To address mechanistic issues, we assessed the binding of StAR to artificial membranes by fluorescence resonance energy transfer using endogenous StAR tryptophan residues as the donor and dansyl-phosphatidylethanolamine in the bilayer as the acceptor. Mixing StAR with dansyl-labeled vesicles composed of phosphatidylinositol increased the fluorescence intensity of dansyl emission excited at 280 nm by 10–40%. This interaction was dependent on pH, with a maximum at pH 3.0–3.5 and essentially no change above pH 5. Binding experiments at different temperatures and various combinations of phosphatidylcholine, phosphatidylylycerol, cardiolipin, and cholesterol showed that binding involves an electrostatic step and one or more other steps. Although binding prefer a thermodynamically ordered bilayer, the rate-limiting step occurs either when the bilayer is in a fluid state or when there is cholesterol-induced membrane heterogeneity. Experiments with fluorescence and light scattering indicate that StAR binding promotes ordering and aggregation of anionic membranes. The inactive StAR mutant R182L had lower affinity for the membrane, and the partially active mutant L275P had intermediate affinity. Far-UV CD spectroscopy of StAR in PC membranes show more β-structure than in aqueous buffers, and the presence of cardiolipin or cholesterol in the membrane fosters a molten globule state. Our data suggest that StAR binds to membranes in a partially unfolded molten globule state that is relevant to the activity of the protein.

The steroidogenic acute regulatory protein (StAR)1 rapidly increases the movement of cholesterol into adrenal and gonadal mitochondria (1, 2) for conversion to pregnenolone by cytochrome P450sc to initiate steroidogenesis (3). Although low levels of steroid biosynthesis can occur in the absence of StAR (4), the StAR protein is required for the rapid gonadotropin-induced and ACTH-induced steroidogenic responses necessary for reproduction and responses to stress (2). The crucial role of StAR in human physiology is best illustrated by the findings in congenital lipid adrenal hyperplasia, which is caused by mutations in the StAR gene (4, 5). Affected individuals have female external genitalia irrespective of chromosomal sex and die in infancy from mineralocorticoid and glucocorticoid deficiency if not diagnosed and treated with hormonal replacement therapy (6).

The mechanism of StAR’s action is unknown. StAR is synthesized as a 37-kDa phosphoprotein that is cleaved to a 30-kDa form upon mitochondrial entry (1, 2). However, StAR’s action requires new protein synthesis, and the 15–30-min time course of StAR’s action correlates more closely with the presence of the cytoplasmic 37-kDa precursor than with the 2–3-h half-life of the intramitochondrial 30-kDa form (7–9). Furthermore, deletion of 62 amino-terminal residues (N-62 StAR), which includes the mitochondrial leader peptide, results in an extramitochondrial protein that is fully active both when expressed in living cells and when expressed in bacteria and added to isolated mitochondria in vitro (10–13). By contrast, StAR is inactivated by deleting just 28 C-terminal amino acids (5), and all StAR mutations that cause congenital lipid adrenal hyperplasia are found in C-terminal domains, suggesting that this region is responsible for StAR’s activity (4, 5). In addition, biophysical studies indicate that StAR acts on the outer mitochondrial membrane (OMM) while undergoing a pH-dependent transition to a molten globule (14). Thus, it appears that the 37-kDa “preprotein” acts on the OMM as a molten globule and is then inactivated by mitochondrial import and cleavage to generate the inactive 30-kDa intramitochondrial protein (13).

Recently, Tsujishita and Hurley (15) suggested a different model based on the 2.2-Å resolution structure of the StAR-like domain of MLN64 (MLN64 residues 216–445), a protein that has 35% amino acid sequence identity with StAR (16) and has StAR-like activity in vivo and in vitro (12, 17). This structure showed that StAR-like proteins have a large hydrophobic tunnel that binds one molecule of cholesterol. The data were interpreted to mean that StAR acts in the mitochondrial intramembranous space (IMS) to shuttle cholesterol one molecule at a time from the OMM to the inner mitochondrial membrane. This “IMS/shuttle” model of StAR’s action was viewed as being inconsistent with the interpretation that N-62 StAR acts on the OMM and forms a molten globule (15).
StAR is active with isolated mitochondria in which proteins associated with the OMM have been inactivated by heat denaturation or partial proteolysis with trypsin, and StAR can transfer cholesterol to intracellular membranes other than OMM. These results suggest that StAR interacts directly with membrane phospholipids and does not require a specific receptor protein (18). Therefore, we sought to study the interactions of biosynthetic N-62 StAR with lipids in synthetic unilamellar vesicles of known composition. By measuring resonance energy transfer to synthetic dansylated membranes, we now show that N-62 StAR interacts with membrane lipids in a pH-dependent fashion that supports the molten globule hypothesis. CD spectroscopy of N-62 StAR in lipid environments also indicates a pH-dependent molten globule. Features of both the OMM/molten globule hypothesis and the IMS/shuttle hypothesis appear to be necessary to account for StAR's behavior.

EXPERIMENTAL PROCEDURES

Reagents—Wild type and mutant 6-His-N-62 StAR proteins were expressed in Escherichia coli and purified as described (19). Dansyl-phosphatidylethanolamine (dansyl-PE) and laurdan were obtained from Molecular Probes, Inc. (Eugene, OR). Egg phosphatidylcholine (PC), dimyristoylphosphatidylcholine (DMPC), tetradecylcardiolipin, and 1,2-dipalmitoyl-sn-glycero-3-phosphatidylglycerol (DPPG) were purchased from Avanti Polar Lipids (Birmingham, AL). All other reagents were obtained from standard sources.

Vesicle Preparation—Vesicles were prepared by mixing phospholipids with or without 2 mol % of (the final lipid concentration) dansyl-PE and with or without 50 mol % cholesterol (dissolved in chloroform). The lipids were dried under N2 to remove chloroform and then hydrated with 20 mM acetate buffer (pH 4) containing 150 mM KCl to give a 10 mM final bulk lipid concentration. The hydrated lipids were heated to 37 °C to ensure that the mixture was above the lipid phase transition temperature and incubated for 1 h with intermittent mixing by rapid agitation. The resulting multilamellar vesicles were sonicated three times for 3 min each using a probe sonicator to obtain small (100–200-Å diameter) unilamellar vesicles (SUV). Ambient temperature was maintained constant in the sample during sonication with a water bath. DMPC vesicles were sonicated at 35 °C to ensure that SUV formation occurred above the phase transition temperature.

Fluorescence Measurements—The binding of wild type and variant StAR proteins to phospholipid vesicles was assessed by fluorescence resonance energy transfer using tryptophan residues in the protein as the donor and dansyl-PE in the membrane as the acceptor. Energy transfer was assayed using a photon-counting spectrofluorometer (Instruments SA) (20). Excitation was set at the maximum for Trp (280 nm), and the emission of dansyl-PE was measured at 510 nm (4.25-nm band pass). Simultaneous assessment of fluorescence intensity at multiple excitation and emission wavelengths was obtained by rapid shimming of monochromator mirrors using control software provided with the instrument. Alternatively, energy transfer was also assessed from excitation spectra with emission at 510 nm. Measurements of the physical state of membranes containing the bilayer probe, laurdan, were obtained using a FC1 fluorometer from ISS (Urbana, IL). Diluents ranged in pH from 2.0 to 7.0 and contained 150 mM KCl with 20 mM phosphate, citrate, acetate, or HEPES buffers as appropriate for the target pH. The temperature was maintained at 37 °C or as indicated in the relevant figure using circulating water baths. Continuous gentle magnetic stirring preserved sample homogeneity in both instruments.

For binding experiments by energy transfer, SUV (50 μM lipid) were equilibrated to the temperature in the sample compartment of the fluorometer, and base-line fluorescence intensity was recorded. The procedure was then repeated following incremental additions of StAR protein. Energy transfer was observed as an increase in fluorescence intensity (excited at 280 nm) upon the addition of protein. Some direct excitation of dansyl-PE also occurs at 280 nm, and this excitation may likewise be affected by the binding of protein. To distinguish these effects from true energy transfer, the direct fluorescence of dansyl-PE was measured simultaneously (excitation = 340 nm, emission = 510 nm). The energy transfer was then corrected for changes in dansyl intrinsic fluorescence by calculating the ratio of fluorescence excited at 280 nm to that excited at 340 nm. When experiments involved the pH dependence of energy transfer with charged lipids, the results were normalized to the intensity at 300 nm. This change in analysis procedure was required by the presence of an optical artifact at 340 nm with anionic membranes at low pH.

Laurdan (1.2 μM) dissolved in Me2SO was incorporated into liposomes prior to sonication, and SUV were prepared as described above without dansyl-PE. SUV were equilibrated in the fluorometer sample chamber to the indicated temperatures, and emission spectra were acquired (excitation = 350 nm). The procedure was then repeated at each temperature in the presence of N-62 StAR. Effects of temperature and the binding of the protein on the emission spectrum were quantified by calculating the value of generalized polarization (GP) from the spectra as described (21).

CD Spectroscopy—Far UV (200–250 nm) CD measurements were carried out in a 1.0-mm path length flat cuvette in a Jasco-715 spectropolarimeter equipped with a Peltier-controlled temperature system. All lipids and protein samples were mixed with the same buffer system that was used for the fluorescence resonance energy transfer measurements and were incubated at 37 °C for 10 min prior to recording the spectrum. Appropriate base lines were obtained under the same experimental conditions and were subtracted from the sample spectra. In some spectra, the noise was reduced by application of a Savitsky-Golay filter.

RESULTS

Binding of StAR Proteins—The apparent binding of N-62 StAR to vesicle surfaces was readily detected by resonance energy transfer. The fluorescence excitation spectrum of SUV containing 2% dansyl-PE has a minimum at 280 nm and a peak at 340 nm, representing intrinsic dansyl fluorescence. The addition of StAR elicits a small decrease in dansyl intrinsic fluorescence and a substantial increase in fluorescence intensity at 280–300 nm (Fig. 1A). The ratio of the two spectra from Fig. 1A shows a peak at 280 nm consistent with a tryptophan absorption spectrum and indicating resonance energy transfer between one or more StAR tryptophans and dansyl-PE in the membrane (Fig. 1B).

Increasing concentrations of N-62 StAR enhanced dansyl fluorescence of SUV made of either PC or 1:1 PC/cholesterol (Fig. 2A). The effect was saturable and fit a simple binding model with an apparent KD of ~0.2 μM in each case. The binding was very sensitive to pH; no binding was detected at pH 7.0 (not shown), and binding was maximal at pH 3.0–3.5 (Fig. 2B), which corresponds to the pH range at which StAR

![Figure 1: Resonance energy transfer](http://www.jbc.org/)

![Figure 2: Binding of StAR to Membranes](http://www.jbc.org/)
forms a molten globule structure (14). This ability of STAR to bind to unilamellar lipid bilayers lacking intrinsic membrane proteins supports the view that STAR acts directly on phospholipid membranes and does not require a receptor.

The binding of STAR was also critically dependent on small variations in the sequence of the STAR protein. The STAR missense mutant R182L causes severe congenital lipid adrenal hyperplasia and is wholly inactive in transfected cells, whereas the mutant L275P causes less severe disease and is wholly inactive in transfected cells (4). Spectroscopic analyses have shown that the R182L mutant is grossly misfolded, while the L275P mutant has a nearly normal fold (19). The R182L and L275P mutants of N-62 STAR bound to SUV composed of either PC or 1:1 PC/cholesterol but differed from wild-type N-62 STAR and from each other in their affinities for the bilayer (Fig. 3). The average values for KD measured between pH 4.0 and 2.0 were 0.16 ± 0.02 μM for wild type, 1.03 ± 0.26 μM for L275P, and 3.1 ± 1.2 μM for R182L (mean ± S.E., n = 28, 8, and 10, respectively). These differences were statistically significant by two-way analyses of variance (p < 0.0001) at all pH values tested with or without the presence of cholesterol.

pH Effect—The statistical analysis of KD for the binding of STAR to PC SUV detected a major effect of pH and differences in the effect of pH on the wild-type and mutant STAR sequences (Fig. 4). Below pH 4, wild-type N-62 STAR bound more efficiently to SUV composed of PC alone, whereas the two mutants showed little difference in binding between PC and PC/cholesterol SUV as a function of pH (Fig. 4A). These results were confirmed statistically by two-way analysis of variance for both the effects of pH (p < 0.0001) and cholesterol content (p = 0.002) as well as the interaction between the two variables apparent for wild type protein in Fig. 4A (p = 0.011). A similar analysis revealed no statistical significance for either mutant (Fig. 4, B and C). Cholesterol tends to aggregate into cholesterol-rich domains when mixed 1:1 with PC (22), therefore creating domains relatively deficient in dansyl groups. The data in Fig. 4 suggest that wild type, but not the mutant forms of N-62 STAR, binds with increasing avidity to cholesterol-rich domains as pH decreases so that N-62 STAR is sequestered away from regions of the membrane that contain dansyl groups. This interpretation is consistent with the effect of N-62 STAR to make the environment of dansyl-PE more rigid in the absence of cholesterol but more fluid in the presence (Fig. 5). Thus, although the presence of cholesterol is not required for STAR to interact with PC membranes, it substantially alters STAR’s binding characteristics.

Effects on Dansyl Intrinsic Fluorescence—The STAR-induced reduction in dansyl intrinsic fluorescence (340 nm) (Fig. 1A) suggested that the binding of STAR alters the physical state of the membrane. Because CD data show that changes in pH affect STAR’s conformation (14), we considered whether changes in pH would also affect the physical state of SUV and their interaction with STAR. In the presence or absence of N-62 STAR, the fluorescence was stable at pH ≈ 4.0 and decreased proportionately as pH was reduced (Fig. 5A). The fractional decrement in fluorescence intensity was greater for SUV containing cholesterol (Fig. 5A, triangles) than for SUV composed of PC alone (circles). Although STAR influenced the pH dependence of dansyl-PE in the two forms of SUV only slightly, this influence was reproducible in opposite directions (Fig. 5B) and required the same concentrations of STAR at which binding occurred (Fig. 5C). Similar results were obtained with the R182L and L275P mutants (data not shown). The dimethylamino group in the dansyl fluorophore titrates between pH 2.0 and 4.0 and no longer absorbs at 340 nm in the protonated state (23). The pK for this reaction depends on the surrounding lipids; a lower pK corresponds to increased membrane fluidity, and higher pK corresponds to increased membrane structure, order, and rigidity (23). The data in Fig. 5A suggest a pK of <2 for dansyl-PE in a fluid PC bilayer and a pK between 2.0 and 2.5 in a more rigid PC/cholesterol membrane. Thus, the binding of STAR increased the pK of dansyl-PE in PC membranes, suggesting that those membranes had become more rigid (Fig. 5A). This effect is common for proteins that bind to the surfaces of lipid membranes (24). By contrast, STAR decreased the pK of dansyl-PE in PC/cholesterol membranes (Fig. 5A), indicating increased fluidity. This enhanced fluidity is consistent with aggregation of cholesterol away from phospholipid-rich regions of the bilayer where dansyl-PE would reside. This result would be predicted thermodynamically if STAR binds preferentially to and thus stabilizes cholesterol-rich regions.

Effects of Membrane Order—To pursue the suggestion that STAR binding might affect membrane order, we varied the fluidity of the bilayer by altering the temperature. Because the phase transition of PC from gel to liquid crystalline phases occurs at temperatures below 0 °C, we instead prepared vesicles from DMPC, whose phase transition occurs at ambient temperature. This choice of lipid allowed us to test the binding of STAR to both the liquid crystalline and the gel phases of SUV without freezing the sample. It also permitted us to reach the liquid phase at temperatures low enough to avoid denaturing the protein.

As with PC vesicles, increasing concentrations of N-62 STAR enhanced dansyl fluorescence in DMPC and 1:1 DMPC/cholesterol SUV in a saturable manner (Fig. 6A). The binding of N-62 STAR to DMPC SUV was similar to its binding to PC or PC/cholesterol SUV (compare with Fig. 2A; maximum Iprot/Iinitial = 0.16–0.19 for PC, PC/cholesterol, and DMPC). However, the binding to DMPC/cholesterol SUV was more than doubled.

Increasing the temperature from 15 to 25 °C increased energy transfer, but there was little if any effect of increasing the
temperature further to 35 °C (Fig. 6B). Thus the binding of N-62 StAR appeared favored by increased membrane fluidity.

The binding of N-62 StAR to SUV of various compositions suggests that StAR should prefer a more ordered membrane over one that is fluid (Figs. 5A and 6A); however, binding apparently preferred the more fluid liquid crystalline state of DMPC above the phase transition temperature of 22 °C (Fig. 6B). To explore this apparent inconsistency, we assessed bilayer fluidity in DMPC and DMPC/cholesterol SUV as a function of temperature, with or without StAR, using the fluorescent membrane probe laurdan. The emission spectrum of laurdan is very sensitive to the polarity of the membrane environment; the spectrum displays large red shifts when the number and/or mobility of water molecules partitioned at the level of the phospholipid glycerol backbones has increased (21). Thus, enhancing bilayer fluidity allows water to have greater access to laurdan and thus shifts the emission spectrum toward longer wavelengths. These spectral shifts are quantified by calculation of GP, which varies from $-1$ (most disordered) to $+1$ (most ordered) (21). Laurdan readily detected the normal DMPC phase transition near 22 °C, whereas vesicles composed of 1:1 PC/cholesterol remained ordered throughout the temperature range (Fig. 6C). The addition of N-62 StAR had little effect on the fluidity of DMPC SUV containing cholesterol, but in the absence of cholesterol N-62 StAR raised the phase transition temperature 5 °C. These data indicate that StAR stabilizes the gel state of the bilayer, thus corroborating the observations in Fig. 5.

Thus, the temperature dependence results (Fig. 6B) required further analysis. One possibility is that the binding results obtained with DMPC membranes represent kinetic effects rather than the thermodynamics of the binding interaction. To test this possibility, we assayed the binding of StAR to DMPC SUV by increasing the temperature incrementally from 15 to 35 °C and then decreasing the temperature back to 15 °C (Fig. 6A).
6D). Consistent with the data in Fig. 6B, binding increased as the temperature was raised, but instead of returning to the original level when the sample was cooled, the binding increased. Thus, the system was not at equilibrium at each temperature because the binding of StAR to lipid in the gel state depended on sample history; heating the vesicles to a temperature above the phase transition apparently removed a kinetic barrier to binding.

The increased order of DMPC SUV when they contain cholesterol helps to explain the effect of cholesterol to enhance binding above 22 °C. However, differences in bilayer fluidity cannot account for the differences in StAR binding between DMPC SUV with and without cholesterol at lower temperatures, because the fluidity of the two types of vesicles detected by laurdan was similar at 15–20 °C (Fig. 6C). These observations may be explained by the tendency of cholesterol to form domains in membranes (22). The kinetic barrier evidenced in Fig. 6D is probably created by a homogeneous and well ordered membrane, such as that formed by DMPC SUV in the gel phase. The domain structure of DMPC/cholesterol vesicles may behave like the fluid phase of DMPC membranes by disrupting the regular surface structure and thus removing the kinetic barrier. Similar phenomena have been observed with other proteins where kinetic barriers to binding and/or function are removed either by temperature-dependent changes in the physical state of the membrane or by promoting structural heterogeneity through compositional diversity (20, 25–27). In addition to explaining the apparent inconsistencies in the data of Figs. 5 and 6, A–C, the data of Fig. 6D show that the binding of StAR occurs in multiple steps. If binding were a simple adsorption reaction, it would be limited kinetically only by diffusion of the protein and vesicles. However, the observed kinetic limitation dependent on physical properties of the bilayer indicates that there must be at least one additional step following the initial collision of the protein with the membrane.

Effects of Membrane Charge—StAR's apparent preference for binding to ordered membranes suggests that electrostatic interactions might be involved in the binding reaction (24). Such an electrostatic mechanism would be consistent with the binding of N-62 StAR to the mitochondrial outer membrane, which contains about 18% cardiolipin (28), a polyanionic phospholipid that is essentially a dimer of two phosphatidylglycerol molecules.

To determine whether the binding of StAR might be assisted by the presence of charged lipids in the membrane, we prepared PC SUV with 18% tetraoleyl cardiolipin. As with SUV composed of PC (Fig. 2A) or DMPC (Fig. 6A), N-62 StAR bound well to vesicles containing cardiolipin, but the energy transfer at saturating protein concentrations (0.5–0.75 μM) was about 5-fold greater (Fig. 7A). Consistent with the data in Figs. 3 and 4, STAR mutant R182L bound with lower affinity than the wild type, and L275P bound with intermediate affinity. However, unlike the results with either PC or DMPC vesicles, both wild type and L275P N-62 StAR appeared to bind cooperatively to PC/cardiolipin SUV. The very low affinity of binding with R182L made it difficult to assess whether cooperativity was present. Measurements of laurdan GP showed that N-62 StAR increased the order of membranes containing cardiolipin (Fig. 7B).

To determine whether the effect of cardiolipin was due to electrostatic charge rather than a specific effect of cardiolipin, we assayed the binding of N-62 StAR to PC SUV containing 18 or 30 mol % DPPG. Vesicles with a ratio of 82:18 PC/DPPG contain a molar concentration of DPPG equal to the molar concentration of cardiolipin in the PC/cardiolipin vesicles; those containing 30 mol % DPPG contain the same number of ionizable species as the PC/cardiolipin vesicles. Both the magnitude of N-62 StAR binding and the apparent cooperativity to DPPG vesicles (Fig. 7C) was very similar to that observed with PC/cardiolipin SUV (Fig. 7A), suggesting that membrane surface charge is the important element in the effect of cardiolipin to enhance binding of N-62 StAR.

The pH dependence of the interaction of N-62 StAR with membranes containing cardiolipin showed a maximum at pH 3.0–3.5 (Fig. 8A). This is similar to the pH dependence of interaction with PC/cholesterol vesicles (Fig. 4A) and argues that the pH effect is a function of the protein rather than the properties of the bilayer and may be associated with StAR's transition to a molten globule at this pH (14). Consistent with this, analysis of the effects of pH on membrane fluidity as assessed by laurdan GP shows essentially no pH-dependent changes in the fluidity of vesicles containing cholesterol and no changes above pH 3 in those without cholesterol (Fig. 8B). Thus, all of the data assessing the interaction of StAR with lipid membranes favors a pH-dependent interaction with membranes containing cardiolipin at a composition approximating that of the OMM.
Increasing concentrations of StAR caused aggregation of vesicles containing cardiolipin or DPPG. To quantify the amount of aggregation, we fit the dansyl region of the excitation spectra (310–400 nm) to a Gaussian curve and calculated the S.D. of the data from the curve. Comparing the standard deviation (i.e. the noise) as a function of StAR concentration demonstrated a systematic trend in vesicles containing cardiolipin or DPPG with increasing concentrations of protein (Fig. 8C). This effect was absent in vesicles composed of DMPC or PC vesicles devoid of either cardiolipin or DPPG. The tendency of the anionic vesicles to aggregate in the presence of StAR may be responsible for the apparent cooperativity of binding. If the binding of a single protein molecule to the surface of one vesicle increases the likelihood of a second vesicle to adsorb to the complex, then StAR would bind with higher affinity when the two vesicles are aggregated. Hence, the binding of a second protein would be enhanced, and repetition of the process would lead to both the high order aggregates and the cooperative binding. This phenomenon of protein binding causing aggregation of anionic vesicles has been reported with other proteins such as the plant toxin thionin (25) and cytochrome c (29).

Conformational Changes of StAR in Lipid Environments—In aqueous Tris or phosphate buffers, the CD spectra of N-62 StAR show minima at the 208 nm (α-π*) and 222 nm (n-π*) positions, which is typical of α-helical proteins. In contrast, N-62 StAR shows relatively weak signals at 215–218 nm, corresponding to parallel β-sheets, or at 225–230 nm, corresponding to antiparallel β-sheets (30). In the presence of PC at pH 4.0, the far UV CD spectrum of N-62 StAR showed an increased signal at 218 nm, suggesting an increase in β-sheet; this pattern was only minimally changed in the presence of cholesterol (Fig. 9A). At pH 3.5, the spectrum in PC without cholesterol was essentially the same as at pH 4.0, but in the presence of cholesterol the curve shows an increase in the β-sheet contribution, about equal to that of α-helix (Figs. 9, A and B). Thus, the presence of cholesterol fosters the pH-dependent molten globule transition described previously in aqueous buffers (14).

In contrast to the relatively modest effect elicited by adding cholesterol to PC membranes, adding 18% cardiolipin has a profound effect (Fig. 9C). At pH 3.5–4.0, the CD signal is reduced dramatically, suggesting that the StAR protein is buried more deeply in the lipid, thus attenuating its signal, and the broad spectral minima at 220–230 nm indicate a predominance of stable β-sheet structures (31). Thus, cardiolipin strongly favors the association of N-62 StAR with a PC mem-
binding to artificial membranes. First, StAR binds to membranes without assistance of other proteins. Second, binding involves at least two steps; the first step is the initial collision of the protein with the bilayer surface, and the second step appears to be limited by the structure of the bilayer. The second step is facilitated by heterogeneity of membrane surface structure induced by compositional diversity or by increased membrane fluidity; thermodynamically, the binding prefers an ordered membrane. Third, in addition to promoting bilayer heterogeneity, cholesterol also appears to exert a specific effect to promote the binding of StAR to cholesterol-rich domains. This interaction may involve StAR’s cholesterol-binding site, since the mutant StAR proteins did not display this effect. Fourth, StAR’s preference for an ordered membrane reflects, at least in part, an electrostatic component to the binding. Apparently, the preference for negative charge in the bilayer is sufficient to compensate for the kinetic subtleties and cholesterol dependence of binding observed in the absence of anionic lipids. Fifth, StAR undergoes a conformational change to a molten globule while interacting with membranes, especially when cardiolipin is present.

Two apparently conflicting models of StAR’s action have been proposed, the OMM/molten globule model (14) and the IMS/shuttle model (15). Our fluorescence energy transfer experiments are compatible with StAR acting either on the OMM or in the IMS, but they also strongly support the concept that StAR undergoes a pH-dependent transition to a molten globule state while interacting with cholesterol-rich membranes, such as the OMM. The CD spectroscopy in membrane environments strongly supports the pH-dependent molten globule model. There can be little doubt that the x-ray crystal structure of 216–444 MLN64 (15) corresponds closely with the as-yet-un-determined structure of N-62 StAR, since these two proteins share about 35% amino acid sequence identity and >50% amino acid similarity (16), and the two proteins behave similarly in vivo and in vitro (12, 17). However, the IMS/shuttle model of StAR’s action (15) is based on parsimony and analogy with the action of other lipid transfer proteins. The molten globule data were considered inconsistent with the crystal structure, because StAR appeared to have evolved a fold ideally suited to transporting lipids, and hence it seemed unlikely that it would then function in vivo only in its molten globule form (15). Nevertheless, the crystal structure also shows that the two potential openings of the cholesterol-binding tunnel are too small to admit a cholesterol molecule. Hence, StAR must undergo a conformational change while binding cholesterol. This required conformational change may correspond to the pH-dependent structural transition to a molten globule that is detected spectroscopically. Similar pH-dependent transitions to a molten globule structure have been observed with other proteins that interact with membranes (32–34). Our fluorescence energy transfer experiments corroborate the suggestion that StAR undergoes a pH-dependent transition to a molten globule while active. Furthermore, our data suggest that this molten globule transition favors binding to cholesterol-rich membranes at pH 4 and that single amino acid replacement mutants that decrease or ablate StAR’s activity also inhibit membrane binding and transition to the molten globule. Thus, while our studies do not establish the specific mitochondrial site of StAR’s action, both the spectroscopic evidence and our fluorescence energy transfer data provide strong evidence for the changes in the shape of the StAR protein that appear to be mediated by the crystallographic data.

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