FRET Study of Membrane Proteins: Determination of the Tilt and Orientation of the N-Terminal Domain of M13 Major Coat Protein

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ABSTRACT A formalism for membrane protein structure determination was developed. This method is based on steady-state FRET data and information about the position of the fluorescence maxima on site-directed fluorescent labeled proteins in combination with global data analysis utilizing simulation-based fitting. The methodology was applied to determine the structural properties of the N-terminal domain of the major coat protein from bacteriophage M13 reconstituted into unilamellar DOPC/DOPG (4:1 mol/mol) vesicles. For our purpose, the cysteine mutants A7C, A9C, N12C, S13C, Q15C, A16C, S17C, and A18C in the N-terminal domain of this protein were produced and specifically labeled with the fluorescence probe AEDANS. The energy transfer data from the natural Trp-26 to AEDANS were analyzed assuming a two-helix protein model. Furthermore, the polarity Stokes shift of the AEDANS fluorescence maxima is taken into account. As a result the orientation and tilt of the N-terminal protein domain with respect to the bilayer interface were obtained, showing for the first time, to our knowledge, an overall α-helical protein conformation from amino acid residues 12–46, close to the protein conformation in the intact phage.

INTRODUCTION

M13 major coat protein is a small protein composed of 50 amino acid residues. The protein is involved in the membrane-bound assembly and disassembly of the phage M13 in the *Escherichia coli* host cytoplasmic membrane and has been the subject of several biophysical studies (for a recent review see (1)). Generally it is believed that approximately half of the protein is located in the membrane, whereas the remaining N-terminal residues are sticking out of the membrane. Despite intensive studies, the topology of the coat protein in lipid bilayers is still a matter of debate. This is mainly due to biophysical inabilities to study the structure and dynamics of the N-terminal domain of the protein in detail. Models for the overall topology of the protein varied 90° from an L-shape to an I-shape (1). This arises because the protein is a single membrane-spanning system that has no internal stability based on segment-segment interactions (1). This means that there is no tertiary structure to hold the protein together. Also, recently it was suggested that the protein is strongly affected by the environment into which it is inserted, i.e., micelles, vesicles, liposomes, or oriented membranes (2). These factors are most important for the N-terminal domain of the protein that emerges from the membrane.

To resolve this problem we have produced several cysteine mutants in the N-terminal domain of the protein and specifically labeled them with the fluorescence probe AEDANS. Analysis of the energy transfer data from the natural Trp-26 to AEDANS using a two-helix protein model and the application of the polarity Stokes shift of the AEDANS fluorescence maxima results in a low-resolution structure of the entire protein, including the tilt and orientation of the N-terminal domain with respect to the transmembrane domain.

EXPERIMENTAL METHODS

Sample preparation

Lipid bilayer systems were prepared from DOPC and DOPG lipids in a 4:1 molar ratio, denoted as DOPC/DOPG, as described before (3). Site-specific cysteine mutants of M13 major coat protein were prepared, purified and labeled with AEDANS as described previously (4). Wild-type protein and AEDANS-labeled M13 coat protein mutants were reconstituted into phospholipid bilayers as reported earlier (5).

Protein titration experiments were carried out using the same protocol as described previously (3). AEDANS-labeled cysteine mutants of M13 coat protein were used with the cysteine residue at positions 7 (A7C), 9 (A9C), 12 (N12C), 13 (S13C), 15 (Q15C), 16 (A16C), 17 (S17C), and 18 (A18C). Titration experiments were performed in which the wild-type protein concentration was increased whereas the mutant concentration was kept constant. The sample conditions for these titrations are given in Table 1. The labeling efficiencies were determined as reported previously (6) and are given in Table 1 as well. The labeling efficiency is explicitly taken into account in Table 1 in the ratio of the number of unlabeled to labeled proteins (r_{ul}), as it affects the acceptor concentration and therefore the energy transfer efficiency.

For the fluorescence experiments, stock solutions of protein mutants and wild-type protein solubilized in cholate buffer were mixed with solutions of lipids in the same buffer, as described previously (5). Repeated dialysis of the mixtures in cholate-free buffer was performed to remove the cholate in the sample. The lipid loss during dialysis can vary between 20–30% (3,5) and is accounted for in the analysis of the experimental data.

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Abbreviations used: FRET, Förster (or fluorescence) resonance energy transfer; DOPC, dioleoylphosphatidylcholine; DOPG, dioleoylphosphatidylglycerol; AEDANS, *N*-(acetylaminoethyl)-5-naphthylamine-1-sulfonic acid; r_{LP}, lipid/protein molar ratio; 22:1PC, 1,2-dierucoyl-*sn*-glycero-3-phosphocholine; 14:1PC, 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine.

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TABLE 1 Sample composition of M13 major coat protein incorporated into DOPC/DOPG vesicles

Mutant	A7C	A9C	N12C	S13C	Q15C	A16C	S17C	A18C
n_A	7	9	12	13	15	16	17	18
Acceptor fluorescence maximum λ_{max}, nm	497.6	496.5	496.7	499.5	493.6	495.1	494.2	491.1
Labeling efficiency	0.44	0.78	0.79	0.55	0.53	0.85	0.54	0.56
r_{LP}	336.0	217.0	276.9	561.5	422.6	239.7	267.7	248.6
r_{ul}	1.27	0.28	0.27	0.82	0.89	0.18	0.85	0.79
E	0.172	0.463	0.505	0.338	0.443	0.880	0.448	0.488
r_{LP}	213.2	158.2	184.8	286.2	245.1	169.9	180.7	171.8
r_{ul}	2.58	0.76	0.90	2.57	2.25	0.66	1.74	1.58
E	0.119	0.366	0.360	0.184	0.261	0.650	0.323	0.354
r_{LP}	156.2	124.5	138.7	192.0	172.6	131.6	136.3	131.2
r_{ul}	3.89	1.24	1.53	4.32	3.62	1.14	2.64	2.38
E	0.095	0.307	0.279	0.129	0.200	0.513	0.261	0.286
r_{LP}	123.2	102.6	111.0	144.5	133.2	107.4	109.5	106.2
r_{ul}	5.20	1.71	2.16	6.07	4.99	1.63	3.53	3.18
E	0.084	0.260	0.238	0.102	0.152	0.436	0.217	0.248
r_{LP}	101.7	87.2	92.5	115.8	108.5	90.7	91.5	89.1
r_{ul}	6.51	2.19	2.79	7.82	6.35	2.11	4.42	3.98
E	0.076	0.231	0.199	0.086	0.129	0.383	0.191	0.225
r_{LP}	86.6	75.9	79.3	96.6	91.5	78.5	78.5	76.8
r_{ul}	7.81	2.67	3.42	9.57	7.72	2.59	5.31	4.78
E	0.067	0.207	0.184	0.075	0.111	0.340	0.175	0.198
r_{LP}	57.0	52.2	52.9	61.2	59.1	53.4	52.6	51.8
r_{ul}	12.4	4.33	5.63	15.69	12.50	4.28	8.43	7.57
E	0.055	0.160	0.137	0.055	0.079	0.254	0.138	0.149

Values are given in terms of r_{LP} and r_{ul}, labeling efficiencies, and observed acceptor fluorescence maxima and energy transfer efficiencies E for mutants with acceptor positions n_A at 7, 9, 12, 13, 15, 16, 17, and 18.

Fluorescence experiments

Fluorescence emission and fluorescence excitation measurements were performed at 20°C as described elsewhere (2,3). The position of the AEDANS emission maximum was different for different labeled mutants because the Stokes shift of AEDANS fluorescence significantly depends on the local polarity of the environment of the label and thus on the distance between the label and the center of the lipid bilayer (7,8). The position of the AEDANS emission maxima was determined using a polynomial approximation of the top part of the emission peak as in (7) and given in Table 1.

For fluorescence excitation measurements, the detection wavelength was set at the maximum of the acceptor (AEDANS) fluorescence of a particular mutant, and the excitation wavelength was scanned from 260 to 400 nm. The resulting AEDANS emission spectra for all mutants and examples of excitation spectra for mutant N12C are presented in Fig.1, *A* and *B*, respectively.

The energy transfer efficiency E (which is an average efficiency for all donors in the system and includes both intra- and intermolecular energy transfer) was calculated from the fluorescence intensities by

$$E = \frac{1}{1 + r_{ul}}\left(\frac{F^{290}}{F^{340}} - \frac{\varepsilon_A^{290}}{\varepsilon_A^{340}}\right)\frac{\varepsilon_A^{340}}{\varepsilon_D^{290}}, \quad (1)$$

where r_{ul} is the ratio of the number of unlabeled to labeled proteins. The derivation of this equation is described in detail elsewhere (3). For every sample the ratio of the fluorescence intensity at 290 nm, F^{290}, (mainly donor excitation) to that at 340 nm, F^{340}, (exclusively acceptor excitation) was calculated as a measure of the donor-to-acceptor energy transfer. The ratio F^{290}/F^{340} was corrected for direct excitation of AEDANS at 290 nm by subtracting the ratio of the extinction coefficients $\varepsilon_A^{290}/\varepsilon_A^{340} = 0.20$ (calculated using mutant Y21A/Y24A/W26A/G23C). The ratio of the extinction coefficients of the acceptor at 340 nm (ε_A^{340}) and donor at 290 nm (ε_D^{290}) is 1.2.

METHODOLOGY

Model for M13 major coat protein incorporated into a lipid bilayer

In this study we will extend our previous single-helix model for the M13 major coat protein (3) to a two-helix model. This model consists of two flexibly linked helical domains connected via a kink (Fig. 2). One domain reflects the transmembrane protein part, and the other domain is the N-terminal protein part that is supposed to stick out of the membrane (1,7,9–11). The conformation of each domain is assumed to be a perfect α-helix. The main axis of the protein O is parallel to the transmembrane protein domain and defines the z axis of the axes system of the protein. The orientation of the x axis is defined by the location of the C_α of Trp-26 (donor), which is used as the reference amino acid residue. The complete set of structural parameters that determines the location and conformation of the protein is presented in Table 2. The protein parameters related to position, orientation, and tilt of the transmembrane domain are taken from a previous study (3). The parameter ranges given in Table 2 indicate the range of values considered in the simulations. It should be noted that