LETTER TO THE EDITOR

The Transmembrane Helices of Beef Heart Cytochrome Oxidase

ABSTRACT The locations of the transmembrane helices in the 12 subunits of beef heart cytochrome oxidase were predicted with a modified form of the von Heijne-Blomberg hydrophobicity scale. Based on ~20 residues per transmembrane helix, about 480 of the estimated 660 helical residues (36.8% of 1,793 total residues) are expected to be in transmembrane helices that have their axes tilted by a small angle α from the normal to the plane of the membrane. This angle is calculated to be ~30°, based on the observed overall tilt angle θ of 39° obtained from circular dichroism (CD) measurements on multilamellar films, or about 25°, based on the observed tilt angle θ of 36° obtained from the infrared linear dichroism of films. For 21 residues per transmembrane helix, the calculated values of α become 32° and 28°, respectively, depending upon the value of θ used. Thus, a transmembrane helical tilt angle of ~30° accounts for the predicted transmembrane stretches in cytochrome oxidase if 20–21 residues are sufficient to span the membrane. Additional helical residues in the lipid head region may deviate by a larger angle from the normal to the plane of the membrane in cytochrome oxidase.

INTRODUCTION

A recent paper by Bazzi and Woody (1) calculates α-helix and β-sheet content in cytochrome *c* oxidase (COX) from the circular dichroism (CD) of the solution and from the infrared linear dichroism (IRLD) of multilamellar films. They calculate the orientation of the α-helices and β-sheets in COX based on CD of multilamellar films and IRLD of amide I and II bands in the IR region. They suggest that the average orientation of an α-helical segment in Cox is 39° (CD) or 36° (IRLD). Bazzi and Woody conclude that a tilt angle of ~10° or 20° from the normal to the plane of the membrane of an estimated 400 out of a total of ~500 α-helical residues would not be sufficient to explain the observed average tilt angle of 39° (CD) or 36° (IRLD).

METHOD

The hydrophobicity profiles of all 12 subunits of COX from beef heart (2) were obtained using the scale shown in Table I (3) modified from von Heijne and Blomberg (4). Hydrophobicity sums were obtained for a moving window of 19-residue segments using an adapted computer program from Kyte and Doolittle (5). The transmembrane helices predicted using this scale correspond to the lipid-buried segments of the two transmembrane helices in subunit II and the single transmembrane helix in subunit IV of beef heart COX located by the arylazidophospholipid labeling experiments of Buse et al. (6, 7).

RESULTS AND DISCUSSION

Twelve different subunits present in a 1:1 ratio (with the exception of subunit VIIIb, which is present in a 2:1 ratio) to all other subunits that constitute beef heart COX (2). These 12 subunits are isolated from beef heart COX in all preparations of Buse and coworkers (2, 8) and appear to be present in stoichiometric ratio in beef heart COX prepared by different methods. Thus, the molecular formula of the subunits of beef heart COX corresponds to 1,793 amino acid residues per monomer (2) and not to ~1,400 residues as was assumed by Bazzi and Woody (1). Of these 1,793 residues, 36.8 ± 2.9% are α-helical and probably >20% are in β-sheets with the rest of the residues in other conformations (beta turn or coil) according to the percentage estimates of α-helix and β-sheet content in beef heart COX of Bazzi and Woody (1).

The peaks in the hydrophobicity profiles of subunits of beef heart COX that correspond to transmembrane helices are listed in Table II. We predict a total of 24 transmembrane helices. Erdweg and Buse (9) suggest that the 20-residue long hydrophobic stretches encountered in some of the smaller subunits may not span the membrane. For example, the segment we predict as a transmembrane helix at 21–39 in subunit VIc based on its average hydrophobicity per residue of 1.35 kcal has, at each end, clusters of charged groups (RRLR at 15–18 and EKRKK at 41–45) that, they suggest, may prevent transmembrane penetration (9). This segment does not react with arylazidophospholipids and may be a cardiolipin binding site (9). But note that the H subunit of the photosynthetic reaction center of *Rhodopseudomonas viridis*, whose crystal structure was recently reported to 3-Å resolution (10), contains a single transmembrane helix at 12–37 that has a cluster of charged residues (RREDRRE) at 33–39.

Blaisie et al. (11, 12) have observed bundles of α-helices in COX that were approximately normal to the plane of the membrane. We used 19-residue segments to locate these transmembrane helices. With one or two edge residues remaining accessible to solvent in the lipid head region, 20–21 residues appear to be frozen in the lipid on the nuclear magnetic resonance (NMR) time scale in bacteriorhodopsin with about two other residues on each side of the membrane being somewhat less rigidly held in position (13, 14). von Heijne (15) points out that a "simple" integral membrane protein with a single transmembrane segment will be ~21 residues long to get across the nonpolar part of the membrane ($\gtrsim$30 Å long). For 24 transmembrane helices in beef heart COX with ~20–21

TABLE I
PROPERTIES OF TRANSMEMBRANE HELICES

Residue	ASA(A^2)	H-Bond	Charge	H'	ΔG_H	H
					kcal	*kcal*
ala	40	0	0	−1.0	−1.0	+1.0
arg	150	+3.9	+6.4	−3.8	+6.5	−6.5
asn	85	+3.9	0	−2.1	+1.8	−1.8
asp	75	+2.6	+6.4	−1.9	+7.1	−7.1
cys	60	0	0	−1.5	−1.5	+1.5
gln	105	+3.9	0	−2.6	+1.3	−1.3
glu	115	+2.6	+6.4	−2.9	+6.1	−6.1
gly	0	0	0	0	0	0
his	120	+2.6	+1.4	−3.0	+1.0	−1.0
ile	100	0	0	−2.5	−2.5	+2.5
leu	95	0	0	−2.4	−2.4	+2.4
lys	125	+2.6	+6.4	−3.1	+5.9	−5.9
met	110	0	0	−2.8	−2.8	+2.8
phe	135	0	0	−3.4	−3.4	+3.4
pro	70	+2.6	0	−1.8	+0.8	+0.8
ser	40	+1.3	0	−1.0	+0.3	−0.3
thr	65	+1.3	0	−1.6	−0.3	+0.3
trp	180	+1.3	0	−4.5	−3.2	+3.2
tyr	155	+2.6	0	−3.9	−1.3	+1.3
val	80	0	0	−2.0	−2.0	+2.0

The estimated hydrophobic free energy gain (H') when a side chain in a protein in random coil conformation is taken out of contact with aqueous solution is based on 25 cal/A^2 of accessible surface area (ASA) gain with appropriate corrections for hydrogen-bond loss (H-bond) and electrostatic charge neutralization (Charge) contributions (3, 4). The assigned hydrophobicity value H in the last column has the opposite sign from the estimated free energy change (ΔG_H, kcal/residue) that is shown. Using this scale we predict the following transmembrane helices in the L, M subunits of the photosynthetic reaction center of *Rhodopseudomonas viridis* (10): 30–48, 56–74 (32–55, 52–78 observed), 84–102, 111–129 (84–112, 110–139); 116–134, 143–162 (115–140, 142–167); 177–197, 196–214 (170–199, 197–225); 232–250, 266–284 (225–251, 259–285).

TABLE II
THE MOST HYDROPHOBIC 19-RESIDUE SEGMENTS IN SUBUNITS OF BEEF HEART COX THAT ARE PREDICTED TO BE TRANSMEMBRANE HELICES AND THEIR HYDROPHOBICITY INDICES (*HI*)

	Segment	*HI*		Segment	HI
Subunit I	19–37	1.59	Subunit II	28–46	1.99
	57–75	1.97		63–81	1.63
	100–118	1.59	Subunit III*	40–58	1.64
	147–165	1.28		83–101	1.87
	183–201	1.80		158–176	1.55
	184–202	1.80		202–220	1.55
	243–261	1.43		240–258	1.60
	270–288	1.99		241–259	1.60
	338–356	1.83	Subunit IV	80–98	1.89
	379–397	1.68	Subunit VIb	18–36	1.31
	414–432	1.47	Subunit VIc	21–39	1.35
	455–473	1.85	Subunit VIIIa	21–39	1.82
			Subunit VIIIb	17–36	1.56

*Alternatively, the first transmembrane helix in subunit III is at 19–37 (HI = 1.39) or at 31–49 (*HI* = 1.51), but only the segment chosen at 40–58 is superimposable in subunits III from seven different species (M. Lundeen and B. Chance, unpublished work).

residues each, we calculate ~480–504 α-helical residues in transmembrane helices that are tilted by some angle α from the normal to the plane of the membrane. These transmembrane α-helical residues make up about three-fourths of all the α-helical residues in beef heart COX. If all other α-helical segments are randomly oriented on the average with respect to the plane of the membrane, an average tilt angle α of 25° or 30° is calculated for the transmembrane helices. This is based on an observed tilt angle, θ, of 36° (IRLD) or 39° (CD) if the fraction of transmembrane α-helical residues, f_{tm}, is 0.73 (20 residues per transmembrane helix). For 21 residues per transmembrane helix, $f_{tm} = 0.76$ and an average tilt angle α of 28° or 32° are calculated based on an observed tilt angle θ of 36° (IRLD) or 39° (CD), respectively.

As Bazzi and Woody (16) point out in their accompanying reply, a tilt angle α of ~30° is consistent with the observed tilt of the transmembrane helices in L, M, and H subunits of the photosynthetic reaction center of *Rhodopseudomonas viridis*. Its crystal structure, reported by Deisenhofer et al. (10), showed tilts of <25° for many of the transmembrane helices though two of the helices (the D helix in the L and M subunits) appeared to be tilted by ~38°. Thus, despite earlier suggestions that the transmembrane helices in COX were approximately normal to the plane of the membrane (within ~10°) (11, 12), probably the tilt angle α is close to ~30°. This is necessary to account for the fraction of transmembrane helical residues in this protein. There is room in the cytochrome oxidase molecule for this many transmembrane helices (17, 18). (Some estimates of the number of transmembrane helical segments in the largest subunits, subunits I and III, differ from ours [3, 5, 18–22]). We predict the same transmembrane helices in all species for which we had sequence information using the scale shown in Table I. These helices are shown for beef heart cytochrome oxidase in Table II. We used our scale to predict the transmembrane helices in the L and M subunits of the photosynthetic reaction center of *Rhodopsomonas viridis* to assure ourselves that we were not grossly over- or underpredicting transmembrane helical segments by our method. We found that we had predicted correctly all ten of the transmembrane helices in these two subunits and that only two residues in the L subunit and only one residue in the M subunit were incorrectly predicted to be a transmembrane helical residue as part of the 19-residue stretch with the highest hydrophobicity (3, 5). These results are shown in the footnote to Table I.

Finally, there is the problem of what the length of a transmembrane helix actually is. We find that 18- or 19-residue segments are more useful in locating the probable transmembrane stretches in a membrane protein than longer segments if hydrophobicity profiles are used because charged residues may be present particularly in the lipid head region (3, 6, 7, 15). The transmembrane helices observed in the crystal structure of the photosyn-

thetic reaction center of *Rhodopseudomonas viridis* range in length from 24–30 residues and, therefore, can not be predicted in their entirety from the hydrophobicity profile of a 19-residue moving window using the hydropathy scale of Kyte and Doolittle (5, 19) or Table I (3). These long stretches appear to have some charged residues close to one or both ends according to the primary structures of the L and M subunits published by Michel et al. (23). Thus, the long stretches observed in the crystal structure with the side chains on the outside may involve some helical residues in the lipid head region in the membrane that deviate from the transmembrane helical axis in this region. As Michel et al. (23) point out, the edges of a transmembrane helical segment are the least well determined regions from the hydrophobicity profile.

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