Response to Lundeen et al.

Dear Sir:

We have recently (1) reported circular dichroic (CD) and infrared linear dichroic (IRLD) studies of the orientation of the α-helical segments in cytochrome oxidase in membrane films. Based on the measured average angle between the normal and the helix axes, we concluded that "... if there are of the order of 20 transmembrane helices in cytochrome oxidase [as predicted by Capaldi et al. (2)], some of them must deviate substantially from a normal orientation. Alternatively, some of the highly hydrophobic stretches identified by Capaldi et al. may be buried α-helices in domains external to the membrane, or may be strands of β-sheet penetrating the membrane." In the accompanying Letter to the Editor, Lundeen et al. (3) conclude that the observed average tilt of 39° (1) would be consistent with ~20 transmembrane helices with an average of 10°, or with all 24 transmembrane helices that they predict, assuming an average tilt of 20°.

Our comments on the letter of Lundeen et al. (3) are as follows: (*a*) Since our paper (1) was submitted, the first high-resolution structure of an integral membrane protein has been reported. The data on the photosynthetic reaction center of *Rhodopseudomonas viridis* (4) indicate an upper limit of ~30° for the average orientation of transmembrane helices, which is larger than the upper limit of 20° that we considered earlier (1), based upon the structure of bacteriorhodopsin (5–7).

(*b*) There is no reason for assuming (3) that helices that do not span the membrane have $\theta = 90°$. A much more plausible assumption would be that helices are randomly oriented, i.e., have $\langle\theta\rangle = 54.7°$. Although Deisenhofer et al. (4) give no data on the reaction center helices that do not span the membrane, their figures are not consistent with an average angle near 90°.

(*c*) Let f_{tm} = fraction of helical residues in transmembrane helices, α_{tm} = the average angle between the transmembrane helix axes and the normal, and θ_{obs} the observed average angle for the protein. Assuming that the other helices have their axial directions distributed randomly,

$$f_{tm} = (3\cos^2\theta_{obs} - 1)/(3\cos^2\alpha_{tm} - 1).$$

Table I shows the results for values of α_{tm} ranging from 0° to 30° and taking θ_{obs} to be 39° as determined by CD or 36° determined by IRLD (1).

Inspection of the results in Table I shows that only if one combines the upper limit of $\alpha_{tm} \simeq 30°$ with the lower estimate of θ_{obs} is the number of predicted transmembrane helices (3) compatible with the experiment. If α_{tm} is significantly less than the upper limit suggested by the reaction center structure (4), several proposed transmembrane helices would have to be deleted, perhaps those in the smaller subunits as suggested by Lundeen et al. (3), and possibly even some from the larger subunits.

In summary, the letter of Lundeen et al. (3), while almost certainly correct in suggesting that our original calculations (1) underestimated the number of subunits in beef heart cytochrome oxidase, does not substantially alter our earlier conclusions. To account for the observed average helix orientation, we must postulate (*a*) a significant deviation of transmembrane helices from an orientation normal to the membrane, or (*b*) an overprediction of transmembrane helices by hydrophobicity profiles.

TABLE I
PERMISSIBLE FRACTION OF TRANSMEMBRANE HELICES IN CYTOCHROME OXIDASE*

α_{tm}	f_{tm}	N_{tm}‡ (residues)	n_{tm}§ (helices)
degrees			
0	0.41	268	13
	0.48	318	16
10	0.43	281	14
	0.50	333	17
20	0.49	325	16
	0.58	386	19
30	0.65	429	21
	0.71	509	25

*For each value of α_{tm}, the upper set of numbers uses a value of $\theta_{obs} = 39°$ (from CD), and the lower set uses $\theta_{obs} = 36°$ (from IRLD).
‡The number of residues in transmembrane helices, assuming a total of 660 helical residues (3), based upon 12 subunits (5).
§The number of transmembrane helices, assuming 20 residues/helix.

Received for publication 12 August 1986 and in final form 7 October 1986.

REFERENCES

1. Bazzi, M. D., and R. W. Woody. 1985. Oriented secondary structure in integral membrane proteins. I. Circular dichroism and infrared spectroscopy of cytochrome oxidase in multilamellar films. *Biophys. J.* 48:957–966.
2. Capaldi, R. A., F. Malatesta, and V. M. Darley-Usmar. 1983. Structure of cytochrome oxidase. *Biochim. Biophys. Acta.* 726:135–148.
3. Lundeen, M., B. Chance, and L. Powers. 1986. The transmembrane helices of beef heart cytochrome oxidase. *Biophys. J.* 51:693–695.
4. Deisenhofer, J., O. Epp, K. Miki, R. Huber, and H. Michel. 1985. Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3Å resolution. *Nature (Lond.).* 319:613–624.
5. Henderson, R. 1977. The purple membrane from *Halobacterium halobium*. *Ann. Rev. Biophys. Bioeng.* 6:87–109.
6. Rothschild, K. J., and N. A. Clark. 1979. Polarized infrared spectroscopy of oriented purple membrane. *Biophys. J.* 25:473–488.
7. Nabedryk, E., and Breton, J. 1981. Orientation of intrinsic proteins in photosynthetic membranes. Polarized infrared spectroscopy of chloroplasts and chromatophores. *Biochim. Biophys. Acta.* 635:515–524.

MOHAMMED D. BAZZI
ROBERT W. WOODY
Department of Biochemistry
Colorado State University
Fort Collins, Colorado 80523