Anomalous Amide I Infrared Absorption of Purple Membrane

Abstract. *Infrared absorption of purple membrane reveals an amide I frequency that falls outside the range normally found for alpha helices. This may indicate along with x-ray diffraction the existence of bacteriorhodopsin alpha helices with unusual structure.*

Much interest has focused on bacteriorhodopsin, the light-driven proton pump in the purple membrane of *Halobacterium halobium* (*1-4*). Understanding how this membrane protein translocates a proton against an electrochemical gradient by transducing the energy from one absorbed photon is a key goal. A major step toward this goal was the reconstruction of a model of bacteriorhodopsin at the resolution of 7 Å by use of electron diffraction (*5*). This picture reveals seven rods of high electron density that run transversely through the bilayer plane. By measuring the infrared dichroism of oriented dried purple membrane, we recently confirmed that these rods are α-helical, with the helical axes arranged on the average less than 26° from the membrane normal (*6*).

It is important to know whether there are any special structural features of bacteriorhodopsin that might provide a clue to its proton pumping mechanism. In the case of dried purple membrane, the infrared absorption reveals an anomalously high C=O stretching frequency (amide I), which could indicate an overall distortion from the normal α-helical structure. However, conformational changes in bacteriorhodopsin may occur on drying, especially since the infrared absorption indicates almost the complete absence of free H_2O in these membrane preparations (*6*).

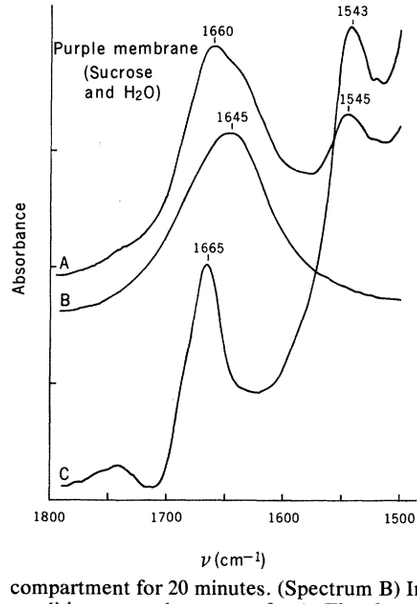
We report here evidence of distorted α-helical conformation in bacteriorhodopsin based on infrared absorption of purple membrane suspended in D_2O or partially dried from sucrose solution. Under these conditions we do not expect conformational changes to be likely. Purple membrane has been shown to be photochemically active in D_2O, with no evidence of conformational change (*7*). Dried purple membrane equilibrated in 94 percent relative humidity exhibits photochemical kinetics identical to the kinetics measured in solution (*8*). Evidence of proton pump activity has also been found in dried purple membrane (*9*). Furthermore, drying purple membrane in the presence of sucrose, as was done for the electron diffraction study (*5*), is believed to stabilize the structure of bacteriorhodopsin.

Purple membrane was prepared according to the method of Becher and Cassim (*10*). Membranes were dried from 0.1*M* sucrose solution onto an Irtran 4 slide (Eastman Kodak, Rochester, New York) by using a continuous stream of N_2, or were suspended after lyophilization in D_2O in a AgCl cell. Concentrations of purple membrane-D_2O solution ranged from 0.1 to 1 m*M*. Infrared measurements were made for both the light-adapted and dark-adapted states of purple membrane by using a dual-beam Fourier transform infrared spectrometer (Digilab FTS-14) (*11*). Frequency calibration was checked by using polystyrene film. Since the Fourier transform spectrometer is also internally calibrated with a He-Ne laser, the frequency of the peaks was found to vary no more than $\pm$ 1 cm^{-1}.

Figure 1A shows the infrared spectrum from 1500 to 1800 cm^{-1} of purple membrane dried from 0.1*M* sucrose solution. Two peaks are found, one at 1660 cm^{-1}, due to the amide I vibration (C=O stretching), and one at 1545 cm^{-1}, due to the amide II vibration (in-plane NH bend) (*12, 13*). The shoulder centered at 1650 cm^{-1} is at least partially due to the presence of H_2O, which is trapped with the sucrose and purple membrane on drying. It is possible to reduce the H_2O contribution to the spectrum by digitally subtracting different amounts of an infrared spectrum of pure H_2O (Fig. 1B). The resulting spectrum (Fig. 1C) displays an amide I frequency near 1665 cm^{-1}. In cases where less H_2O is subtracted, the amide I frequency falls between 1660 and 1665 cm^{-1}.

Infrared spectra of D_2O suspensions of purple membrane have an amide I peak near 1665 cm^{-1} and an amide II peak at 1545 cm^{-1} (Fig. 2). In these cases partial deuteration of the α-helix peptide groups is expected to result in a lowering of the amide I frequency by a few wave numbers and a drop in intensity of the amide II peak due to a shift in frequency to 1450 cm^{-1} (*14*). We also find evidence of a shoulder near 1645 cm^{-1}, which may be due to the amide I vibration of deuterated peptide groups in unordered conformation (*14*).

An amide I frequency of 1665 cm^{-1} is outside the normal range (1650 to 1655 cm^{-1}) reported for α-helical proteins and polypeptides (*12-15*). However, an α-helical conformation that deviates from the standard Pauling-Corey α-helix (*10*) (pitch P = 5.4 Å and unit height h = 1.5



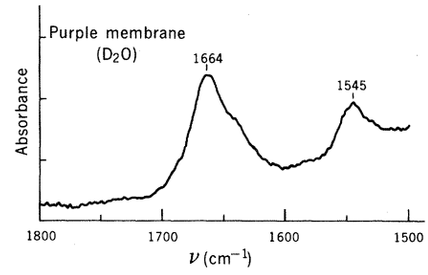


Fig. 1 (left). (Spectrum A) Infrared absorption from 1500 to 1800 cm^{-1} of purple membrane dried from 0.1*M* sucrose solution onto an Irtran 4 slide. Spectra were recorded with 100 scans of sample and 50 scans of reference beam at a resolution of 4 cm^{-1}. The absorbance is 0.3 at 1660 cm^{-1} and 0.1 at 570 nm. Spectra were smoothed using a nine-point Lorentzian smoothing factor. Spectra were measured within 4 minutes after prolonged exposure of the sample to room lights; no change was found in spectra recorded after the sample had dark-adapted in the sample compartment for 20 minutes. (Spectrum B) Infrared absorption of H_2O in AgCl cell. Recording conditions were the same as for A. The absorbance at 1645 cm^{-1} is 0.3. (Spectrum C) Difference spectrum obtained by subtracting half of B from A. The absorbance at 1665 cm^{-1} is 0.023. The rising background is due to sucrose absorption near 1450 cm^{-1}. A 1515 cm^{-1} peak due to tyrosine and a shoulder at 1690 cm^{-1} are also observed. The peak at 1740 cm^{-1} is due to ester carbonyls present in some of the purple membrane lipid. Visual inspection was used to determine the amount of H_2O subtraction necessary to eliminate the shoulder in spectrum A. Fig. 2 (right). Infrared absorption from 1500 to 1800 cm^{-1} of purple membrane suspended in D_2O in a AgCl cell. Spectra were recorded with 100 scans of sample and 50 scans of reference beam at a resolution of 4 cm^{-1}. The absorbance at 1664 cm^{-1} is 0.043 and the concentration of purple membrane is approximately 1 m*M*. The spectrum was corrected for a sloping background due to D_2O by subtraction of a reference D_2O absorption spectrum. The spectrum was recorded 16 minutes after suspension of purple membrane in D_2O.

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Å) might exhibit a shifted amide I frequency. In particular, an increase in hydrogen bond distance of 0.05 Å could produce a rise in the frequency of the amide I band by as much as 10 cm^{-1} (*16*). Two examples of distorted α-helical polypeptides with amide I frequencies near 1665 cm^{-1} are poly-β-benzyl-L-aspartate, which forms a left-handed helix (*17*), and poly-β-benzyl-DL-glutamate, which forms an $\alpha_{L,D}$-helix (*18*). In the case of bacteriorhodopsin, the existence of distorted α-helical conformation is also supported by x-ray scattering on dried purple membrane (*19*), which reveals an anomalous pitch $P = 5.05$ Å. A second possibility that cannot be excluded is that factors other than α-helix structure, such as the two-dimensional crystalline arrangement of bacteriorhodopsin in the purple membrane, give rise somehow to the anomalous amide I frequency.

Other measurements have revealed features of bacteriorhodopsin that may lead to distortion in the α-helices. First, partial amino acid sequencing of bacteriorhodopsin (90 residues) (*20*) shows that at least 50 percent of the sequence that is expected to be α-helical based on the electron diffraction model (*5*) is not α-helical (*20*). Second, electron diffraction shows the existence of supercoils in the α-helices of bacteriorhodopsin (*19, 21*). It is not known whether the degree of supercoiling is sufficient to account for the observed shift in the amide I frequency. Infrared spectroscopy of highly supercoiled proteins such as fd phage coat protein might help shed light on this question.

In summary, infrared absorption measurements of purple membrane in D_2O suspensions and partially dried in sucrose, considered together with x-ray data, indicate that there is distortion in the structure of bacteriorhodopsin α-helices. It will be important to know whether this feature is common to other membrane proteins, or is related in some specific way to the proton transport function of bacteriorhodopsin.

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Prolongation of Islet Allograft Survival Following in vitro Culture (24°C) and a Single Injection of ALS

Abstract. *Isolated rat islets remain morphologically and functionally intact during a 7-day period of in vitro culture at 24°C. In vitro culture of islets at 24°C for 7 days prior to transplantation, in conjunction with a single injection of antiserum to lymphocytes into the diabetic recipient, results in islet allograft survival of 100 days when the islets are transplanted across a major histocompatibility barrier.*

Lafferty *et al.* (*1*) obtained prolonged survival of allografts of the thyroid gland by in vitro culture of the donor thyroid in the presence of 95 percent O_2 and 5 percent CO_2 for 26 days prior to transplantation. They suggested that organ culture of the thyroid removed passenger lymphoid cells and that these lymphoid elements played a major role in the sensitization of the host to foreign antigens of the thyroid cells. We attempted to utilize this in vitro approach for prolongation of islet allograft survival and found that 95 percent O_2 produced disintegration of isolated islets after 4 to 5 days of in vitro culture. Because of this limitation, we determined the effect of prior treatment of donor rats with total body irradiation and intravenous silica in conjunction with either in vitro culture of the islets in the presence of rabbit antiserum to rat lymphocytes (ALS) for 1 to 2 days or a single injection of ALS into the recipients at the time of transplantation (*2*). Prolongation of islet allograft survival was obtained across a minor histocompatibility barrier (Fischer to Lewis) by pretreatment of the donors and in vitro culture of the islets with ALS and across a major histocompatibility barrier (ACI to Lewis) by pretreatment of the donors in conjunction with a single injection of ALS into the recipients.

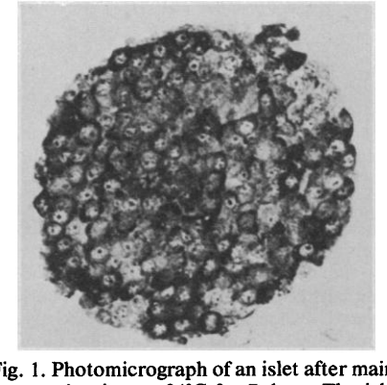

Fig. 1. Photomicrograph of an islet after maintenance in vitro at 24°C for 7 days. The islet cells appear normal and the beta cells have a normal degree of beta granulation. Small groups of alpha cells are present in the upper right and lower left portion of the photograph. Aldehyde fuchsin stain; magnification, × 300.

Opelz and Terasaki (*3*) reported that in vitro maintenance of lymphocytes at a low temperature (22°C) for more than 4 days resulted in loss of the ability of the lymphocytes to stimulate allogeneic lymphocytes in mixed lymphocyte cultures. Since we were attempting either to diminish or to alter the lymphoid elements in the islets prior to transplantation, we determined whether isolated islets would survive for 7 days when they were incubated in vitro at room temperature (24°C). Surprisingly, the islets not only survived but remained morphologically and functionally intact at the end of the 7-day incubation period. Figure 1 illustrates a normal degree of beta granulation and the presence of alpha cells at the periphery of an islet after 7 days of culture at 24°C. Insulin secretion during in vitro culture (24°C) was 16 μU per islet per hour in the presence of glucose (1.5 mg/ml) and increased to 60 μU per islet

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