The data are consistent with either Eq. 3, $k_1' = k_2' = 14\ s^{-1}\ mol^{-1}$; or with Eq. 2, $k_1 = k_4 = 3 \times 10^{-3}\ s^{-1}$. The two mechanisms have different implications for the physiology of the albumin-fatty acid complexation, but the experiments with a protein concentration series which can distinguish the two have not yet been done.

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TIME-RESOLVED RESONANCE RAMAN CHARACTERIZATION OF THE INTERMEDIATES OF BACTERIORHODOPSIN

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In an attempt to determine eventually the structure of the chromophore of the intermediates involved in the primary process of the photosynthetic proton-pumping cycle of bacteriorhodopsin, new simple techniques have been developed (1–4) to obtain time-resolved resonance Raman spectra of this system. The techniques used in this report involve chopping continuous wave (CW) laser light to produce pulses of variable width and separation to obtain temporal information on the microsecond and millisecond time scales. An optical multichannel analyzer (Princeton Applied Research Corp., Princeton, N.J.) with a Dry Ice-cooled silicon-intensified vidicon is used for detection. By using these techniques as well as different laser frequencies to take advantage of differing resonance enhancements of the intermediates, flow techniques (5–7), and computer subtraction methods, the resonance Raman spectra of the retinal chromophore of the individual intermediates are extracted.

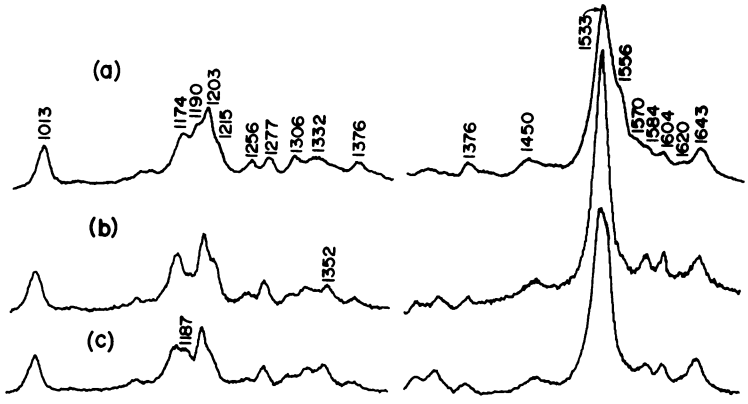

FIGURE 1 (*a*) 100 mW CW excitation from a Spectra-Physics model 165 argon ion laser at 5,145 Å of 100 μM bacteriorhodopsin in a melting point capillary (Spectra-Physics Inc., Laser Products Div., Mountain View, Calif.). (*b*) Light-adapted recirculated flow spectrum of bacteriorhodopsin with 4 mW of 5,145 Å excitation. (*c*) Same as in (*b*) but dark-adapted.

Resonance Raman spectra of bacteriorhodopsin are shown in Fig. 1. A CW spectrum is shown in Fig. 1 *a*. This spectrum is a superposition of several intermediates, bR_{570}, bL_{550}, bM_{412}, and at least one other intermediate, possibly bO_{640}. A flow spectrum, shown in Fig. 1 *b*, contains bR_{570} with minimal contributions from the other intermediates. A dark-adapted (DA) flow spectrum is shown in Fig. 1 *c*, giving the resonance Raman spectrum of bR_{560}^{DA}. Some conclusions about the various forms of bacteriorhodopsin are given below.

bR_{570}. The so called "fingerprint region" (1,100–1,400 cm^{-1}) has been shown to be very sensitive to the isomeric configuration of the retinal (7). The similarity between the fingerprint regions of bR_{570} and the protonated Schiff base of all-*trans* retinal is not as close as that between the fingerprint regions of the unphotolyzed rhodopsin and the protonated Schiff base of 11-*cis* retinal (5). The fingerprint region of bR_{570} bears a closer resemblance to that of the protonated Schiff base of 13-*cis* retinal (2).

bL_{550}. We have recently reported the observation of a band at 1,620 cm^{-1} (3) that grows at a similar rate as the C=C stretch at 1,556 cm^{-1} assigned to bL_{550} (2). If this band is the same as that assigned for the unprotonated C=N vibration (8), the results suggest that deprotonation might occur with the appearance of bL_{550} or earlier. The fingerprint region of bL_{550} is characterized by a strong band at 1,190 cm^{-1}. Other bands are too weak to report conclusively at this time.

bR_{560}^{DA}. This form is presently thought to be an equilibrium between 13-*cis* and all-*trans* retinal (9). The resonance Raman spectrum of bR_{560}^{DA} in Fig. 1 *c* confirms the existence of two isomers as evidenced by the broadening of the C=C stretch at 1,533 cm^{-1}. An examination of the fingerprint region reveals the presence of another isomer in addition to the isomer present in bR_{570} form (see Fig. 1 *b*).

Possible conclusions are: (*a*) the exact isomeric form of the chromophore in bacteriorhodopsin (as concluded from resonance Raman studies) is not firmly established.

The fingerprint regions of bR_{570}, bL_{550}, bR_{560}^{DA}, and bM_{412} (2, 6) are all different. Whether these changes are due to differences in isomeric configuration, state of protonation, or other changes in the electronic structure of the olefinic system of the retinal chromophore is not yet clear. (*b*) Deprotonation of the retinal Schiff base may occur earlier than previously thought from optical absorption data.

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STUDIES ON PROTEINS AND tRNA WITH TRANSIENT ELECTRIC BIREFRINGENCE

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Transient electric birefringence was used in this study for the determination of size and shape of several native and sodium dodecyl sulfate (SDS)-denatured proteins, yeast and rat liver bulk tRNA, and purified yeast $tRNA^{Phe}$. An instrument constructed by R. C. Williams (1) with a resolution time of 8 ns for propylene carbonate allowed the observation of birefringence decay phenomena previously masked by instrumental noise. The light source was a 5 mW HeNe laser with a wavelength of 623.8 nm. The Kerr cell was designed to fit a 1 × 1 cm spectrophotometer cuvette and consisted of platinum sheet electrodes spaced 1.5 mm apart in a Teflon support. The high-voltage source was a 20 kV, 1.5 mA power supply. The high-voltage pulser used a switched charged line of 500 ft of RG-8A/U cable short-circuited by a triggered spark gap to effect a very fast decay time. A thin film high-frequency resistor capable of withstanding high