The fingerprint regions of bR_{570}, bL_{550}, bR^{DA}_{560}, 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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REFERENCES

1. CAMPION, A., J. TERNER, and M. A. EL-SAYED. 1977. Time-resolved resonance Raman spectroscopy of bacteriorhodopsin. *Nature (Lond.)*. **265**:659.
2. TERNER, J., A. CAMPION, and M. A. EL-SAYED. 1977. Time-resolved resonance Raman spectroscopy of bacteriorhodopsin on the millisecond time-scale. *Proc. Natl. Acad. Sci. U.S.A.* **74**:5512.
3. CAMPION, A., M. A. EL-SAYED, and J. TERNER. 1977. Resonance Raman kinetic spectroscopy of bacteriorhodopsin on the microsecond time-scale. *Biophys. J.* **20**:369.
4. MARCUS, M. A., and A. LEWIS. 1977. Kinetic resonance Raman spectroscopy: dynamics of deprotonation of the Schiff base of bacteriorhodopsin. *Science (Wash. D.C.)*. **195**:1328.
5. MATHIES, R., R. B. FREEDMAN, and L. STRYER, 1977. Resonance Raman studies of the conformation of retinal in rhodopsin and isorhodopsin, *J. Mol. Biol.* **109**:367.
6. ATON, B., A. G. DOUKAS, R. H. CALLENDER, B. BECHER, and T. G. EBREY. 1977. Resonance Raman studies of the purple membrane. *Biochemistry*. **16**:2995.
7. RIMAI, L., D. GILL, and J. L. PARSONS. 1971. Raman spectra of dilute solutions of some stereoisomers of vitamin A type molecules. *J. Am. Chem. Soc.* **93**:1353.
8. LEWIS, A., J. SPOONHOWER, R. A. BOGOMOLNI, R. H. LOZIER, and W. STOECKENIUS. 1974. Tunable laser resonance Raman spectroscopy of bacteriorhodopsin. *Proc. Natl. Acad. Sci. U.S.A.* **71**:4462.
9. SPERLING, W., P. CARL, CH. N. RAFFERTY, and N.A. DENCHER. 1977. Photochemistry and dark equilibrium of retinal isomers and bacteriorhodopsin isomers. *Biophys. Struct. Mech.*, **3**:79.

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

current and voltage was mounted in a logarithmically tapered cavity designed for correct impedance match for the charged line. The photo detector was a RCA 8644 photomultiplier tube (RCA Solid State, Somerville, N.J.) with a Zener diode divider. A Tektronix type 454 oscilloscope was used to observe the voltage or optical pulse (Tektronix, Inc., Beaverton, Ore.). With this apparatus, two relaxation times were detected for a number of interesting proteins. The semimajor and minor axes of prolate ellipsoids may be determined from this data by using the numerical inversion procedure described by Wright (2, 3). This technique has also been shown to allow the rapid estimation of molecular weight of proteins in SDS solution.

Bovine serum albumin (BSA) obtained from a commercial supplier was shown to be homogenous by Sephadex chromatography, ultracentrifugal analysis, and gel electrophoresis. The two relaxation times observed were 28 and 78 ns for a 0.75% solution. The relaxation times extrapolated to zero concentration were 26.9 and 75.5 ns. These data, applied to the inversion procedure of Wright, yield the following dimensions for the equivalent prolate ellipsoid model for BSA: $2\ a_3 = 140.9 \pm 4.9$ Å and $2\ a_1$ 41.6 ± 3.6 Å. These values are in good agreement with data obtained by other procedures. Other proteins examined were human transferrin, ovalbumin, chymotryptsinogen A, lactoglobulin, lysozyme, cytochrome *c*, and rhodopsin. SDS-denatured proteins gave a slow relaxation time linearly related to log molecular weight (4).

Total tRNA and purified species of tRNA gave similar decay curves. tRNA in buffer, in high Mg^{++}, and in solutions of EDTA exhibited a decrease in relaxation time with increasing tRNA concentration, possibly indicating a more compact structure at higher concentrations. A 0.5% solution of purified yeast $tRNA^{Phe}$ gave relaxation times of 18 and 44 ns, corresponding to major and minor axes of 54.1 and 18.7 Å at 20°C. The magnitude and sign of the birefringence signal and the relaxation time for tRNA was found to vary considerably with temperature. Between 20°C and 35°C a dramatic drop in birefringence occurred with an increase in size of the molecule. This change in tRNA structure was also observed at similar temperatures with acridine conjugates of tRNA by Millar and Steiner (5) and was not altered by higher concentrations of Mg^{++}. Examination of the hyperchromicity curve shows only a few hydrogen bonds had been broken during this temperature change. At 38–40°C the birefringence disappears. Between 40 and 60°C another birefringent species of opposite sign appears, most probably due to the denatured tRNA. The birefringence of tRNA at room temperature is negative and can be obscured by the positive birefringence of water at low tRNA concentrations.

A 0.5% solution of total yeast deacylated tRNA in 0.001 M EDTA had a slow relaxation time of 84 ns at 20°C. The addition of Mg^{++} to a concentration of 0.5 mM decreased the relaxation time to 71 ns. After incubation of this solution at 60°C for 3 min, the relaxation time at room temperature fell to 51 ns. These changes are consistent for a molecule going to a more compact structure. Total yeast tRNA in 0.001 M Mg^{++} gave calculated semi-major and minor axes of 55 and 17 Å at 20°C, compared to 53 and 21 Å for purified acylated $tRNA^{Phe}$.

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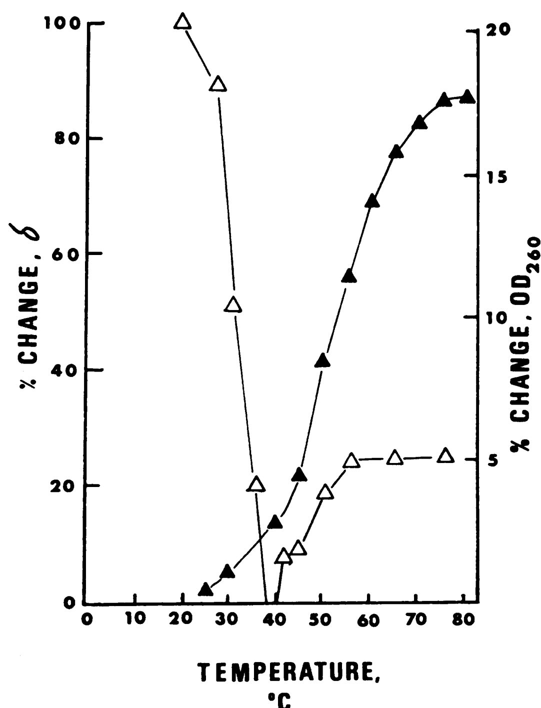

FIGURE 1 Birefringence △ and optical density ▲ as a function of temperature for yeast $tRNA^{Phe}$.

REFERENCES

1. WILLIAMS, R. C., W. T. HAM, and A. K. WRIGHT. 1976. Ultra high speed electro-optical system for transient birefringence studies of macromolecules in solution. *Anal. Biochem.* **73**:52–74.
2. WRIGHT, A. K., R. C. DUNCAN, and K. A. BECKMAN. 1973. Numerical inversion of the Perrin equation for rotational diffusion constants for ellipsoids of revolution by iterative techniques. *Biophys. J.* **13**: 795–803.
3. WRIGHT, A. K., and M. R. THOMPSON. 1975. Hydrodynamic structure of bovine serum albumin determined by transient electric birefringence. *Biophys. J.* **15**:137–141.
4. WRIGHT, A. K., M. R. THOMPSON, and R. L. MILLER. 1975. A study of protein-SDS complexes by transient electric birefringence. *Biochemistry.* **14**:3223–3228.
5. MILLAR, D. B., and R. F. STEINER. 1966. The effect of environment on the structure and helix-coil transition of sRNA. *Biochemistry.* **5**:2289–2301.

METAL ION INTERACTIONS WITH FLUORESCENT DERIVATIVES OF NUCLEOTIDES

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The 1, N^6-ethenoadenosine phosphate derivatives have been shown to be useful probes of nucleotide binding sites in a variety of enzymes (1). Perturbation of the etheno-