Photoelectric Potential from Photoreceptor Cells in Ventral Eye of Limulus

Abstract. *Intense colored light from a gas laser evokes a photoelectric potential in the photoreceptor cells of the ventral eye of* Limulus. *This potential has two components, both of which have the action spectrum of a rhodopsin with an absorbancy maximum at 530 nm. The evidence is consistent with the hypothesis that the photoelectric potential arises directly from the orderly array of rhodopsin molecules which are an integral part of the photoreceptor cell membrane.*

When the lateral eye of *Limulus polyphemus* is stimulated with very intense lights, a short-latency response can be recorded, with intracellular electrodes, from the eye's photoreceptor cells (the retinular cells) (*1*). This electrical response, which we called a photoelectric potential (PEP), has a number of characteristics similar to the so-called early receptor potential (ERP) studied in vertebrate eyes with extracellular electrodes (*2, 3*). In addition, the observations that the polarity of the PEP reversed across the photoreceptor cell membrane and that the amplitude and the polarity of the PEP could be altered by changing membrane potential indicated that the generator of the PEP was intimately associated with the photoreceptor cell membrane (*1*). We present evidence indicating that the *Limulus* PEP, like the ERP, has two components, and the spectral sensitivity of these components closely resembles the absorption spectrum of *Limulus* rhodopsin.

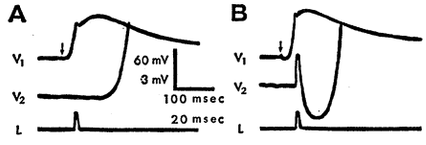

Fig. 1. Intracellular responses of *Limulus* ventral-eye cells to light. In both A and B, V_1 is a direct current-coupled recording. Arrow indicates onset of light stimulus for the V_1 trace. Calibration bar of 60 mv and 100 msec applies to V_1. (V_2) A high-gain, capacitance-coupled recording of a portion of V_1 shown on an expanded time scale. Calibration bar of 3 mv and 20 msec applies to V_2. (L) Light monitor synchronized only with V_2 (at the same sweep speed); L indicates onset of the light stimulus for the V_2 trace. The light intensity in B is several hundredfold greater than that in A.

The photoreceptor cells of the ventral eye of *Limulus* have many anatomical properties, membrane characteristics, and responses to light which are essentially the same as those found in the retinular cell of the lateral eye (*4, 5*). The methods used in these experiments were those used previously (*1*), with two additions. The action spectra of the PEP were determined with a continuous wave (CW) gas laser (argon or krypton) (*6*). With this laser, five lines of sufficiently intense monochromatic light within the spectral region of interest were available. The action spectrum of the generator potential was determined with a CW xenon arc source, narrow-band interference filters, and neutral-density filters.

When a pulse of white light of moderate intensity (in the range of microwatts) is focused onto a ventral-eye photoreceptor cell, the membrane potential undergoes a transient depolarization with the characteristic waveform of the generator potential (Fig. 1A, V_1) and with a latency of several milliseconds (Fig. 1A, V_2 and L). As was observed in the lateral-eye retinular cells (*1*) when the light intensity is increased several hundred-fold, another response appears whose onset is coincident with the light pulse (Fig. 1B, V_2 and L). This response is the PEP. It has two components, a depolarizing potential change which is coincident with the light pulse and a hyperpolarizing potential change which outlasts the light pulse (Fig. 1B, V_2).

Monochromatic light from the laser produced the same PEP's as those evoked by white light. The action spectra of both components of the PEP fit reasonably well with the absorption spectrum of rhodopsin (predicted by Dartnall's nomogram) with the absorption maximum taken at 530 nm (*7*; Fig. 2). Previous studies have shown by microspectrophotometry (*8*) and with electrophysiological techniques (*4*) that the absorption spectrum and generator potential action spectrum, respectively, peak near 530 nm. We confirm that the action spectrum of the potential of the ventral-eye cells peaks near 530 nm (Fig. 2).

In conclusion, we have found that a PEP can be recorded with intracellular electrodes in the photoreceptor cells of the *Limulus* ventral eye. This PEP is similar, in two more respects, to the ERP recorded extracellularly from vertebrate eyes, namely, there

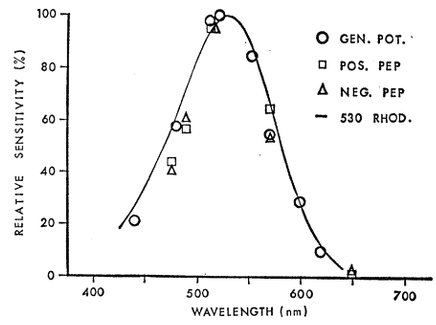

Fig. 2. Action spectra of the PEP and generator potential. Solid line taken from Dartnall nomogram for pigment with maximum absorbancy at 530 nm. Open circles show the action spectrum of generator potential. Squares and triangles indicate the action spectra of the depolarizing and hyperpolarizing components, respectively, of the PEP.

are two components to the response and both components have action spectra which are the same as both the absorption spectrum of rhodopsin and the action spectrum of the generator potential.

We and others (*2, 3*) interpret the available evidence to indicate that the PEP and ERP represent an early, and perhaps direct, electronic change in rhodopsin molecules. Recent evidence suggests that the generation of the ERP is dependent on preservation of an ordered array of rhodopsin molecules (*3*). Moreover (*5*) the properties of the ventral-eye PEP, like those of the lateral-eye PEP (*1*), indicate that the PEP generator is an integral constituent of the photoreceptor membrane. Hence, the generators of the ERP and PEP may well be an ordered array of rhodopsin molecules lying in the cell membranes of photoreceptors.

Whether the PEP and ERP are in the direct chain of events leading to the permeability changes associated with the generator potential and ERG, respectively, and if so, how they are coupled to these permeability changes, remain open questions.

J. E. Brown
J. R. Murray
Departments of Biology and *Physics, Massachusetts Institute of Technology, Cambridge*

T. G. Smith
National Institutes of Health, Bethesda, Maryland

References and Notes

1. T. G. Smith and J. E. Brown, *Nature* **212**, 1217 (1966).
2. K. T. Brown and M. Murakami, *ibid.* **201**, 626 (1964); *ibid.* **204**, 739 (1964); R. A. Cone, *ibid.*, p. 736; W. L. Pak and R. A. Cone,

ibid., p. 836; W. L. Pak and T. G. Ebrey, *ibid.* **205**, 484 (1965); K. T. Brown, K. Watanabe, M. Murakami, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 457 (1965); R. A. Cone, *ibid.*, p. 483; W. L. Pak, *ibid.*, p. 493; K. T. Brown, *Nature* **207**: 1249 (1965); G. B. Arden and H. Ikeda, *ibid.* **208**, 1100 (1965); G. S. Brindley and A. R. Gardner-Medwin, *J. Physiol.* **182**, 185 (1966); K. T. Brown and P. W. Gage, *Fed. Proc.* **25**, 329 (1966); R. A. Cone, *Science* **155**, 1128 (1967).
3. R. A. Cone, *Science* **156**, 536 (1967).
4. R. Millecchia, *ibid.* **154**, 1199 (1966).
5. T. G. Smith and J. E. Brown, in preparation.
6. J. R. Murray, thesis, (1965). J.R.M. developed and built the laser used by us.
7. H. J. A. Dartnall, *Brit. Med. Bull.* **9**, 24 (1953).
8. G. C. Murray, *Science* **154**, 1182 (1966).
9. Partly supported by a Grass Foundation Fellowship to J.E.B., by grant 5 RO1 NB-06251-02, by USAF contract AF 33(615)-3885, by Bell Telephone Laboratories, Inc., and by ONR (Nonr 3962(22)). We thank Prof. Ali Javan for his kindness and assistance in the laser work. Part of the research was carried out at the Marine Biological Laboratory, Woods Hole, Mass.

10 August 1967

Chemical Modification of Yeast Alanine-tRNA with a Radioactive Carbodiimide

Abstract. *Yeast alanine-tRNA was reacted with 1-cyclohexyl-3-[2-morpholinyl-(4)-ethyl] carbodiimide ^{14}C-methoiodide in the presence of magnesium ion. The carbodiimide formed addition products with bases in the sequences ψpGp, UpCp and UpUpIpGpCp. The expected bases in the sequence TpψpCpGpApUp did not react, although this region is postulated to be in a loop that is not hydrogen-bonded. The capacity of the alanine-tRNA to accept amino acids decreased after reaction with the carbodiimide.*

Although the primary sequences of several transfer RNA molecules have been determined (*1*, *2*), the secondary and tertiary structures of these molecules and the relationship which these structures have to biological activity need further investigation. For this reason, a tRNA (*3*) of known sequence —yeast alanine-tRNA—was treated with 1-cyclohexyl-3-[2-morpholinyl-(4)-ethyl]carbodiimide methoiodide (CM CMI), a reagent which reacts with the bases Up, ψp, Gp, and Ip, but not with Ap and Cp (*4*). The reactivity of the CMCMI with these bases in the intact tRNA molecule depends upon the conformation in the presence of Mg^{++} (*5*). The reacted tRNA was digested with pancreatic ribonuclease, which normally cleaves polyribonucleotides at the 3′-phosphoryl bond of the pyrimidine bases, and ribonuclease T_1, which cleaves at the 3′ phosphoryl bond of the bases G and I; two-dimensional oligonucleotide patterns (fingerprints) were made of the digests. Since the CMCMI derivatives of bases are not susceptible to the action of pancreatic ribonuclease (*6*), the oligonucleotides containing CMCMI could be located by their new positions in the fingerprint as well as by their radioactivity. We also measured the effect which the chemical modification of these bases had on the ability of the tRNA to accept alanine.

The CMCMI was synthesized by treating excess 1-cyclohexyl-3-[2-morpholinyl-(4)-ethyl]carbodiimide (redistilled, Aldrich Chemical Co.) with ^{14}C-methyl iodide (New England Nuclear Corp.). The CMCMI was then crystallized from a mixture of acetone and ether. The purified alanine-tRNA was obtained by the method of Apgar *et al.* (*7*). The purity of the alanine-tRNA is thought to be greater than 80 percent (*8*).

In one series of experiments, mixed yeast tRNA (General Biochemicals Corp.) was treated with the radioactive CMCMI under the following conditions: 25 mg of CMCMI, and 2.5 mg of tRNA per milliliter in 0.01*M* borate, *p*H 8.0, and 0.02*M* $MgCl_2$. The reaction was stopped by precipitating the tRNA with two volumes of cold 95 percent ethanol.

To determine the quantity of ^{14}C-CMCMI attached to the tRNA, 20-μl portions of an aqueous solution of the precipitate were spotted onto washed Whatman DE 81 paper and eluted with *p*H 1.9 buffer (*9*) for 3 hours to remove excess reagent. The paper was dried; the spots were cut out, placed in scintillation vials, moistened with seven drops of NE219 liquid scintillator (Nuclear Enterprises), and counted on an Ansitron scintillation counter.

At 38°C (Fig. 1) nearly 2 moles of CMCMI attach per molecule of tRNA in the first 60 seconds. The reaction then slows down so that after 8 to 10 hours 6 to 7 moles of reagent have

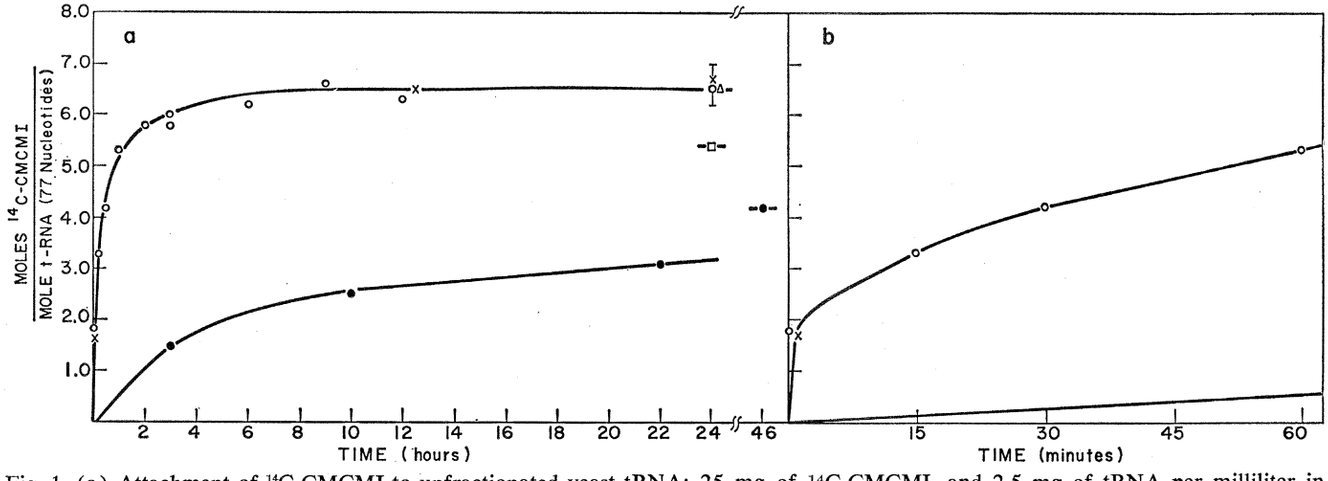

Fig. 1. (a) Attachment of ^{14}C-CMCMI to unfractionated yeast tRNA; 25 mg of ^{14}C-CMCMI, and 2.5 mg of tRNA per milliliter in 0.01*M* borate, *p*H 8.0, 0.02*M* $MgCl_2$. Solid circles, 2°C; open squares, 16°C; open triangles, 30°C; open circles 38°C. Attachment of ^{14}C-CMCMI to alanine-tRNA (conditions as above); crosses, 38°C. (The 24-hour point of alanine-tRNA is average of four experiments at 38°C, the range being indicated by the extent of the vertical lines.) (b) Expansion of the 0 to 1-hour segment of Fig. 1a.