BRIEF COMMUNICATION

VOLTAGE TRANSIENTS FROM PHOTO-ISOMERIZING AZO DYE IN BILAYER MEMBRANES

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ABSTRACT Voltage transients are induced by brief light flashes on bilayer membranes with absorbed 3,3'-bis(α-(trimethylammonium)methyl)azobenzene (Bis-Q). The voltages are positive for *trans*-to-*cis* photo-isomerization, and negative for *cis*-to-*trans* photo-isomerization. The risetimes in phosphatidylethanolamine-decane bilayer membranes indicate that absorbed *trans*-Bis-Q is photo-isomerized to *cis* within 2 μs, and that *cis* is photo-isomerized to *trans* within 15 μs.

INTRODUCTION

Photo-isomerizable agonist molecules have made kinetic studies of the opening and closing of acetylcholine receptor channels possible (1,2). Nass et al. (3) recently reported that channels in voltage-clamped *Electrophorus* electroplaque close within 100 μs after a light flash alters the potency of the agonist, 3,3'-bis (α-(trimethylammonium) methyl) azobenzene, or Bis-Q. The times required for absorbed Bis-Q to photo-isomerize and for the receptor channels to respond are not separately known.

Photo-voltages are induced across bilayer membranes by a number of electrically charged cyanine dyes (4,5), probably by physical movements of dye in the membrane's surface layer after photo-isomerization (5,6). Bis-Q has two stable isomeric forms (1–3), illustrated in Fig. 1. The data reported here indicate that (*a*) photo-isomerization causes Bis-Q to move within the surface layer of bilayer membranes, (*b*) absorbed *trans*-Bis-Q normally is located closer to the center of the membrane than is *cis*-Bis-Q, (*c*) absorbed *trans*-Bis-Q photo-isomerizes and moves to the *cis* position within 2 μs, and (*d*) absorbed *cis*-Bis-Q photo-isomerizes and moves to the *trans* position within 15 μs.

METHODS

Bilayer membranes were prepared by the syringe method using 10 mg lipid/ml decane. The lipids were phosphatidylethanolamine (P-3511, Sigma Chemical Co., St. Louis, Mo.), glycerol monoolein (4-4102, Supelco, Inc., Bellefonte, Pa.) and a mixture of air-oxidized cholesterol and phosphatidylcholine (5,6). All experiments were performed at 22 ± 1°C, in 0.01 M NaBr unless otherwise noted. Detailed procedures are described elsewhere (6,7). Light flashes of 9 ns duration were provided by a Molectron SP-10 tunable dye laser (Molectron Corp., Sunnyvale, Calif.). The laser with a UV beam extractor provided ~15 $\mu J/mm^2$ per flash of 337 nM light to the membrane, or with Molectron laser dye 70354-2 in *p*-dioxane, it provided ~10 $\mu J/mm^2$ per flash of 421 nM light.

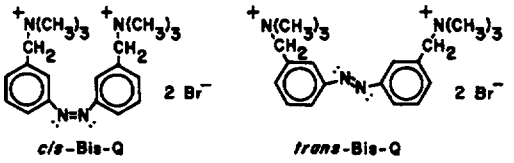

FIGURE 1 The two stable geometrical isomers of Bis-Q. Each isomer has several conformers.

Pure *trans*-Bis-Q was obtained from both Dr. Henry Lester and Dr. Norbert Wassermann and by synthesis using procedures previously described (1). Aqueous solutions of pure *trans*-Bis-Q were prepared in the dark just before use. Aqueous solutions containing *cis*-Bis-Q were prepared by illuminating Bis-Q solutions with 337 nM laser light (inside the laser cavity). Nuclear magnetic resonance integration (on a Hitachi Perkin Elmer R-24 spectrometer, Perkin Elmer Corp., Norwalk, Conn.) in D_2O indicated the *cis*-Bis-Q solutions were 94% *cis* and 6% *trans* (±4%). Measurements over 60 d indicate that ~1/2% of the *cis* isomerizes to *trans* per day during storage at ~22°C in D_2O in the dark. Aqueous *cis*-Bis-Q solutions were stored for 2–4 h in the dark until used. Bis-Q was added to solution on the positive electrode side of the bilayer membrane, after the membrane had stabilized, usually 5 min after it had become black.

RESULTS

Fig. 2 A illustrates the photo-voltage waveforms obtained from phosphatidylethanolamine membrane with single laser flashes, 1 mM *trans*-Bis-Q, and 337 nM light. The waveforms had a positive 0.15-mV amplitude, 2-μs risetime, and a falltime of approximately the membrane's RC time constant. The polarity is established by noting that the dye was added to the solution on the side of the membrane in contact with the positive electrometer electrode, and that moving a positive charge through the membrane toward the positive electrode creates a positive voltage change. Fig. 2 C illustrates the photo-voltage induced by 2 mM *cis*-Bis-Q and 421 nM light. Those waveforms had a negative 0.04-mV amplitude, 15-μs risetime, and a

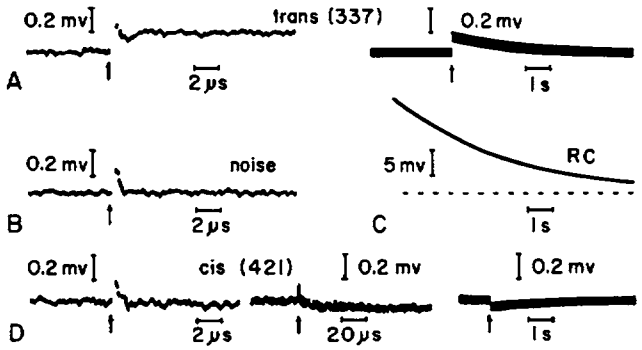

FIGURE 2 Photo-voltage waveforms induced by single laser flashes and phosphatidylethanolamine-decane bilayer membranes. (A) The wave-form induced by 1 mM pure *trans*-Bis-Q and 337 nM light, at 2 different oscilloscope sweep speeds. (B) The noise trace was recorded after rupture of the membrane used in A. Similar noise traces were recorded before forming the membrane and with a bilayer membrane present but before the Bis-Q addition. (C) The waveform resulting after application of a current pulse to the membrane through the 10^9 Ω shunt resistor. Similar time constants resulted from all bilayer membranes used in this work. (D) The waveforms induced by 2 mM *cis*-Bis-Q and 421 nM light, at 3 sweep speeds. Vertical arrows mark the time of the flashes.

falltime of the membranes' RC time constant. The photo-voltages obtained with 1 mM *trans*-Bis-Q and 421 nM light, and 2 mM *cis*-Bis-Q and 337 nM light were too small to be detected above the noise shown in Fig. 2 B.

The photo-voltage amplitudes were increased at reduced aqueous solution salt concentrations, and were reduced with increased salt concentrations, as has been observed with cyanine dyes (6). With phosphatidylethanolamine-decane bilayer membranes, 1 mM *trans*-Bis-Q, and 337-nM light flashes, the photo-voltage amplitudes were +0.2 mV in 1-mM NaBr solutions, and +0.02 mV in 0.1-M NaBr solutions. Unfortunately, reduced apparatus resolution occurs with lower aqueous solution conductance, as is discussed in detail elsewhere (7). The risetime limit imposed by the solution conductance and cell geometry used here was ~1 μs for 1-mM NaBr solutions and ~100 ns for 10^{-2}-M NaBr solutions.

Fig. 3 illustrates the waveforms obtained with multiple flash illumination. Turning the laser flasher on again after the membrane voltage had returned to the base line from the trace shown in Fig. 3 B (after a 13-s delay), for example, produced a voltage excursion of only +0.8 mV. Turning the flasher on again after a 5-min delay, however, produced a +2.3-mV excursion. The positive photo-voltage excursion obtained with *cis*-Bis-Q solution and the laser flashing at 337 nM, shown in Fig. 3 A, was increased to +1.0 mV by ~700 flashes of 421 nM light, provided the 337-nM flashes followed the 421-nM flashes within 30 s. 5 min after the 700 flashes of 421-nM light, however, 337-nM flashes would only reproduce the +0.2-mV excursion shown in Fig. 3 A.

Bis-Q solutions also induced photo-voltage transients across glycerol monoolein and oxidized cholesterol/phosphatidylcholine bilayer membranes. The amplitudes were a factor of 10 smaller for both *cis* and *trans*-Bis-Q in glycerol monoolein membranes, so risetime measurements were not possible. With oxidized cholesterol/phosphatidyl-choline membranes, the amplitudes induced by *trans*–Bis-Q and 337 nM light were several times smaller than in phosphatidylethanolamine membranes, whereas the photo-voltages induced by *cis*-Bis-Q and 421 nM light were comparable in amplitude and risetime to those induced in phosphatidyl-ethanolamine membranes. In all cases, converting absorbed *trans*-Bis-Q to *cis* with multiple 337-nM light flashes gave positive voltage transients, whereas converting absorbed *cis*-Bis-Q

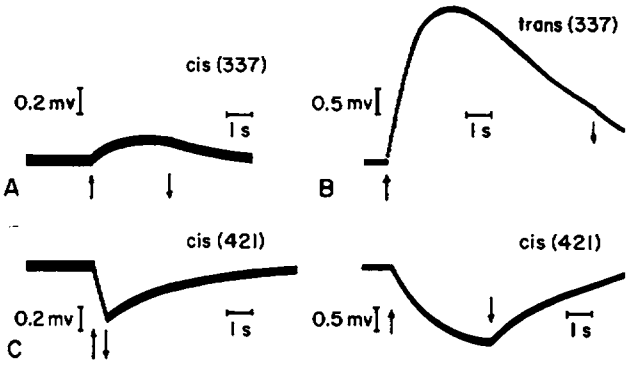

FIGURE 3 Photo-voltage waveforms induced by multiple laser flashes (34 flashes per s) and phosphatidylethanolamine-decane bilayer membranes with (A) 2 mM *cis*-Bis-Q and 337 nM light, (B) 1 mM *trans*-Bis-Q and 337 nM light, and (C) 2 mM *cis*-Bis-Q and 421 nM light. Vertical arrows mark the beginning (up) and end (down) of illumination.

to *trans* with 421-nM light flashes gave negative voltage transients, both of which fell with approximately the membranes' RC time constant.

The stability of phosphatidylethanolamine bilayer membranes was not modified by 1 mM *trans*-Bis-Q. With 2 mM *cis*-Bis-Q, however, the bilayer membranes typically ruptured within 10 min after the dye was added.

DISCUSSION

The voltage transients described here can be explained by assuming that (*a*) both isomers of Bis-Q absorb into the membrane's surface layer, (*b*) the equilibrium position of absorbed *trans*-Bis-Q is closer to the center of the membrane than is the equilibrium position of absorbed *cis*-Bis-Q, and (*c*) absorbed Bis-Q changes position in the membrane after photo-isomerization, Point *b* is consistent with the structures shown in Fig. 1, in that the *cis* isomer has a higher charge density than *trans*. When absorbed *cis* photo-isomerizes to *trans* it moves to the *trans* position producing a charge movement into the membrane (away from the positive electrode), resulting in a negative voltage transient. When absorbed *trans* isomerizes to *cis*, it moves out to the *cis* position, producing a positive voltage transient. Electrical equivalent circuits and voltage waveforms for such charge movements have been presented by Huebner et al. (5,6). Because both isomers are stable on the time scale of these experiments the photo-voltages are expected to decay with the membranes' RC time constants.

Multiple flashes are expected to summate these events. For example, Fig. 3 B indicates that ~2 s (or ~70 flashes) are required to convert all of the absorbed *trans*-Bis-Q to *cis*. A few seconds later, additional 337-nM flashes produced only a small voltage excursion, whereas 5 min later, a much larger voltage excursion resulted. Apparently, additional *trans*-Bis-Q can be absorbed from the solution in 5 min. The fact that the *cis*-to-*trans* photo-voltage risetime was much faster than the time required for additional *trans* to absorb from solution indicates that absorbed *cis* was responsible for the *cis*-to-*trans* photo-voltage, rather than the photo-voltage being the result of the photo-isomerization of dye in solution. This is consistent with the fact that phosphatidylethanolamine bilayer membranes had a reduced stability in the presence of *cis*-Bis-Q. Apparently absorbed *cis*-Bis-Q destabilized these membranes.

The reduction in photo-voltage amplitude upon increasing the salt concentration from 0.01 to 0.1 M NaBr (where the Debye lengths are ~30 and ~10 A, respectively) is consistent with the view that absorbed Bis-Q is normally 10 A or so out from the hydrocarbon membrane core. The smallest amplitude for glycerol monoolein membranes may result from the smallest lipid head group size, or from the absence of phosphate groups. The position normally occupied by azo dyes in membranes may vary with dye structure, as has been observed with cyanine dyes (5). The synthesis of additional azo dye structures, which is in progress, may make it possible to probe various parts of the membrane surface. At present, it is clear that these procedures make possible kinetic studies of intermolecular rearrangements in membranes, and provide an upper limit for the time required for photo-isomerization of the two isomers of Bis-Q in bilayer membranes.

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