R. japonicum (strains 3I1b138, 505W, and 61A72) was evaluated by use of greenhouse-grown plants potted in washed sand. Each *Rhizobium* strain produced numerous healthy-appearing pink nodules on all six soybean lines within 3 weeks of inoculation. Thus soybean lines whose seeds lack SBL retain their ability to be nodulated by symbiotic *R. japonicum*.

The remaining unanswered question for symbiosis is whether lines of *G. max* whose seed lack SBL are also without the lectin in their roots. Seedling roots of the line Beeson contain small amounts of SBL, but the lectin decreases to an undetectable level when the cotyledons abscise, about 2 weeks after planting (*11*). The origin of the root SBL in seedlings is uncertain, but it probably represents mobilized reserve SBL that had been stored in the cotyledons. If the genes controlling the absence of SBL in seeds of the five lines are structural, it follows that SBL is not involved in recognition of rhizobial symbionts by *G. max*. If, on the other hand, the genes are regulatory genes that control the levels of SBL in soybean tissues, it is plausible that SBL could be present in the roots of the five lines. Both possibilities, as they relate to soybean-*Rhizobium* symbiosis, should be examined.

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14. Each extract was diluted in twofold increments to a final dilution of 1:2048 in (i) PBS and (ii) in PBS containing 5 m*M* *N*-acetyl-D-galactosamine. Portions of each dilution (25 μl) were transferred to wells in a microtiter plate (Abbott Laboratories), and 25 μl of a 3 percent suspension of trypsinized type O human erythrocytes were added to each well. The following titers were recorded for these lines of *G. max* after 3 hours at room temperature: Ada, 32; Amsoy, 32; Chippewa, 64; Columbia, 0; Early White Eyebrow, 8; Fabulin, 32; Flambeau, 32; Funk Delicious, 64; Kanro, 32; Kent, 64; Kim, 16; Kura, 64; Manitoba Brown, 64; Norredo, 0; Peking, 64; Sato, 64; Scott, 64; Sooty, 0; T102, 0; Wea, 64; Wilson, 128; Wilson-5, 0; Wilson-6, 64; Wisconsin Black, 32; and Yellow Marvel, 16. There was no agglutination in any well containing *N*-acetylgalactosamine.
15. Purified SBL and dialyzed protein extracts were labeled with fluorescein isothiocyanate by the method of S. Udenfriend [*Fluorescence Assay in Biology and Medicine* (Academic Press, New York, 1962), p. 221]. Details of the culture method for rhizobia and the assay procedure are in T. V. Bhuvaneswari *et al.* [*Plant Physiol.* **60**, 486 (1977)]. *Rhizobium japonicum* (strains 3I1b38, 3I1b138, and 3I1b143) bound both fluorescent-labeled SBL and the 20 fluorescent-labeled control extracts, but did not bind fluorescent-labeled extracts of the five lines. *Rhizobium japonicum* (strains 505W and 61A72) and *Rhizobium* sp. (strains 3G4b10 and 3G4b19) did not bind SBL or any extract.
16. For the procedure for protein extraction from soybean seed and for staining and destaining methods, see T. Hymowitz and H. H. Hadley [*Crop Sci.* **12**, 197 (1972)]. The 10 percent polyacrylamide gels were made by using the catalyst system of E. M. Jordan and S. Raymond [*Anal. Biochem.* **27**, 205 (1969)] and a gel buffer of 0.25*M* tris adjusted to *p*H 2.4 with citric acid. The electrode buffer was 0.37*M* glycine adjusted to *p*H 3.7 with citric acid. The lysozyme (muramidase) was purchased from Sigma Chemical Company.
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Subpicosecond Spectroscopy of Bacteriorhodopsin

Abstract. *Subpicosecond pulses have been used to study the ultrafast dynamics of the photochemistry of bacteriorhodopsin. An optically induced absorption that appears in about 1.0 picosecond at physiological temperatures has been resolved in time. The data can be interpreted in terms of the photochemical formation of bathobacteriorhodopsin and provide support for an excitation mechanism involving molecular rearrangement in the protein induced by electron redistribution in the chromophore.*

Newly developed techniques for the generation and utilization of subpicosecond optical pulses (*1*) have been applied to the study of systems ranging from relatively simple organic molecules (*2–4*) to complex proteins (*5*). We have employed these techniques to study the ultrafast photochemistry of bacteriorhodopsin at physiological temperatures. Bacteriorhodopsin is of interest because of its similarities to visual pigment rhodopsins and its biological role as an energy converter. Time-resolved studies play an important role in distinguishing between different types of molecular transformations involved in its photochemistry.

Bacteriorhodopsin develops in the plasma membrane of *Halobacterium halobium* when this bacterium is grown in light and at low oxygen tension (*6*). It acts as a light-driven proton pump (*7*), converting light energy into chemical energy and subsequently into a proton gradient across a cell membrane. This gradient is then used to generate adenosine triphosphate, in agreement with the Mitchell hypothesis (*8*).

The chromophore in both rhodopsin and bacteriorhodopsin is retinal, complexed to an ϵ-amino group of the lysine residue (*6*) through a protonated Schiff base linkage (*9*). It has been shown that light absorption in both these pigments produces a new species, called the batho intermediate, which has an absorption maximum ~ 40 to 45 nm to the red of that of the rhodopsin from which it is produced. The batho intermediate then thermally relaxes to a species with an absorption that is once again similar to that of the parent pigment. These similarities may indicate that the primary mechanism of light excitation is the same in both rhodopsin and bacteriorhodopsin.

Figure 1 shows the photochemical cycle of light-adapted bacteriorhodopsin as outlined by other workers (*10*). We are interested in the dynamics of the primary step: bacteriorhodopsin $bR_{570} \rightarrow$ bathobacteriorhodopsin K_{610} at physiological temperatures. Previous studies (*11, 12*) indicated very rapid ($\leq$ 6 psec) formation of the batho species at higher energy (*13*). The results (*11, 12*) were limited in resolution by the duration of the excitation and probing pulses. In the work reported here we were able to resolve the formation time of K_{610} by using subpicosecond optical pulses.

The experimental apparatus we used has been described (*1, 4*). The pulse train consists of subpicosecond optical pulses at 615 mm, near the absorption maximum of K_{610}, from a passively modelocked continuous-wave dye laser. The pulses are obtained by acousto-optic dumping from the laser resonator at a repetition rate of 10^5 per second. After compression with a grating pair, the puls-

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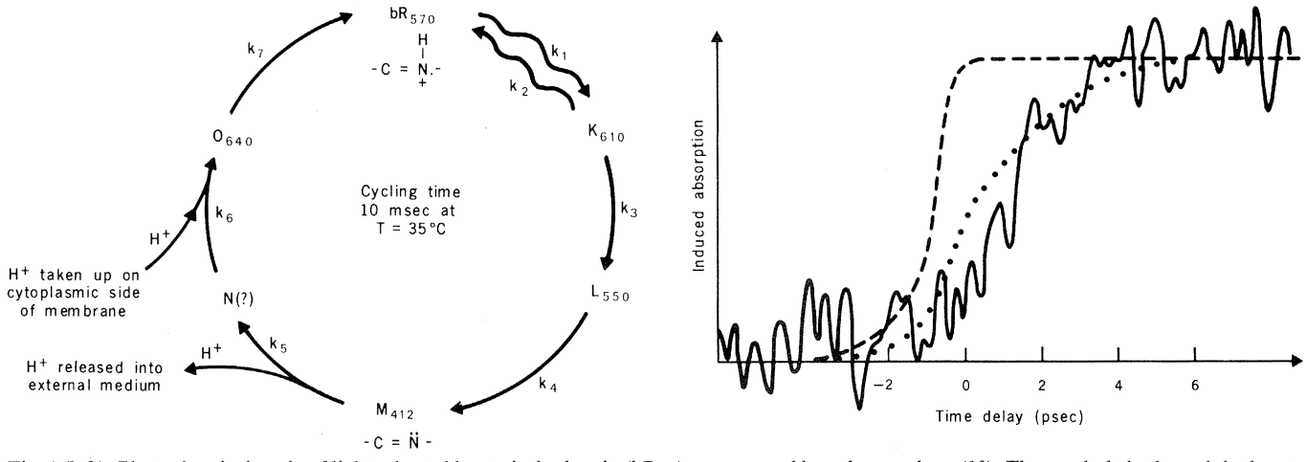

Fig. 1 (left). Photochemical cycle of light-adapted bacteriorhodopsin (bR_{570}) as proposed by other workers (*10*). The symbols k_1 through k_7 denote rate constants; K, L, M, N, O, and bR refer to distinct molecular species, and the subscripts indicate their respective absorption maxima. The state of protonation of the Schiff base has been determined by resonance Raman spectroscopy. Fig. 2 (right). Induced absorption at 615 nm plotted against the delay between the probe and excitation pulses. (Dashed curve) Experimentally determined instantaneous response of the system; (dotted curve) computed response for a 1.0-psec exponential rise.

es have a duration of 0.5 psec each (full width at half-maximum) and an energy of 5×10^{-9} J. The pulse train is split into two beams at the beam splitter, with the probe beam being weaker by at least a factor of 10. A digitally controlled, stepper motor–driven, translation stage is used to vary the time delay between the probe pulses and the stronger excitation pulses. The pump (excitation) beam and the probe beam are focused by a lens to the same 10-μm spot in a thin sample cell containing a suspension of membrane fragments with $7.5 \times 10^{-4}M$ bacteriorhodopsin. The sample was flowed through this thin cell to remove the photochemical products from the laser beam before the next pump and probe pulses reached the sample. A similar flowing sample was used in a previous picosecond experiment to measure the lifetime (*14*) of the emission observed at room temperature in purple membrane (*15*). A light source was used to illuminate the sample reservoir to ensure that all bacteriorhodopsin molecules were in the light-adapted bR_{570} form. At a sample concentration of $7.5 \times 10^{-4}M$ about 50 percent of the light is absorbed in a 0.1-mm path length. After passage through the flow cell the pump beam is blocked, and the probe beam passes to a photomultiplier tube. Modulation of the probe beam by the chopped pump is detected with a lock-in amplifier whose output is recorded as a function of delay time in the multichannel analyzer. Averaging is achieved by repetitive scanning of the desired delay interval. In these experiments averaging was performed over periods of 5 to 10 hours to obtain the time-resolved response of bathobacteriorhodopsin. During this time the laser pulses themselves change in duration by less than 0.1 psec. Figure 2 shows the induced absorption at 615 nm as a function of relative-time delay between the excitation and probing pulses for a suspension of membrane fragments with $7.5 \times 10^{-4}M$ bacteriorhodopsin in doubly distilled water, *p*H 6.6. An increase in absorption is observed after the excitation pulse, as indicated by the solid experimental curve. The maximum change is less than 1 percent.

The dashed curve in Fig. 2 is the instantaneous response of the measurement system. It is obtained from autocorrelation measurements of the pulses used in the experiment and includes the appropriate coherence contribution (*1*). Note that the coherence between pump and probe causes the predicted response for an instantaneous process to reach its full value at zero delay. The dotted line in Fig. 2 is the calculated response of the system to a simple 1.0-psec exponential rise. The actual signal appears to deviate slightly from this simple curve near zero delay. This may be due to contributions from depletion of the original ground state and from excited state absorption. Nevertheless, the time constant with which the system approaches its final level falls in the range 1.0 ± 0.5 psec.

We suggest that the induced absorption that we observe is due to the formation of the first photochemical intermediate in the bacteriorhodopsin photocycle (K_{610}). In agreement with this suggestion is our observation that the induced absorption remains constant for the 50-psec delay interval of our experiments. This is to be expected since K_{610} has a lifetime of about 2 μsec at room temperature (~ 20°C) (*10*). The induced absorption observed at 635 nm and assigned to the batho state by Kaufmann *et al.* (*11*) occurred in < 6 psec and remained constant for > 300 psec. At 615 nm we did not observe the additional 15-psec transient detected by Kaufmann *et al.* at 580 nm. It seems plausible that at 635 and 615 nm the response is dominated by the increased absorption of bathobacteriorhodopsin, whereas at 580 nm the signal is also due to depletion and partial recovery of the ground state. Pertinent to this point are recent measurements (*16*) indicating that the photochemical quantum yield of $bR_{570} \rightarrow K_{610}$ is only 0.3.

It is interesting to compare our observed formation time of bathobacteriorhodopsin with the lifetime at room temperature (*14*) of the near-infrared emission observed by Lewis *et al.* (*15*). Using an emission-gating technique, Hirsch *et al.* (*14*) measured an emission lifetime at room temperature at 780 nm of 15 ± 3 psec, which Alfano *et al.* (*17*) had previously been unable to resolve. The onset of fluorescence was instantaneous within their experimental resolutions (*17*). With the results reported here it is apparent that photochemical formation of bathobacteriorhodopsin and relaxation to such an emitting state would have to occur along different, albeit competing, pathways.

Now that the formation time of a batho intermediate has been resolved at physiological temperatures, one wonders what photon-induced molecular rearrangement can occur in 1.0 ± 0.5 psec and also store a considerable fraction of the photon energy (*13*). There have been at least three suggestions for this molecular rearrangement: (i) isomerization

(*18, 19*), (ii) a change in the state of protonation of the Schiff base (*20*), and (iii) a molecular rearrangement in the protein induced by electron redistribution in the chromophore (*21*).

Isomerization is an unlikely candidate in our case. Not only is the bacteriorhodopsin chromophore initially all *trans*, but 13-*cis* is the only other isomer that can combine with the bacteriorhodopsin protein matrix, and it leads to a blue shift of the absorption maximum relative to bR_{570} (*22*). Time-resolved measurements also indicate that such molecular rearrangements occur on a slower time scale (*23, 24*).

The second suggestion can be discounted on the basis of experiments by Marcus and Lewis (*25*). With kinetic resonance Raman studies they demonstrated that the state of protonation of the Schiff base changes on a time scale of 10 to 30 μsec following photoexcitation. This is certainly too slow for the intermediate formation we observed.

Our results are consistent with the third possibility, proposed by Lewis (*21*). In this proposal, light absorption results in a significant electron redistribution in the retinylidene chromophore (*26*). This, in turn, induces a protein conformational transition. One possibility might be proton movement from one amino acid to another. Such a rearrangement could introduce the appropriate absorption changes (*26, 27*), would be sensitive to deuteration (*20*), and could also excite the proton translocating function of this biological light-driven proton pump.

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Thrombin-Induced Platelet Aggregation Is Mediated by a Platelet Plasma Membrane-Bound Lectin

Abstract. *Thrombin-activated human platelets cause agglutination of trypsinized, formalinized bovine erythrocytes. This lectin activity of stimulated platelets was blocked by galactosamine, glucosamine, mannosamine, lysine, and arginine, but not by N-acetylated sugars, other neutral sugars, or other amino acids. Inhibitors of the thrombin-induced lectin activity also blocked thrombin-induced platelet aggregation. It appears that a membrane surface component that has lectin activity mediates platelet aggregation.*

The mechanisms underlying membrane-membrane interaction are important for understanding many problems that are central to cell and developmental biology, such as differentiation, cell aggregation, viral infectivity, and fertilization. The systems that have been useful in identifying membrane components responsible for direct cell-cell interaction involve experimental manipulation of the membrane surface. Starvation of slime mold amoebas causes an alteration of the membrane surface with a concomitant increase in cell-cell interaction (*1, 2*); similarly, differentiation of myoblasts appears to alter the cell membrane to facilitate cell fusion (*3, 4*). In these examples, the membrane surface components implicated in cell-cell interaction have lectin activity; that is, they can agglutinate trypsinized, formalinized erythrocytes. In the lectin model of cell-cell interaction (*5*), cell contact is mediated by a lectin molecule on one cell that specifically attaches to receptors on an adjacent cell.

Platelet aggregation is also a process that involves interaction of membrane surfaces. This process causes the primary arrest of blood flow (hemostasis) and the formation of arterial thrombi (thrombosis). The most potent physiological agent for inducing platelet aggregation is thrombin. Thrombin activation of platelets causes a change in platelet shape and an alteration in the surface membrane, resulting in the platelets becoming cohesive and aggregated (*6*).

We have previously reported that platelet plasma membranes have lectin activity (*7*). We now report that thrombin stimulation of intact cells causes the expression of a lectin activity not seen with unstimulated cells. This activity appears to mediate at least the initial stages of platelet aggregation.

The lectin activity of intact, washed human platelets was measured by their ability to agglutinate trypsinized, formalinized erythrocytes from different species. The erythrocytes had no lectin activity, but as the source of receptors could be cross-linked by appropriate lectins. In this assay, washed platelets agglutinated erythrocytes from species such as cow, electric eel, rabbit, catfish, and sheep.

After stimulation by the thrombin, platelets had enhanced lectin activity (Fig. 1). Unlike the activity of unstimulated platelets that caused the agglutination of erythrocytes from many species, the enhanced lectin activity was detected only with cow or sheep erythrocytes and not with rabbit erythrocytes as a source of receptor. This erythrocyte species specificity is similar to that seen in the lectin activity of isolated platelet

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