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THE ROLE OF WATER ON PHOTOCHEMICAL ACTIVITIES OF MEMBRANE PROTEIN COMPLEXES OF BACTERIA

BY

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ABSTRACT

The role of water on photochemical activities of membrane complexes of bacteria. - Cells of purple bacteria (*Rhodospirillum rubrum*) transferred into HTS (Hexane, Tween and Span)- reverse micelles displayed the primary photochemical reactions of photosynthesis measured as fast bacterichlorophyll (BChl) fluorescence induction kinetics and photooxidation of P800 (measured as light induced absorption changes at 820 nm). The cells in HTS-reverse micelles showed water dependent increases in variable fluorescence for extended periods of time. Cells in HTS-reverse micelles without water totally lost the variable fluorescence after 24 h, while in the presence of 6% water they behaved almost like the normal cells maintaining their activity even after 24 hr. ^{1}H-Nuclear-Magnetic-Resonance (^{1}H-NMR) studies shows that the bound/free water ratio decreased with increasing concentration of water in HTS-reverse micelles.

The obtained results, altogether, clearly demonstrate that the amount and physical state of water determine the primary photosynthetic activity of bacteria in HTS-reverse micelles.

Key-words: Bacteriochlorophyll, ^{1}H-NMR, reverse micelles.

INTRODUCTION

Water plays a fundamental role in determining the reaction rates and the thermodynamics of biological systems. Cellular organization, from macromolecular assembly to enzyme catalysis, depends on water, although exactly how is not known. Reverse micelles or water in oil microemulsions, offer the possibility of modulating the amount of water to which enzymes and multienzymatic complexes are exposed.

Reverse micelles are formed in apolar solvents with synthetic detergents (or natural surfactants like phospholipids) with or without a cosurfactant (WOLF & LUISI, 1979; MENGER & YAMADA, 1979; MARTINEK *et al.*, 1986; DARSZON & SHOSHANI, 1992). Many enzymes entrapped in reverse micelles exhibit catalytic activity which is strongly affected

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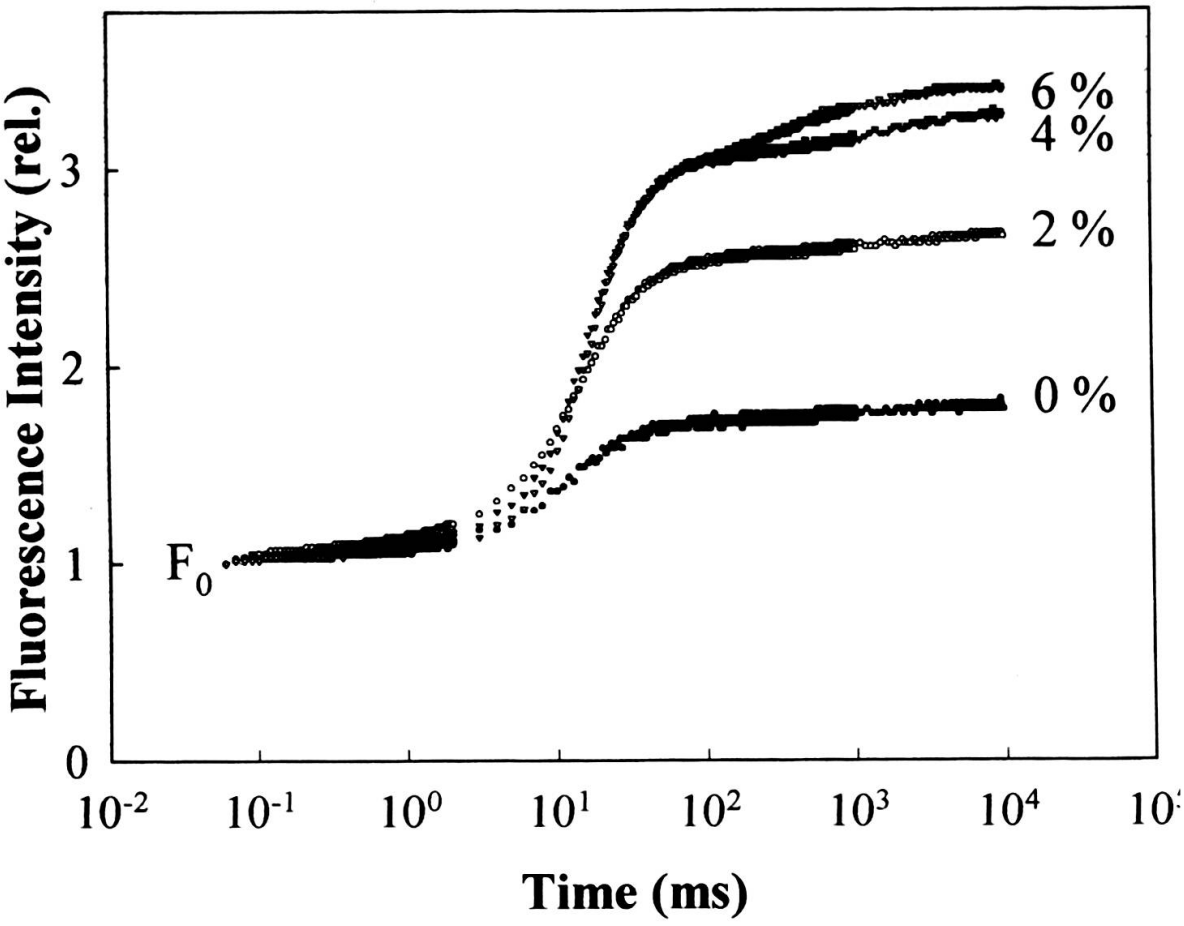

FIG. 1.

Effect of different amount of water on the BChl fluorescence induction kinetics. Water concentration in the HTS-reverse micelles were pre-adjusted and then cells were added. Measurements were done after 1 hour of incubation as described in material and method.

by the ratio of water to surfactant molecules (MENGER & YAMADA, 1979; BARBARIC & LUISI, 1981; GOMEZ-PUYOU, 1992). At low water content enzyme activity is significantly lower than in aqueous media, and the activity increases as the amount of micellar water is raised (LUISI *et al.*, 1988; GARZA-RAMOS *et al.*, 1992).

Many enzymes become very thermostable in reverse micelles, and more resistant to denaturants (GARZA-RAMOS *et al.*, 1992; SHOSHANI *et al.*, 1994). Under low water conditions some enzymes can perform reactions that are difficult in all water media. Both polar and apolar substrates can be used in reverse micelles, which are even able to house organelles and whole cells (reviewed in PFAMMATTER *et al.*, 1992; ESCAMILLA *et al.*, 1992). Thus, reverse micelles represent an attractive tool to probe the role of water in protein structure and function (LUISI *et al.*, 1988, DARSZON & SHOSHANI, 1992; GARZA-RAMOS *et al.*, 1992).

Most of the work in reverse micelles has focused on water-soluble proteins. However, a few membrane proteins have been transferred into ternary systems (for

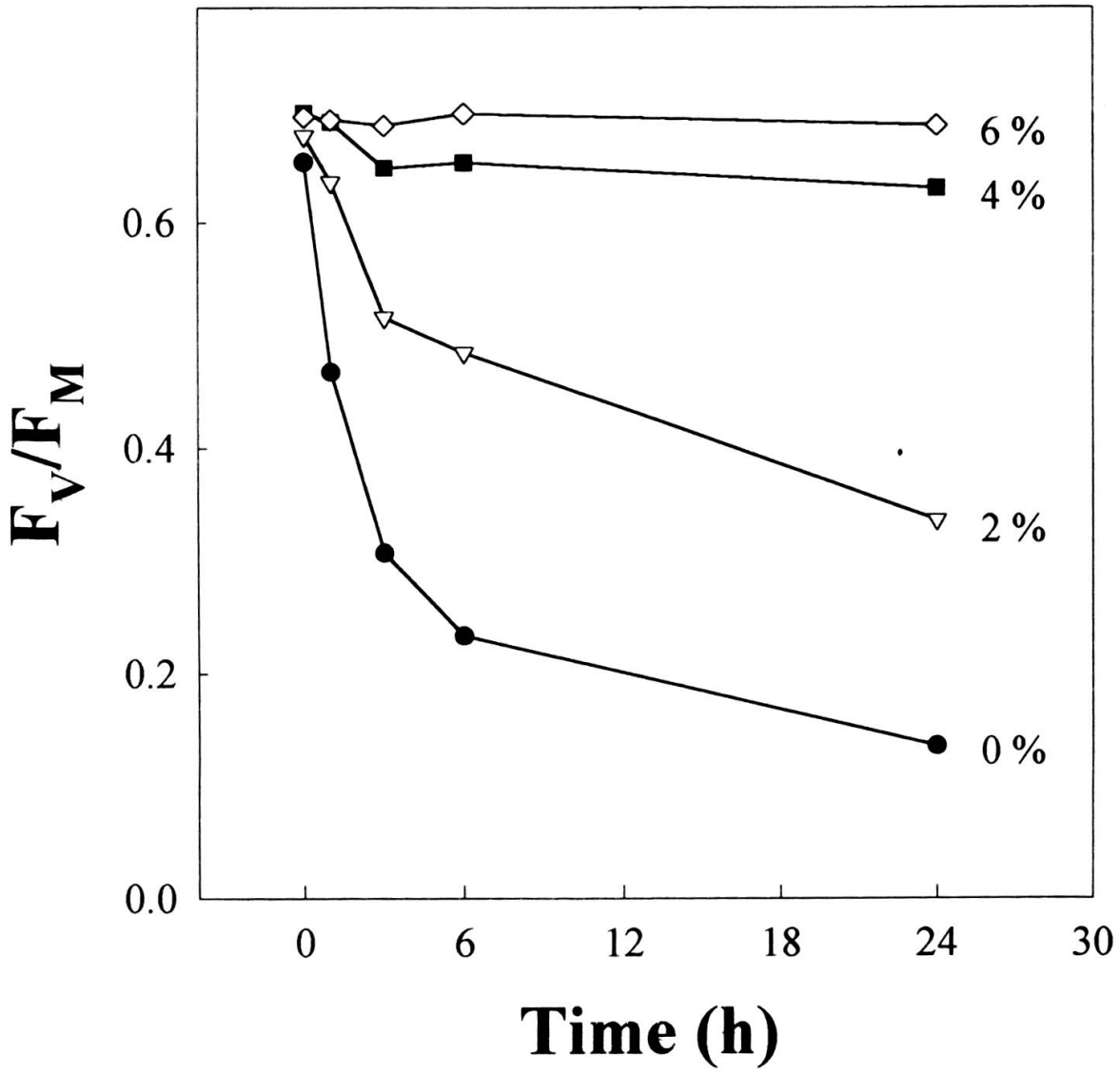

FIG. 2.

Effect of water on the maximum quantum yield of primary photochemical activity (F_V/F_M) of bacterial cells incubated in HTS-reverse micelles for different time.

review see DARSZON & SHOSHANI, 1992). Membrane proteins in reverse micelles have been used as building blocks for model membranes (MONTAL *et al.*, 1981), and to estimate the functional state of certain enzymes as they may exist in the inner mitochondrial membrane (DARSZON & GOMEZ-PUYOU, 1982; SANCHEZ-BUSTAMANTE *et al.*, 1982). Some membrane bound enzymes also exhibit a striking thermostability in ternary systems (AYALA *et al.*, 1986). Activity studies in reverse micelles have been reported for: rhodopsin (DARSZON *et al.*, 1979), *Rodospirillum sphaeroides* reaction centers (SCHOENFELD *et al.*, 1980), succinate dehydrogenase (AYALA *et al.*, 1985), cytochrome oxidase (ESCAMILLA *et al.*, 1989), the ATPase from mitochondria (GARZA-RAMOS *et al.*, 1990, 1992) and chloroplast (KJERNEN *et al.*, 1997). Although *R. sphaeroides* reaction

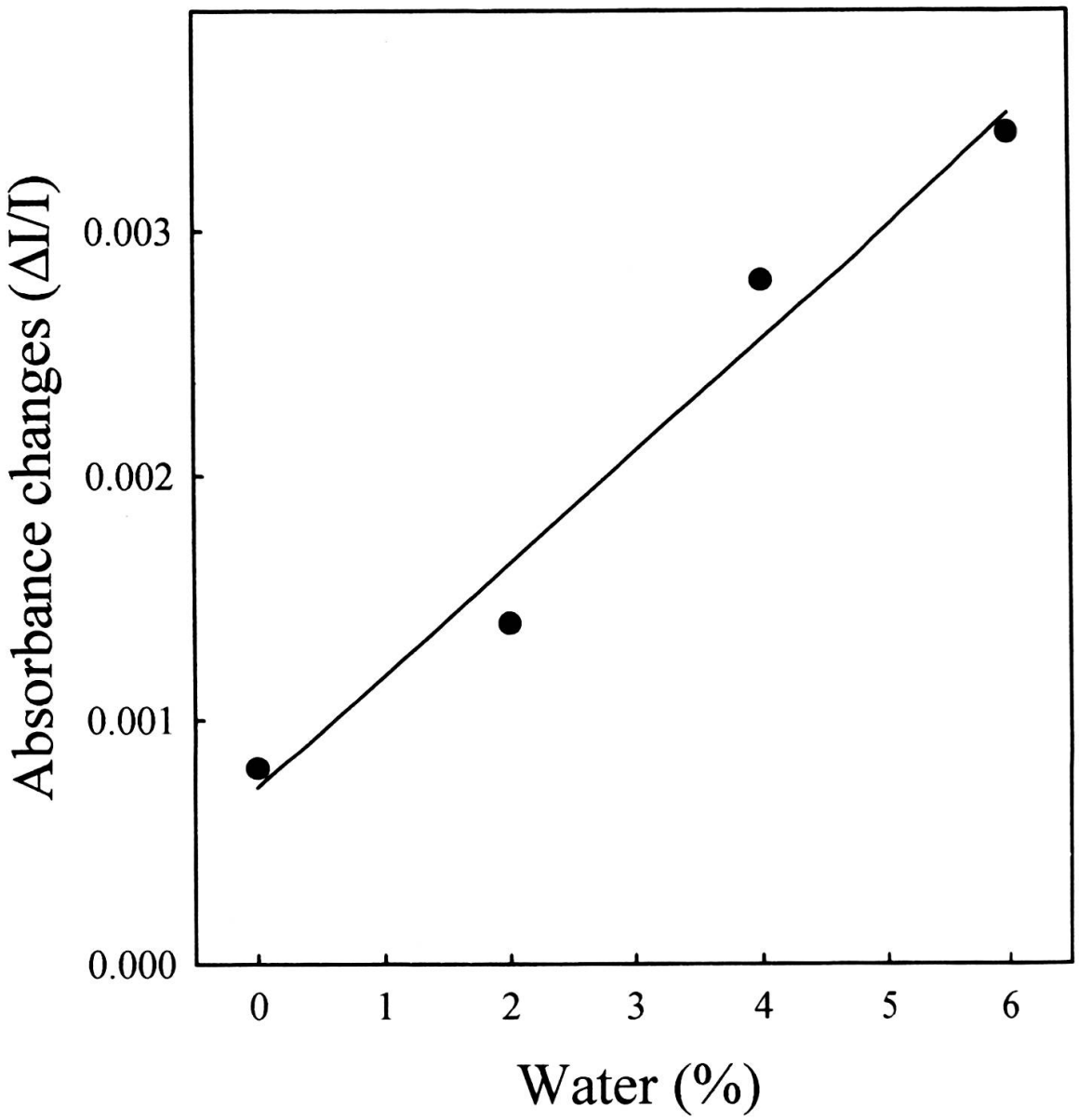

FIG. 3.

Effect of water on photooxidation of the reaction center, P, measured as light induced absorption changes after 1 hr of incubation in HTS-reverse micelles with different amount of water.

centers were studied in phospholipid reverse micelles, the water dependence of their activity, and stability were not characterized (SCHOENFELD *et al.*, 1980). Furthermore their variable bacterial chlorophyll fluorescence was not studied.

Considering the above stated information, we have used anoxygenic purple bacterial cells, *Rhodospirillum rubrum* in HTS-reverse micelles to understand the effect of water on photosynthetic activity and stability. Despite some basic differences in the structure and function of the purple bacteria, the general principles of energy transduction are the same in anoxygenic and oxygenic photosynthesis.

Chlorophyll *a* fluorescence kinetics of higher plants, measured over many orders of magnitude in time, provide an experimental approach to visualize and to analyze PSII reactions leading to the filling up of the PQ pool (GOVINDJEE, 1995; STRASSER *et al.*, 1995). It has been used extensively as a sensitive tool to investigate the photosynthetic apparatus *in vivo* under physiological conditions (STRASSER *et al.*, 1999). In this paper bacterial chlorophyll (BChl) fluorescence has been used as a tool to monitor the rate of primary photochemistry.

We have shown clearly that water affects the rate of primary photochemistry of purple bacteria, *Rhodospirillum rubrum*. The NMR studies indicate that the photosynthetic activity is affected by the availability of water in reverse micelles. We have also shown that the bacterial cells could be kept viable for many hours in HTS-reverse micelles. The availability of stable bacterial system in reverse micelles open interesting new perspective in biotechnology.

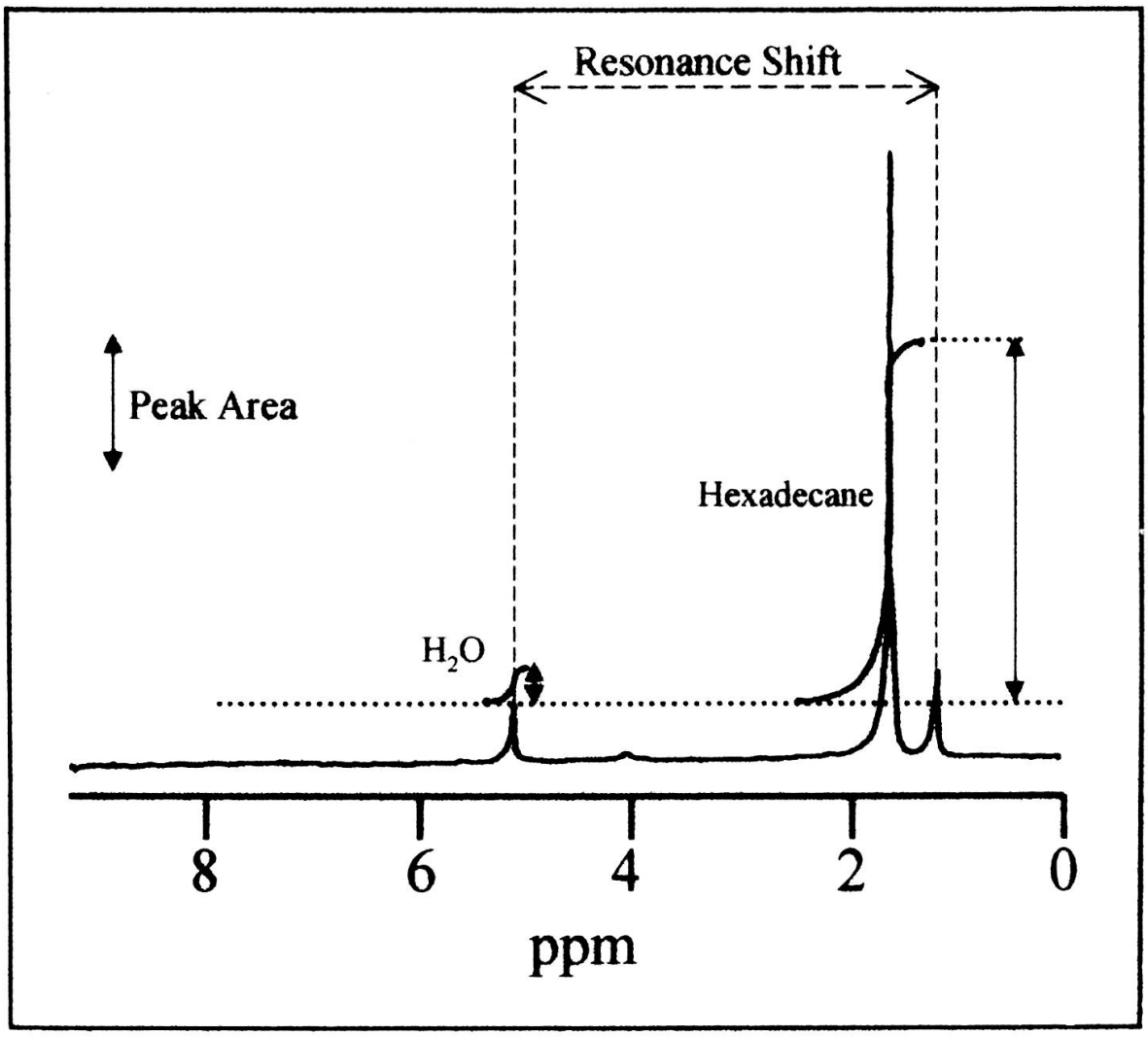

FIG. 4.

Original ^{1}H-NMR traces showing the water and hexadecane peaks (solid lines). The peak distance (dashed line) indicate the proton resonance shift of H-O-H from the main -CH_3 resonance band.

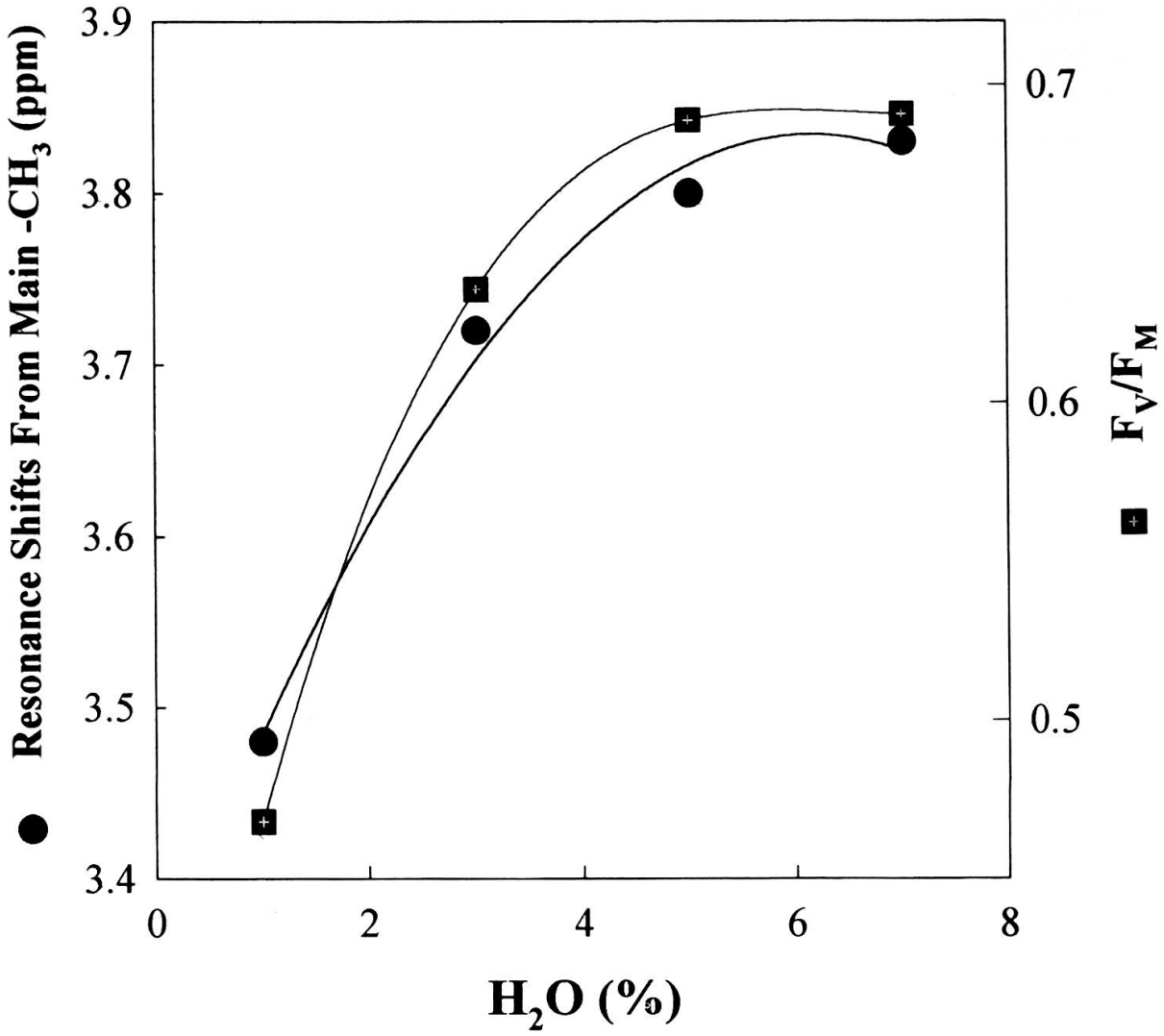

FIG. 5.

Effect of water content in HTS-reverse micelles on water resonance shifts (circles) and maximum quantum yield of primary photochemical activity (square) of bacteria cells.

MATERIAL AND METHODS

Wild type cells of *Rhodospirillum rubrum* were grown anaerobically in Sistrom medium (SISTROM, 1960) photo-heterotrophically with succinate as carbon source as described earlier (GHOSH *et al.*, 1994).

Bacterial chlorophyll (BChl) fluorescence induction kinetics were measured with a fluorometer, Plant Efficiency Analyzer (PEA, Hansatech Ltd., King's Lynn, Norfolk, England) with 650 nm of 600 Wm^{-2} light intensity. Light was provided by an array of 6 LED (peak, 650 nm), focused on to the sample surface to provide a homogeneous illumination over the exposed area (4 mm diameter). BChl fluorescence signals were detected using a PIN photocell after passing through a 890 nm filter (Kodak 87C).

To detect the primary photochemistry of the reaction center, changes in the absorption at 820 nm were measured by combining the Hansatech $P700^+$ measuring system with PEA. This was done by replacing one of the red LED (out of 6) from the PEA head with a broad far red LED, which was covered by an interference filter (peak 820 nm). Measuring beam was provided through this far red LED modulated with 4 KHz. The transmitted light was monitored on opposite side of the leaf by a photodiode screened as well by a 820 nm interference filter. Actinic light was provided by red LEDs through PEA. The signal from the detector was recorded with a chart recorder and simultaneously digitized and stored with a resolution of 12 bit in a computer.

RESULT AND DISCUSSION

Effect of Water on the BChl Fluorescence Transient of R. rubrum

R. rubrum suspended in buffer showed a fast BChl fluorescence induction when exposed to actinic light. The fluorescence signal starts from initial level (F_0) and reaches to its maximum level (F_M) within 4-5 sec. Cells incubated in HTS-reverse micellar system without water lost some of the variable fluorescence ($F_V = F_M-F_0$) within 1 hr and almost totally lost their activity after 24 hrs of incubation (see Fig. 1 and 2). The kinetics of the effect of water content in HTS-reverse micelles on maximum quantum yield of primary photochemical activity, F_V/F_M ratio is shown in more detail in Fig. 2. In contrast, the cells incubated in HTS-reverse micelles containing 6% water behaved almost like the normal cells. The photochemical activity was totally preserved; no difference in the fluorescence induction kinetics were observed when the cells incubated in HTS-reverse micelles with 6% water were exposed to light for several times at an interval of 10 sec (data not shown). These results clearly demonstrate that water plays an important role for the primary photochemistry of bacterial cells.

Effect of Water on Light Induced Absorption Changes at 820 nm

Like BChl fluorescence induction kinetics, cells incubated in HTS-reverse micelles showed a clear effect of water on photooxidation of the reaction center, P, measured as absorption changes at 820 nm (Fig. 3). A linear increase in the rate of photooxidation of P was observed by increasing the water content in the HTS-reverse micelles. This observation further confirms that water plays an important role for primary photochemical activities.

Behavior of Water in HTS-reverse Micelles

The concentration of water was monitored in HTS-reverse micelles using NMR. Water can be easily quantified using this technique which also allows an estimation of the bound to free water ratio. The integral of the water peak area at about 5 ppm was normalized with the integral peak for Haxadecane at 1.6 ppm (Fig. 4). This gives the quantity of water in the system. However, the water resonance shift (measured from the

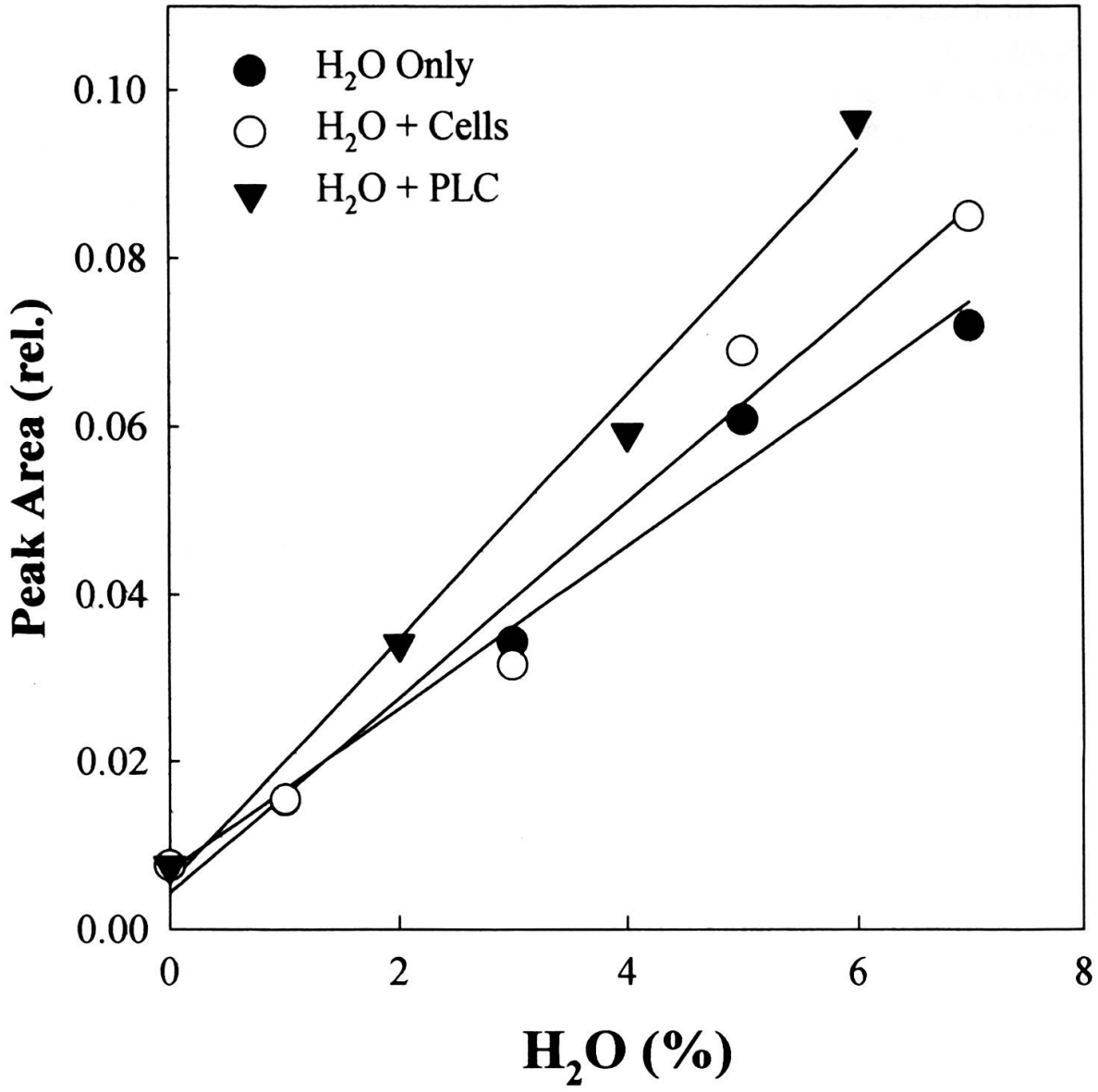

FIG. 6.
Peak area of water, in arbitrary units, as a function of water content in HTS-reverse micelles.

main -CH_3 at 1.17 ppm) gives a general idea about the behavior of water in the reverse micellar system. As observed earlier (KERNEN *et al.*, 1997), the bound to free water ratio in reverse micelles changes as a function of the total amount of water in the system. This can be seen in Fig. 5 where the resonance shifts of water protons are plotted against the water content of the reverse micelles. The water resonance increases from 3.4 to 3.8 as the water concentration was raised to 6% (v/v). Fig. 6 illustrates that all the water added to the HTS-reverse micelles is accounted for in the NMR measurements as a linear increase in the integral area of the water peak.

A sharp increase in the resonance shift was seen by adding 2% of water (v/v) to reverse micelles and saturation occurred between 4 and 5% water in the system. The maximum quantum yield of primary photochemical activity measured as BChl fluorescence kinetics of bacterial cells incubated in HTS-reverse micelles displayed a very similar water dependence (Fig. 5). A direct correlation was observed between the water resonance shift and the primary photosynthetic activity of cells. Though the total water content increases linearly as water is added to reverse micelles, the availability of free water is limited and does not appear linearly.

The obtained results clearly demonstrate that the amount and physical state of water determine the primary photosynthetic activity of bacteria in HTS-reverse micelles. Our results with the bacterial system indicate that it is worth while to explore how water influences photosynthetic activity in low water system to better understand the molecular mechanisms involved in the process.

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RÉSUMÉ

LE RÔLE DE L'EAU SUR L'ACTIVITÉ PHOTOCHIMIQUE DES COMPLEXES PIGMENTS-PROTÉINES DANS LES MEMBRANES PHOTOSYNTHÉTIQUES DES BACTÉRIES ROUGES

Les réactions photochimiques primaires de la photosynthèse, mesurées par l'intermédiaire des cinétiques rapides de l'induction de la fluorescence bactériochlorophyllienne (BChl) et par la photooxidation du P800 (mesuré par les changements d'absorption à 820 nm induits par la lumière), ont été examinées dans des cellules de bactéries rouges (*Rhodospirillum rubrum*) placées dans des micelles inversées composées d'HTS (Héxane, Tween et SPAN). Dans les cellules placées dans les micelles l'amplitude de la fluorescence variable était dépendante de la concentration en eau et ceci même après une période d'incubation prolongée. Sans adjonction d'eau, les cellules placées dans les micelles perdaient toute fluorescence variable en l'espace de 24 h., alors qu'en présence de 6% d'eau elles se comportaient comme des cellules normales qui montraient une forte activité même au-delà de 24 h. Des mesures par Résonance-Magnétique-Nucléaire (^{1}H-RMN) ont montré que le rapport entre l'eau liée et l'eau libre diminuait avec l'augmentation de la concentration en eau dans les micelles. Les résultats obtenus montrent clairement que la concentration et l'état physique de l'eau déterminent l'activité photosynthétique primaire des bactéries placées dans des micelles inversées composées d'HTS.

Mots-clés: Bactériochlorophylle, ^{1}H-RMN, micelles inversées.

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