Protein Structure Alteration Induced by Light-Activated Water Absorption. A Study with Bacteriorhodopsin

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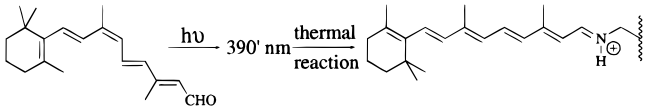
Water is a crucial component in determining the structure of proteins. Nonetheless, it has been difficult to address the problem of the role of water in protein structure in terms of both experiments and theory. Therefore, the general understandings that have been developed concerning the structure of proteins have emphasized the sequence of amino acids as the sole determinant in the structure of proteins. In this paper the development of a system is reported in which light regulates the amount of water that is absorbed by a protein structure.

The system we report in this paper is the ligand binding reaction of the protein bacteriorhodopsin.[1] The ligand is a retinal chromophore characterized by an all-trans configuration, covalently bound to the protein via a protonated Schiff base.[2] It is known that 9-*cis*-retinal binds with the apoprotein bacterioopsin. Nevertheless, formation of the covalent linkage via a protonated Schiff base between the retinal and the protein is prevented until light alters the retinal configuration.[3,4] Recently it was shown that a crucial component in this reaction was the amount of absorbed water in the protein.[5] In this paper we focus on this interrelationship among the absorption of light and water by the protein structure in terms of monitoring a specific protein reaction.

The reaction we use to monitor this interrelationship among protein structure, water, and light is described below. The sequence of absorption changes that occur after light-induced covalent ligand binding of retinal by bacterioopsin are

$$\underset{(9\text{-}cis\text{-retinal})}{390\text{ nm}} \xrightarrow{h\nu} 390'\text{ nm} \rightleftharpoons 470\text{ nm} \rightleftharpoons 470'\text{ nm} \rightarrow 570\text{ nm}$$

In this reaction sequence the light reaction isomerizes the retinal complexed with bacterioopsin from the 9-cis configuration to a retinal isomer that can covalently bind with the apoprotein (Figure 1). Despite the isomerization that occurs in this first step, the absorption of the retinal is not altered by this photochemical reaction. Subsequent to the photochemical step a thermal process occurs. This process is associated with chromophore−protein interaction alteration, as reflected in a red-shifted chromophore absorption maximum. The newly formed species absorbs at 470 nm and probably consists of a mixture of two species, which exist in thermal equilibrium with the 390′ nm species. The formation of a final 570 nm pigment, characterized by a protonated Schiff base (Figure 1), is critically dependent on the amount of absorbed water. In a previous investigation,[5] we showed that the water absorption by the protein can occur in the 470 to 570 nm conformational transition.



9-cis retinal - 390 nm species 570 nm species

Figure 1. Reaction between 9-*cis*-retinal and apoprotein following light absorption.

It is the objective of this paper to probe the initial photochemical event, and to investigate whether protein water absorption can occur even in the light-driven transformation. We conclude that the photochemical reaction can indeed regulate the amount of absorbed water. The diagnostics of this water absorption is the extent to which the 570 nm species is produced.

Using the 570 nm absorption in 100% humidity as a diagnostic of when the production of the 390′ nm species by the photochemistry is maximized, it can be deduced that 10 s of illumination allows the establishment of the maximum production of the 390′ nm species. In order to separate the photochemical transition from the subsequent protein conformation transitions, it was necessary to lower the surrounding humidity in which the reactions take place. Following 10 s of illumination, at a humidity of 51%, not all molecules are transformed into the 570 nm species, even though the same amount of the 390′ nm species was produced (i.e., isomerization was maximized). At this humidity, despite complete isomerization, the absorptions of the 470 nm intermediate and the 570 nm species coexist. Thus, given the complete isomerization in 10 s, but the incomplete conversion to the 570 nm absorption, we used the ratio of the 470 nm intermediate to the 570 nm species as a diagnostic tool to monitor the extent of water absorption by the protein in the photochemical transformation. Specifically, the illumination times are altered under the same 51% conditions of humidity, and the 470/570 nm absorption ratio is changed, indicating the extent of water in the protein. To appreciate this, it is important to note that it takes significantly longer for the 470 nm species to appear relative to the irradiation times that produce the 390′ nm intermediate. Therefore, since the only molecules present during irradiation are those characterized by the 390′ and 390 nm absorptions, it is logical to conclude that the interconversion between these species is a controlling factor in the water absorption. Since this interconversion can only occur with light, it is clear that light must be responsible for the extent of protein water absorption, which controls the final concentration of the 570 nm species.

These conclusions are drawn from the results shown in Figure 2, in which spectrum A produced with 60 s of illumination is compared to curve B, which results from 10 s of illumination. These spectra were obtained 15 min after this irradiation protocol, in order to ensure the production of the 470 nm species and the 570 nm product. In Figure 2 the relative intensity of the shoulder at 470 nm as compared to the peak at 570 nm indicates that the length of irradiation has an effect on the 470 nm intermediate production. In other words, as the length of the irradiation increases the ratio of the 470 nm shoulder to the 570 nm peak decreases. Thus, it can be concluded that, during the irradiation process, the protein absorbs water, and this is reflected in the amount of 570 nm species produced.

To demonstrate that the water absorption process does not occur in the 390 nm species (prior to irradiation), we incubated films in the dark in 51% humidity for 10 s, 3 min, and 14 h. Following these incubations, these films were irradiated for 10 s and produced similar ratios of the 470 to 570 nm intermediates. The same films with 1 min of irradiation had most of the molecules converted to the 570 nm pigment. Since the films equilibrated for 10 sec, 3 min, and 14 h showed similar sensitivity to irradiation time, we can conclude that even after 14 h of dark equilibration the films were essentially not equilibrated

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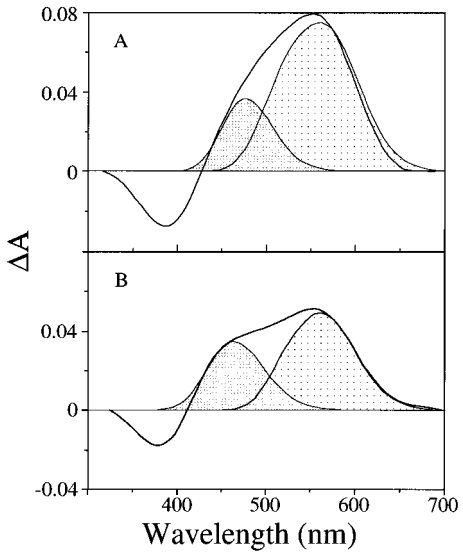

Figure 2. Difference absorption spectra demonstrating the effect of irradiation time on the 470/570 nm ratio at 51% humidity: (A) 60 s and (B) 10 s of irradiation time. The sample was irradiated with white light using a KL1500 electronic halogen lamp. The absorption spectra were measured immediately after irradiation and 15 min later.

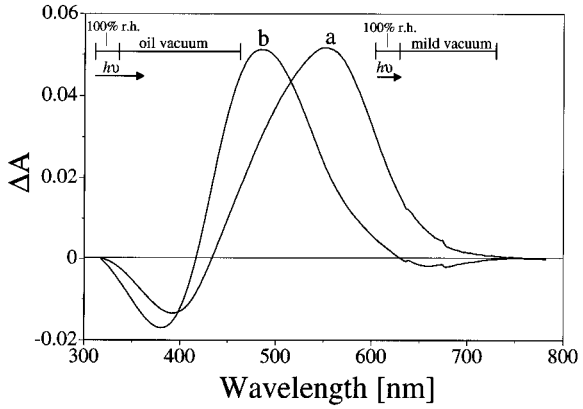

Figure 3. Difference absorption spectra (before and after experiments) demonstrating the effect of vacuum on 470 and 570 nm species production. The spectra were monitored 15 min after the illumination. (a) The 390 nm species was irradiated for 1 min with white light at 100% humidity, followed by vacuum (20 mmHg) treatment in the dark for 15 min before monitoring the spectrum. b) Illumination of the 390 nm species as in (a), but the illumination was continued for an additional minute in vacuum (2 mmHg), followed by vacuum treatment in the dark for 15 min.

with the surrounding water. These experiments highlight the dominant role of irradiation in the water absorption. They show that extensive changes in the dark equilibration have no observable effects on production of the 570 nm species. However, small changes in irradiation time have a significant effect on this transformation. Thus, from these experiments we can conclude that water does not permeate the binding sites that are required for 570 nm production when the 390 nm species is exposed to an aqueous environment.

To further refine our understanding of which step in the above sequence of reactions is associated with the water absorption, we irradiated the 390 nm species in the presence of high (100%) humidity. After such irradiation the chamber was evacuated using a vacuum of 20 mmHg. Thus, after the production of the 390′ nm species by irradiation the subsequent dark reactions proceeded under vacuum. Despite this, the 570 nm species that was produced was identical to what was formed under 100% humidity (Figure 3, trace a). Note that similar results were obtained with a 2 mmHg vacuum, but a 530 nm species was obtained instead of a 570 nm species, as is known for bacteriorhodopsin behavior at high vacuum.[6] This suggests that the water absorption responsible for 570 nm species production occurs as a result of the irradiation that produces the 390′ nm species. Once it occurs, the protein structure prevents the bound water from being extracted by vacuum.

In contrast, if after production of the 390′ nm species in 100% humidity the chamber is evacuated by high vacuum (2 mmHg), but the irradiation is continued (for 1 min), only the 470 nm species would be formed (Figure 3, trace b). This demonstrates that irradiation allows the bound water in the protein to be extracted, preventing formation of the 570 nm species. We note that a mild vacuum (20 mmHg) produced a mixture of 470 and 570 nm species (ca. 1:1), indicating that only part of the bound water was removed.

It is interesting to address the question as to how such water cannot be evacuated from the 390′ nm species in the dark, when we know that the 470 nm species can absorb water.[5] There are two possible explanations for this observation. First, it is possible that once the 390′ nm species is produced with absorbed water, it converts to the 570 nm species directly without passing through the 470 nm intermediate. Alternately, under these conditions the kinetics is such that the 470 nm species is produced and decays in a very short period of time without any appreciable accumulation of this species. These results indicate that after 390′ nm species production, the subsequent thermal transformation to the 470 nm intermediate (which takes 15 min) involves a responding of the protein that allows water absorption by the protein.

The above results raise the intriguing possibility that the photon absorption by the retinal induces a protein conformational change that temporarily opens a crucial cavity in the protein. Water subsequently binds to groups in this cavity, and as the 390′ nm species state is produced the protein closes and prevents equilibration of this water with the surrounding aqueous phase.

In support of such transient openings and closings of cavities in proteins are the fundamental studies by Fraunfelder and coworkers.[7] These studies have shown that heme proteins bind and release ligands by opening and closing the protein cavity. In addition, recent theoretical studies on acetylcholinesterase[8] suggested ligand binding due to transient dipolar characteristics of the protein structure. In terms of such an understanding there are two possible alternatives for the presence of transient dipoles in the transformation of 390 to 390′ nm species. First, following light absorption, the retinal chromophore experiences a very large dipole change,[9] which is enhanced even further by the protein.[10,11] This induced dipole could result in protein conformational alteration.[12] Second, as a result of light-induced retinal conformational changes, the protein may transiently pass through a dipolar state which opens and closes a crucial cavity that needs to be hydrated. Alternatively, dipoles may not be involved at all. Possibly, light absorption by the retinal causes conformational changes in both the retinal and the protein, which may temporarily open a cavity for hydration.

In summary, this method of hydration control, which can be so well defined in time, opens the possibility of a variety of experiments aimed at elucidating the essential role that water plays in defining the structure of proteins.

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