Photorefractive Protein bacteriorhodopsin, BR , as a material for holographic data-recording

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SUMMARY

The search of materials of biological origin with specific estates of potential technologic applications whose structure and functions come out from a process of adaptive evolution experienced by the organisms in extreme environments, is one of the most active fields in the modern biophysics.

In short, the protein bacteriorhodopsin, acts like a protonic bomb whose photocycle has demonstrated interesting implications in the technologies of optic storage of information by means of holographic methods.

In the present work some results of the investigation undertaken by our group are exposed, consistent mainly in the study of the influence that working conditions (pH, state of aggregation of the environment, intensity and time of illumination, etc.) have over photophysical and photochemical states of bacteriorhodopsin as well as in the tracking of the possible presence of bacteriorhodopsin proteins and xantorhodopsin, in the salines of Santa Pola (Alicante, Spain).

1. INTRODUCTION

For the bioengineering of the XXI century, learning from the benefits demonstrated by the adaptive structures generated by natural selection, and using proteins as functional units incorporating them in the modern technological devices, are two of the most important challenges.

In this sense, molecules as photoactive proteins are able to transform the light directly into an electric signal [1]. This process implies the formation of an electric dipole and occur by changing protein spectrum.

Chlorophyll (in vegetables and bacteria) and rhodopsin (in animals and bacteria) are the most extended photosensitive biomolecules in our planet whose role as converters of solar energy make them very well-known.

The basic structure of the rhodopsin consists of retinal molecules connected to proteins (opsins) with a specific structure by means of a Schiff base. In almost all the natural rhodopsins, the cromophore is the portion of retinal in the complex retinal-protein due to its high molar extinction coefficient, its excellent quantum yield and its short isomerization time. The proteins and retinal that separately absorb in the UV range when combining, shift their spectrum of absorption to the visible region. It is necessary to highlight that the protein complexes of the retinal transport ions through the membranes, while chlorophyll produces separations of electric charges.

In 1971 there was discovered a complex protein derivate of the retinal in the external wrapping of one of the archaeobacteria species, inhabitant of strongly saline environments [2]. Named bacteriorhodopsin (from now on, BR) due to their similarity with the visual rhodopsin of the eye, it uses the energy of the solar light to manage an ionic transport of hydrogen through the cellular membrane creating this way an electric potential that allows the synthesis of adenosín triphosphate (ATP).

This is the better studied example of protein photoactive and it presents very diverse technical applications, which in turn have generated an important research activity [3]. BR, forming what is known as purple membrane in archaeobacteria, allows those microorganisms to obtain metabolic energy starting from the light in extreme conditions of high salinity, low oxygen concentration and high solar radiation [4]. In stagnated waters some kinds of bacteria (as Salinibacter ruber) contain in their membrane another protein together with retinal, called xantorrhodopsin (XR) that also works as a protonic pump [5].

2. THE RHODOPSIN EVOLUTION

The evolution of photosensitive molecules as rhodopsins, it is tied mostly to the process of adaptation of living beings to the environments with diffused light. The first vertebrates that appear in the fossil record lived in shallow waters where light arrived muffled. Many of the animals that nowadays inhabit such atmospheres possess rhodopsin molecules with a maximum of absorption around 500 nanometres, what belongs together with the ghastly intensities in the twilight or in situations of veiled light.

In these molecules the maxima of absorption in certain wavelengths can be explained by means of the substitution of 15 amino acids in 12 different places. It seems that these fifteen amino acids were substituted numerous times in the evolutionary course of the rhodopsins. The fact that the maximum of absorption in the ancient rhodopsins locates around the 500 nm, would explain the reason that their current descendants evolved –at least up to 18 different times– in order to comprise a wider range, of among 480-525 nm.

And although the dominat tendency among researchers has pointed to awarding these adaptive changes to a selective pressure of darwinian type, there are also other authors who regard the neutralist theory of molecular evolution as a better explanation [6]. Anyway, it seems beyond all doubt that the statistical tests of positive selection are difficult to obtain in a molecular level. The adaptive phenomena in the evolutionary course of the rhodopsins, must be elucidated through mutagenic analysis using ancient pigments related with them.

Only this way we will be able to understand how the photosensitive molecules have ended up acquiring the absorption spectrum that they in fact possess. In nature diverse visual pigments adapted to different environmental brightness exist. And to determine the molecular mechanisms involved in the adaptive selection of such pigments,we should first identify the aminoacidic changes that are potentially important in the modification of the wavelength for the maximum absorption. In fact the possibility of using directed-mutagenesis methods and growths of cellular fabrics, makes photosensitive pigments an ideal model for the study of adaptive mechanisms at the molecular level.

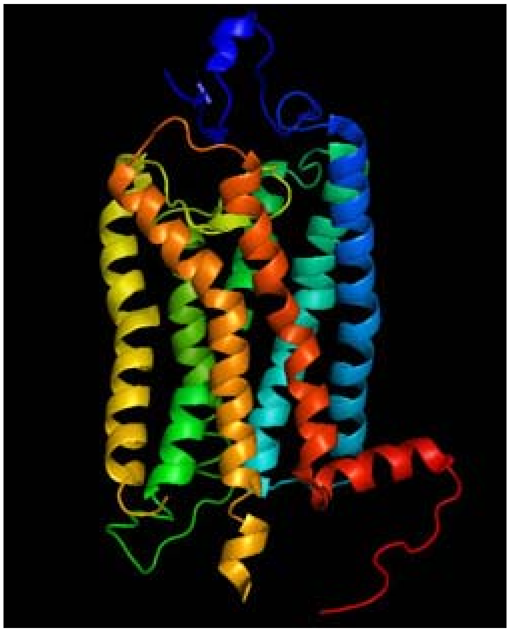

Fig. 1. Rhodopsin molecule. Graphic recreation of the multiple-helix structure of a rhodopsin molecule

3. EVOLUTIONARY ADAPTATION AND TECHNOLOGICAL OPTIMIZATION

Living organisms thoroughly take benefit from the recognition of molecular conformations, what simultaneosuly establishes remarkable similarities and differences with the inert systems. One of the basic component of an electronic device, for example, is a switch; and photosensitive proteins, in turn, can be considered in fact as something similar to a switch. The rhodopsin, let us say, absorbs a photon and it unchains a sequence of biochemical reactions that end in a nervous impulse, a similar performance to that of a phototransistor.

Nevertheless, along evolution, nature has appealed to a probe-and-error method to solve by means of suitable molecules organic problems that are similar to those that we find in inorganic molecules taken advantage to carry out logical functions, of exchange or management of data.

For that reason, when we think in biophysics about the technologic application of biological materials, we must deal with three fundamental questions:

1) Which are the elementary physical features of the process that we are approaching?
2) Is there some biological system that possesses those same features or carry out similar functions with an equivalent purpose?
3) Of all the varying adaptativas that the evolution has been able to generate, according to the different colonized environments, which is the best one that fits our demands?

Since our purpose was the search of photosensitive materials for the digital storage of information by means of optic procedures, it seemed logical to think of the most versatile photosensitive proteins. And particularly in the BR, since it has a great stability against chemist as thermal degradation, its photophysic behavior resembles that of the chlorophyll (but with an inferior yield that does not overcome 15 % on converting light energy in chemical energy), besides being reversible and therefore subjected to repeated uses

The BR is a protein located in some regions of the bacterial membrane collectively denominated purple membrane (MP). Forming a hexagonal crystalline net in the MP, the molecules of BR are not denaturalized until the 80 ºC (or even sometimes until the 160 ºC), and they retain their cromophoric estates in a pH range from 00,0-11,0. Their advantages are:

It shows an extreme simplicity of the photosynthetic system, with the protonic transfer carried out by a single molecule. Thanks to the specific constitution of the bacterial cellular wall and the packaging of BR in the MP, the bacteria survive under extreme environmental conditions (high

temperatures, intense sun radiation, high saline concentration, reactions oxidation-reduction) without loss of its photosynthetic ability.
BR electro-optical states are reversible, for what presents a cycle of recording/reading/erasing longer in many orders of magnitude to those of the current synthetic pigments. Additionally these cycles are muchfaster, and could centuplicate the reading speed and data storage.
The photosensitive proteins are of nanometric size. A biomolecular computer could be fifty times of smaller size than a current computer. They are also controlled by light, what endows them of the advantage that photons can converge to higher densities than in electric currents. These two factors together allow to obtain a bigger resolution and a better storage efficiency.
As BR is assembling in groups inside the bacterial membrane (in form of two-dimensional protein crystals) it is easy to incorporate it in polimeric films with a lot of technical uses. Films of BR are flexible and can be produced with non-toxic and recyclable materials.

The other retinal proteins of the halobacteria, together with the BR, constitute a family of homologous membrane proteins. Concretely they are the photoactive pump of chloride ions, the halorhodopsin (HR), and the well-known phototactic pigment sensoriorhodospin (SR). The sequences of all these proteins have been determined thanks to the modern techniques of DNA sequence. The HR shows 32 % of identity with the BR, and the SR 26 %. For the paraffinic chains a 14 % is identical in the three proteins, including most of those known by its functional importance thanks to the mutagénesis of the BR. Also, there are clear indications of a remarkable homology among the amino-acids pointing toward the membrane inner side, supplying an additional evidence that the linkage area is one of the most closely preserved molecular features during the evolution of this proteinic family.
As it was shown, BR is an example of connection among nature and technology that is being explored as an active element for use in optic devices, including computers memories and optic modulators [7].

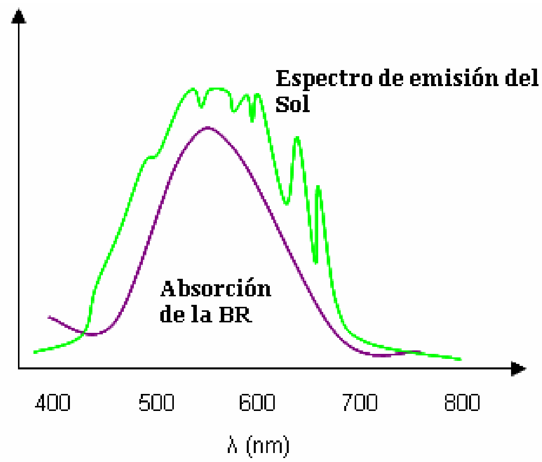

Fig. 2. Sun emission spectrum and absorption spectrum of BR. As it can be observed, a clear correlation exists between both, explained by adaptive evolution of the photosensitive pigments

The retinal of BR is unstable in the form “cis” for what it takes a proton of the cytoplasm to return to their form “trans”. This way it is possible to create a protonic gradient that will be used by a trans-membrane ATPase to obtain ATP. If we assign binary values (0 and 1) to each one of this two states, a group of BR molecules could be used to store data. By means of transparent polymers theer can be produced films that contain molecules of BR as photosensitive material [8]. The relatively small size of the protein could be transformed in digital memories based on BR that could offer enormous storage capacities per volume-unit. Eventually, there can be achieved holographic memories, also called 3D-memories.

The BR also has promising applications in the field of photovoltaic technology. When illuminated, BR directionally carries electric charges and stores electrostatic energy. This mechanism could have application in the construction of photovoltaic cells based on the active center of this reaction. The charge transfer originated by the illumination of the BR can also be used to generate an electric signal. This property can find a relevant use in photoelectric convertors of light signals, as motion-detectors and artificial retinae.

4. **OBJECTIVES**

In the biological aspect, our starting position consists of studying the BR and xantorrhodopsin (XR) diversity in different ponds of the salines of Bras del Port (Santa Pola, Alicante).
The chosen methodology has been the electrophoresis technique in desnaturalizating gradient (DGGE), forit has been designed to amplify the BR and XR gene in different samples. Since the different selected ponds differ in their salinity we will be able to see differences of spatial distribution. In turn we carried out the taking of samples in different times of the year with the purpose of observing variations in the time.
Although the process of biophysic and genetic characterization of the wild-type and mutant BR and XR molecules found in Bras del Port goes on at the present time, our preliminary results come to confirm the already obtained data from similar works on these molecules in similar environments [9]. Therefore the idea of a convergent evolution of the photosensitive pigments that, as the BR, that have adapted in diverse environments of equally extreme physical-chemical conditions is reinforced.
In the technological aspect, another of our objectives has been to obtain and characterize photosensitive layers of BR in a polimeric matrix for information storage. With that aim it is of great importance to optimize the elaboration of films of BR for the study of anisotropy features, using different materials as support of the BR [10]. The chosen films were basically composed of acrylamide and bisacrilamide combinations whose concentration guarantees that the films are not too rigid neither too brittle.
The efficiency of the BR has been only studied in its natural form (wild-type) because the genetic modifications (except maybe D96N, much more expensive) they do not absorb in the range of wave-lenghts that make it interesting for the technologies of information storage by means of holographic methods based on the lasers available at the moment. Also, the geographical vicinity and the possibility of a controlled purification of the production in situ, are the reasons that have taken us to use in fact the BR extracted of the Halobacterium halobium from the salines of Santa Pola. And it is difficult to guess if from now on evolutionary analysis will help the elaboration of photosensitive films. The main reason is that the next research trend is focused on the search of techniques that allow us to join the stability of the recorded information in BR films with the possibility of optional re-recording (the stability of the stored information, therefore, should not be absolute).

5. **RESULTS**

As expected, the time evolution of the film spectroscopic response (time changes of absorbance as function of the wavelength) depends on the quantity of present acrylamide in every case, expressed in percentage form. The behavior of the films can be listed as follows:

Films of 5%. Their absorptions to 400 nm vary between 0,8 and 2 units, and the absorbance maxima are in an interval of wavelengths between 550 and 580 nm.

Films of 10%. Their absorptions to 400 nm vary between 0,8 and 2,4 units, and in a very similar way as the behavior of the previous sample their absorbance maxima also concentrate in the interval of 550-580 nm.

Films of 15%. Their absorptions to 400 nm vary between 0,8 and 2 units, and the maxima of absorption are in an interval of wavelengths between 550 and 580 nm.

Films of 20%. Their absorptions to 400 nm vary between 0,7 and 1,7 units, and the maxima of absorption are also in an interval of wavelengths between 550 and 580 nm.

Films of 40%. they vary their absorbance around 400 nm between 0,7 and 1,2 units, and as the previous ones their maxima of absorption are between 550 and 580 nm.

During the polymerization of the acrylamide and the bisacrylamide, the distribution of the BR clearly shows a dependence of the dynamic variation of the transmitance with the medium in which the BR is emboded. The dynamics of the BR depends equally on the relative humidity in the films, and this in turn depends on the composition of this films.

Diffraction nets were stored with a space frequency of 1700 lines/mm. The experience lasted more than one hour without detecting fatigue in the recording material, since its efficiency in diffraction is the same one in the different periods of irradiation.

6. **CONCLUSIONS**

The utility of using materials of biological origin whose optic estates provide natural photocycles of two or more states by means of which it is possible to digitally code information has been verified. The fact that this biomateriales has reached its current configuration after a process of adaptation through natural selection under extreme environmental conditions (great F.T.C. intensity environmental, high salinity of the media, high temperatures, etc.) guarantees the robustness of their physical-chemical properties, what increases the interest and scope of technologic applications.

It has been demonstrated that diffraction gratings can be stored in a material with holographic recording methods based on the use of bacteriorhodopsin as a photochromic material. Everything allows us to trust their possibilities of technological application, very especially in the field of all-optical information storage.

7. **ACKNOWLEDGMENTS**

We want to thank the support and the professors' supervision Antonio Fimia, of the Department of Science of Materials, Optics and Electronic Technology, and of Immaculate Meseguer, of the Department of Vegetable Production and Microbiology. This investigation has been partially finance for the project FIS2006-09319 of the Ministry of Science and technology of Spain, and the company Bras of Port CORP. Discussions with the group of exobiological systems, in the Agrupación Astronómica de Alicante, also supplied a remarkable help

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