Heat-proof proteins

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PROTEINS are being exploited increasingly for technological ends, in biosensors and bioreactors for example. But some of these applications require high (more than 100 °C) operating temperatures, under which conditions many proteins are likely to denature. A striking exception is bacteriorhodopsin, as Shen *et al.* report on page 48 of this issue[1]. They found that in dry films bacteriorhodopsin was structurally stable up to a temperature of 140 °C. This is an astonishing result and an encouraging one for a new aspect of bacteriorhodopsin research — its use in optical information processing.

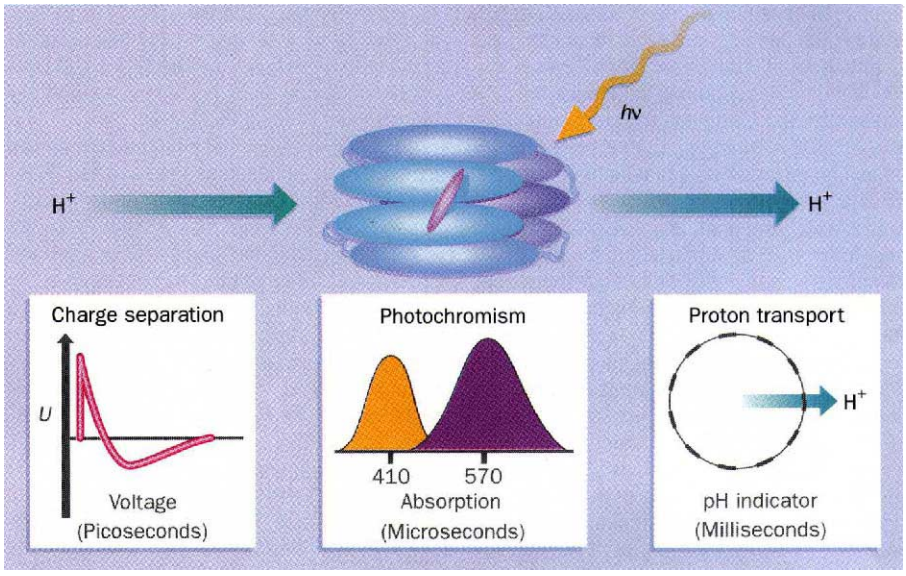
Approaching San Francisco by air, one obtains a spectacular view of the intensely purple basins of the salt works in the bay area. In the 1970s it was shown that the colour was largely due to a retinal-containing protein which could be isolated from bacteria living in the harsh conditions of a saturated solution of common salt, with little dissolved oxygen[2]. Survival in this ecological niche is possible because *Halobacterium salinarium* (formerly *Halobacterium halobium*) developed during evolution a set of proteins that enables it to use sunlight directly as an energy source. The key protein in the halobacterial photosynthesis is bacteriorhodopsin. Several tens of thousands of bacteriorhodopsin molecules form a two-dimensional crystalline lattice in the cell membrane over a region typically 500–1,000 nm in diameter and just 5 nm thick, the length of a single bacteriorhodopsin molecule. From its colour, this membrane fragment is called a 'purple membrane' and may be considered as the biological solar cell of the halobacterium. Each single bacteriorhodopsin molecule in the purple membrane acts as a light-driven proton pump. After absorption of a photon, bacteriorhodopsin transports a proton from the inside of the cell to the outer medium and thereby converts light energy into chemical energy which enables the halobacterial cell to survive.

When we talk of bacteriorhodopsin's inertness towards chemical and photochemical degradation and its remarkable longevity, we should qualify this: it is the purple membrane form that we mean. Purple membranes contain about 10 lipid molecules per bacteriorhodopsin acting like a glue for the bacteriorhodopsin trimers. Solubilization of the membrane and analysis of the isolated trimeric or monomeric bacteriorhodopsin indicates that the intact membrane is crucial for the stability of the material. Shen *et al.*[1] have identified another key aspect, the absence of water, in maintaining stability above 100 °C, extending the utility of purple membranes at the high temperatures demanded for technical applications.

Bacteriorhodopsin combines three functions that in principle are technically useful (see figure)[3]. First, it is a light-driven proton pump, so might be exploited to convert sunlight into chemical or electrical energy. Second, it has photoelectric properties due to the initial charge separation in the molecule after absorption of a photon. Third, it is a photochromic protein: absorption of light leads to a reversible colour change from purple to yellow. It can be switched back and forth with blue and yellow light more often than any other known chemically synthesized photochromic compound. This and the fact that bacteriorhodopsin uses light very efficiently (just 1–2 photons are needed to switch a single molecule from purple to yellow) make it attractive for optical information processing.

Two decades of interdisciplinary research have pinned down the structure[4] and molecular function[5,6] of bacteriorhodopsin fairly precisely. Deliberately mutated bacteriorhodopsin molecules were first obtained in *Escherichia coli*[7]. With the development of a halobacterial transformation system[8] it has become possible to obtain the mutated bacteriorhodopsin molecules in the stable purple membrane form and in bulk. Dry films of some functionally modified bacteriorhodopsins have already proved useful in optical information processing, for example in holographic pattern recognition[9]. The optical properties of dry bacteriorhodopsin films might produce different technical applications[10]. Thermal denaturing has never been a problem, but nobody has tried to push dry bacteriorhodopsin films to the limits. The results of Shen and co-workers place them well into a temperature range that is adverse for most synthetic photochromic materials, especially in the presence of oxygen and light.

With luck, their work will encourage others to transform 'well characterized' biological macromolecules into the solid or glassy state (not, in general, as easy as with purple membrane) and to investigate the conformational stability of the resulting material at higher temperatures. □



Bacteriorhodopsin consists of a single strand of 248 amino acids arranged as seven α-helices (blue) and a covalently attached retinylidene residue (purple). Absorption of a photon leads to the transport of a proton through the proton pore of bacteriorhodopsin. This process divides into (1) an initial isomerization and charge separation on the picosecond timescale, (2) a reversible colour change on the microsecond timescale and finally (3) the proton transport which can be observed by means of pH indicators. A complete proton transport cycle of bacteriorhodopsin takes about 10 milliseconds. Each of these basic phenomena is of potential technical use.

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1. Shen, Y. *et al. Nature* **366**, 48–50 (1993).
2. Oesterhelt, D. & Stoeckenius, W. *Nature new Biol.* **233**, 149–152 (1971).
3. Oesterhelt, D. *et al. Q. Rev. Biophys.* **24**, 425–478 (1991).
4. Henderson, R. *et al. J. molec. Biol.* **213**, 899–929 (1990).
5. Birge, R. R. *A. Rev. phys. Chem.* **41**, 683–733 (1990).
6. Zimanyi, L. *et al. Biochemistry* **31**, 8535–8543 (1992).
7. Braiman, M. S. *et al. J. biol. Chem.* **262**, 9271–9276 (1987).
8. Ni, B. F. *et al. Gene* **90**, 169–172 (1990).
9. Thoma, R. & Hampp, N. *Opt. Lett.* **17**, 1158–1160 (1992).
10. Hampp, N. *et al. Mat. Res. Soc. Bull.* **17**, 56–60 (1992).