TECHNOLOGY OF BIOMOLECULAR DESIGN, WITH EXPERIMENTS ON LIGHT CONTROL OF THE PHOTOCHEMICAL CYCLE IN *HALOBACTERIUM HALOBIUM*

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Although mankind has known how to make use of biological processes such as fermentation since the time of the Pharaohs, only recently has the general recognition of the astounding molecular properties of living systems stirred developmental activities toward a technological application of biological principles and means. Such efforts are strongly supported and motivated by recognition of the restriction of resources and of the time structure of biological cycles on earth. In the concluding remarks of this discussion it seems fitting to summarize a few molecular properties of biological systems that form the basis of their general usefulness.

As a result of evolution and selection, living organisms are perfectly designed for trapping relevant energy and information. I note further, that the molecular trapping devices are amplified by the ability to search and collect 'food' in general. These searching and collection processes that are found in the organisational series from bacteria to humans are recognized as phototropism and chemotaxis. The basis of molecular trapping is the high binding constants of the specific proteins involved be they binding light, oxygen, carbon dioxide, water, carbohydrates, lipids or nitrogen compounds. Mass and energy are taken up and converted. Often this activity triggers a searching mechanism. Usually the trapping systems are in excess allowing collection of whatever appears within reach. Thus, the photoacceptor of the purple membrane in *Halobacterium halobium*, at daylight light-flux, picks up roughly 1 quantum/second at 570 nm with an energy of 50 kcal/mol. Similar computation can be carried out for all structures interacting with biological systems. The binding energies for chemical interactions may be as large as 12 kcal/mol. In addition it should be mentioned that the high quantum efficiency of bacteriorhodopsin, which approaches 1 quantum/molecule turned over, is similar to the reaction observed in the vertebrate eye. The efficiency of energy conversion for biological systems has been computed to be as high as 70%. Usually the conversion is nearer 40%, still much higher than observed in non-biological devices.

One of the most significant properties of biological systems, which lend themselves to technological appreciation is their built-in control mechanisms. Genetic as well as intrinsic control, whether by induction–repression or activation–inhibition, only need relatively few molecules to control cellular processes. Thus, the amplification and restriction of reaction rates is accomplished with high efficiency. We compute a precision of 2–3% in the regulation of biological systems. However, it should be added that cellular control in itself needs some extra energy which usually amounts to 1% of the total energy flux [1].

Of further interest is the competitive utilization of the sources of cellular systems, as was earlier observed by Pasteur. In systems studied up to the present, affinities clearly favour light over oxygen, and oxygen over nitrogen which again offers means of technical control. I would like to stress that light is as useful a control parameter for proton transfer as any physical perturbation. This can be illustrated by the case of the relatively simple proton pump in the purple membrane of *Halobacterium halobium*. The photochemical cycle activation of 570 nm light is followed by proton release that allows a vectorial proton transport. This vectorial transport is primarily controlled by the intensity of 570 nm light as described by the adsorption isotherm [2].

Recently we found that the prominent intermediate (412 nm) of the photochemical cycle that regenerates the 570 nm compound coupled to a vectorial proton

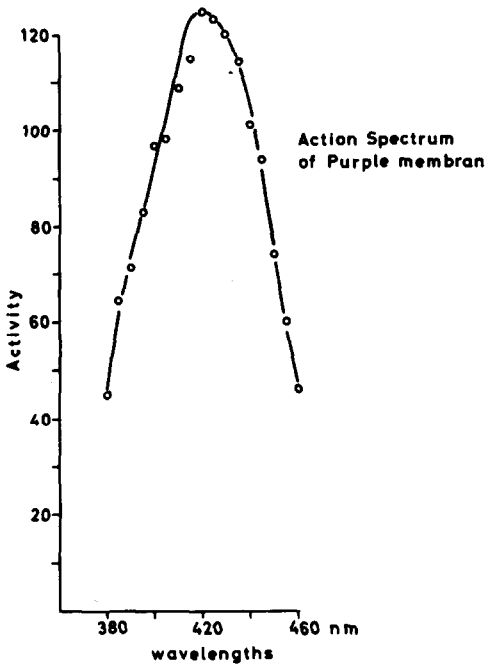

Fig.1. Action spectrum of the blue component of the purple membrane. The activity is given in relative units of extinction change per time, purple membrane concentration 3×10^{-5} M.

binding step, is also light-sensitive. This is shown in the action spectrum of the 412 nm component of a suspension of isolated purple membranes. The measurements were made in the presence of ether at low temperature (see fig.1). Here, light photoactivates the regeneration of the 570 nm purple component of the photochemical cycle with a quantum yield of nearly unity.

Thus, an additional blue light sensitivity of the system allows control of proton pump turnover. This is shown in the pH-record (fig.2) that was obtained at low temperature in the presence of ether using a pH-indicator (methylumbiliferon). The record clearly indicates that two successive laser flashes of 580 nm light eject protons from the system. These are not re-bound during the time of observation. A photo-activation of the 412 nm compound is initiated by a flash of 412 nm light and protons immediately rebind. Finally, in the experiment shown, a third 580 nm laser flash ejects protons again, demonstrating the perfect control exerted on the cycle and proton transport. It should be mentioned that the kinetic constants of the photochemical cycle are almost pH-independent over a large pH range (at least pH 4–8) [2,3].

I do not need to stress here that electron pathways can be regulated perfectly in both directions of the potential scale using light, phosphate- and/or redox potentials in addition to suitable chemical effectors. Slowly, we learn of biological species and systems that are able to operate at large pressure and temperature ranges and realize that membranes in general are remarkably pH insensitive.

The structure and function of the fundamental systems in bioenergetics rely, as generally in biology, on the function of proteins as single species or

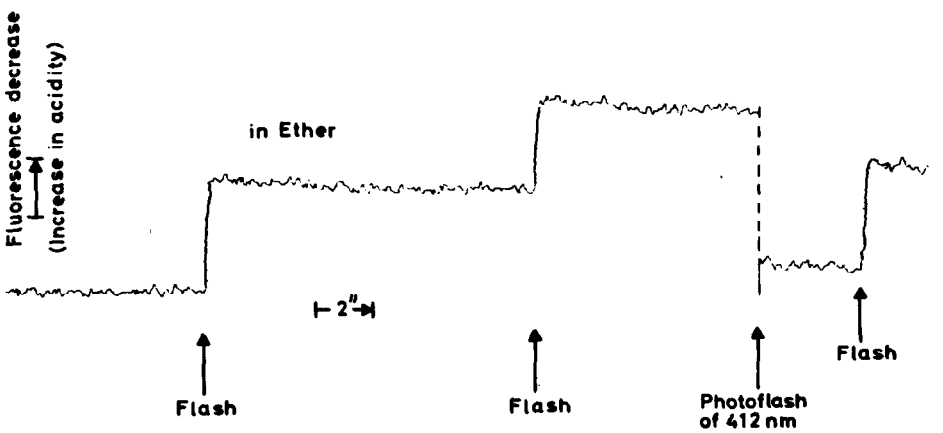

Fig.2. Repetitive laser activation (580 nm) of the purple membrane, pH kinetics detected by methylumbiliferon. Temperature $-10°C$.

assemblies in higher units of aggregation with the membranes serving for insulation of chemicals and charges. It is interesting to note that protonation and deprotonation of proteins are the fundamental covalent steps that follow perturbation of proteins by physical parameters; by large molecules or by 'coenzyme'-mediated interactions with light, electrons, oxygen or other effectors. Thus, an understanding of these mechanisms should point to the possibility of solving the problems of insulation and stabilization of bioenergetic systems, such as those mentioned in this discussion. With all these thoughts and ideas in mind I should like to stress that biological systems interacting with energy sources dissipate energy to maintain multiple steady states which must be sufficiently distant from equilibrium to maintain independent existence and to organize themselves in time and in space to produce phase separation and interfaces, and eventually reproduction phenomena, morphogenesis and development. These phenomena are not known in the technological experience of today and should lead to new aspects of energetics in the future.

References

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