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MEMBRANES AND PHYTOCHROME ACTION

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1. INTRODUCTION

The concept of translocation of phytochrome as an annex to the transformation of P_r into P_{fr} has offered useful viewpoints for a fruitful discussion of certain phytochrome 'paradoxes' (1), as well as for understanding certain aspects of the interesting phenomenon of 'de-etiolation' as recently described by RAVEN (2). Of special interest seems the aggregation which may be connected with translocation and responsible for the inadequately large optical 'disappearance' of phytochrome upon red irradiation, as observed under various conditions (see, *e.g.* RAVEN, *l.c.*, p. 73 and fig. 56).

Since the mechanism of all this seems barely understood, it appears useful to digress a little on some recent discussions of relations of proteins to membranes which may, in part, be applicable to the behaviour of phytochrome, a pigment-protein complex.

2. SOME RECENT IDEAS ABOUT MEMBRANES AND THEIR POSSIBLE RELATION TO PHYTOCHROME ACTION

It is not our idea at this moment to express preference for any specific membrane model proposed in literature, but we will provisionally start our discussion on the basis of the model presented in a recent survey by SINGER and NICOLSON (3).

These authors advocate a 'fluid mosaic' model for functional cell membranes, consisting of a lipid double layer with more or less mobile imbedded proteins. The structure strives towards the lowest free energy level. With respect to the membranes, SINGER and NICOLSON (*l.c.*) distinguish between two types of proteins, *viz.* periferal ones and integral ones. The former are loosely bound to the membranes and are more easily extractable without adhering lipids. The inte-

gral proteins require strong agents, *e.g.*, detergents to dissociate them from the membranes, and they are considered to be critical to the structural integrity of the membranes.

The authors also mention the cooperative phenomena of membranes: 'an effect which is initiated at one site on a complex structure and transmitted to another remote site by some structural coupling between the two sites.'

It may be worth while to give some further quotations from the same article. Arisen from very different fields of research, they seem of great interest to phytochrome students. We will first mention the distinction between 'trans' and 'cis' effects. 'Trans effects' operate at a localised region at one side of the membrane, and transmit an effect to the other side. 'An integral protein may exist in the membrane as an aggregate of two (or more) subunits, one of which is exposed to the aqueous solution at the outer surface of the membrane and the other is exposed to the cytoplasm at the inner surface. The specific binding of a drug or hormone molecule to the active site of the outward-oriented subunit may induce a conformational rearrangement within the aggregate, and thereby change some fundamental property of the aggregate or of its inward-oriented subunit'.

It would seem that a story like this is closely applicable to the transformation $P_r \rightarrow P_{fr}$ in the case of phytochrome, if the primary agent is not considered to be a drug or hormone molecule but a quantum of light of suitable wavelength.

Besides the above discussed 'trans-effects', SINGER and NICOLSON (*l.c.*) distinguish 'cis-effects', as cooperative changes that may be produced over the entire membrane or large areas of it, as a consequence of events primarily occurring at only one or a few localized points on the membrane surface. These effects may involve transmission and amplification of localized events over the entire surface of a membrane. This also seems of importance in the transmission and amplification of stimulus effects so as to ultimately affect entire organs, as happens in the physiology of *e.g.*, phytochrome effects. SINGER and NICOLSON quote some examples from entirely different fields as substantial evidence that long-range cis-type cooperative effects intrinsic to the membranes are involved.

Attention is also being paid to a membrane model proposed earlier by CHANGEUX *et al.* (4) in which a membrane is visualized as an infinite two-dimensional aggregate of identical lipoprotein subunits. These subunits may exist in either of two conformational states, one of which has a much larger binding affinity for a specific ligand than the other. 'The binding of a single lipid molecule to any one subunit then triggers the cooperative conversion of many of the subunits to the ligand-bound conformation in order to maximize the interactions among the subunits.'

Without closely adhering to CHANGEUX's specific membrane concept 'it is possible that a particular integral protein can exist in either of two conformational states, one of which is favoured by ligand binding; in its normal, unbound conformation the integral protein is monomolecularly dispersed within the membrane, but in the conformation promoted by ligand binding, its aggrega-

tion is thermodynamically favoured. The binding of a ligand molecule at one integral protein site, followed by diffusion of the non-liganded protein molecule to it, might then lead to an aggregation and simultaneous change in conformation of the aggregated protein within the membrane. This mechanism could result in a long-range cis-type cooperative phenomenon,...'. (3).

This picture seems particularly attractive to phytochrome students, since the transition P_r–P_{fr} by light, by many authors is brought in connection with a change in the conformational state of the protein moiety (*e.g.* ROUX and HILLMAN (5), HOPKINS and BUTLER (6), KROES (7)).

Another interesting aspect is a remark made by CHANGEUX *et al.* (4) that the ligand molecules may be of very different nature (small ions, protons, or macromolecules, *e.g.* hormones or cellular proteins). We would like to express the view that the 'energy-acceptor' which, in photo-stimulus processes, acts as a mediator between the energy receiving pigment molecule and the subsequent 'biochemical' reaction chain, may have the properties and the position of a 'ligand' molecule.

A further aspect worth considering is the position of P_r and P_{fr} with respect to the cell membranes involved. Arguments may be advanced that P_r is in the position of a (monomolecularly dispersed) periferal protein in the sense of SINGER and NICOLSON. KROES extracted phytochrome from dark-grown oat seedlings, with Tris buffer, EDTA, and β-mercapto-ethanol. These are relatively mild agents, and probably would not extract an 'integral' protein. There are some indications that P_{fr} resembles more an 'integral' protein.

Of interest are observations by RUBENSTEIN, *c.s.* (8) that a fraction of phytochrome is present in pellets centrifuged from homogenates of etiolated, 5-day-old oat seedlings. The homogenates were obtained by grinding the seedlings under fluorescent lighting, in a buffer similar to the one, used by KROES (*cf.* above). The phytochrome in the pellet was not extractable by the buffer solution, it could be removed for the greater part by a detergent (1 % Triton X–100). It is not known whether the pellet fraction of phytochrome is enriched in or consists solely of P_{fr}.

The authors have also investigated the reaction of supernatant and pellet phytochrome to irradiation of the intact seedlings with red light. This causes a massive decrease in phytochrome content. The expectation of the authors that pellet and supernatant phytochrome behaved differently was, according to them, not fulfilled. Looking at their figures, however, shows that the decrease of phytochrome by the red radiation over 3 periods between light and harvest on the average is 67 % for the supernatant phytochrome and 54 % for the pellet phytochrome.

More interesting, perhaps, is that the phytochrome content of the pellet from etiolated seedlings is about 1/30 of that of the supernatant, after red illumination of intact seedlings this is about 1/22. Relatively more thus remains in the pellet. This might indicate that red illumination promotes the association of the pigment with the pellet, probably with membranes, which may be connected with P_{fr} formation. Since the authors extracted also their etiolated seedlings under

fluorescent light, the presence of at least some P_{fr} in this material seems very likely.

After the above parts of this article were written, recent results, especially of MARMÉ *et al.* (9) became available, representing big progress with respect to the relations of phytochrome conversions and membranes.

These authors observed that a brief red illumination of dark-grown squash seedlings (converting P_r to P_{fr}) strongly increased the fraction of phytochrome in the pellet, after a homogenizing and centrifugation procedure. The light treatment and also the magnesium concentration affected the distribution of phytochrome between the pellet and the supernatant; the total was not altered.

In the presence of 10 mM magnesium, binding of phytochrome with pelletable material was also observed *in vitro*. Moreover, the sedimentation properties of the binding fraction were altered by complexing with phytochrome.

Without light treatment (*in vivo*) only 4% of phytochrome was found pelletable, against 40% after 3 min red radiation before homogenation.

The binding of P_{fr} to membranes was partially reversed by subsequent far red irradiation *in vivo*, just before extraction, the pelletable 40% P_{fr} after red irradiation alone being reduced to 12%, which then appears as P_r!

In vitro effects of red – far red sequences proved to be strongly dependent on the time scale of magnesium additions. Starting from low magnesium content, and adding more Mg^{++} to the extract *before* the light treatments, far red following red hardly affected the amount of pelletable phytochrome after the final centrifugation; in fact increased it even a little, whereas far red alone yielded far less pelletable phytochrome viz., 54, 59, and 7% respectively for red, red – far red, and far red treatment respectively.

Increasing Mg^{++} in the extract *after* the various irradiations brought about a clear red-far red reversibility, pelletable phytochrome decreasing from 48 to 15%, and to 2% for far red treatment alone.

It should be observed that in all cases in which red was the last treatment, phytochrome in the pellet was P_{fr}, if far red was the last treatment, it was P_r. The figures discussed showed that, in general, P_{fr} seems to yield a distinctly better pelletable combination with the 'binding material' (i.e. a membrane fraction) than P_r.

Experiments comparing the extractability of phytochrome in etiolated and de-etiolated material (2) appear of great interest.

In this connection, it may be mentioned that the various near-infrared maxima of bacteriochlorophyll in *Chromatium* (10) show marked differences in extractability towards lipophilic solvents. It was suggested that more ready extractability pointed to a closer connection of bacteriochlorophyll with lipids (10). This probably may be interpreted as different modes of binding to membranes which also find their expression in the location of the absorption maxima.

In an aggregation model, very similar to the one proposed by RAVEN (*l.c.*, fig. 56), SINGER and NICOLSON suggest that proteins under certain conditions (which might not be exactly applicable to phytochrome conversion) may loose some of their periferal, more polar peptides, and thus become more hydropho-

bic and become suitable for aggregation in the plane of the membrane.

Whatever this may be, the above remarks do not intend to offer a straightforward explanation for phytochrome action. They only serve to bring out that there recently appears a remarkable convergence in thinking about membranes and about phytochrome action, and that several aspects of this convergence seem very much worth to be persued further, theoretically and experimentally.

3. Summary

Some recent ideas about the structure of biological membranes are discussed in relation to phytochrome action. Special attention is due to statements like: 'it is possible that a particular integral protein can exist in either of two conformational states, one of which is favoured by ligand binding; in its normal, unbound conformation the integral protein is monomolecularly dispersed within the membrane, but in the conformation promoted by ligand binding, its aggregation is thermodynamically favoured...' (3). See further text.

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