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ON THE PRIMARY PHOTOACT IN THE CONVERSION OF PROTOCHLOROPHYLLIDE INTO CHLOROPHYLLIDE

(with some correction of a previous paper, 1).

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The pigment-lipoprotein forms are designated by a capital letter (P = protochlorophyllide; C = chlorophyllide or chlorophyll) followed by the positions of the fluorescence and of the red absorption maximum respectively at liquid nitrogen temperature. The 2 first leaves of germinating beans were used in the experiments.

We previously summarized experiments in which we showed that, in bean, the photoconversion of protochlorophyllide into chlorophyllide involves two steps: the first one leading to the production of an intermediary pigment form P 690-676 as the result of the absorption of photons, and the second one consisting in the dark formation of chlorophyllide from P 690-676.

P 690-676 represents a stable electronic configuration corresponding to a definite type of interaction between chlorophyllide and lipoprotein. The facts lead to the assumption that, in this particular type of interaction, the energy of activation for the reduction of protochlorophyllide is lowered.

The data are tentatively represented by the following provisional scheme:

INITIAL ETIOLATED MATERIAL P 657-647 $\xrightarrow{h\nu}$ P 690-676 (1)

P 690-676 + P 690-676 + 2 H^+ + 2e^- $\longrightarrow$
C 697-682 + P 690-676 (2)

C 697-682 $\longrightarrow$ lipoprotein + free chlorophyllide (3)

Lipoprotein + newly synthesised protochlorophyllide
$\longrightarrow$ P 657-647 (4)

In this scheme, the lipoprotein behaves like an enzyme which "manipulates" properly the electronic configuration of its substrate (protochlorophyllide), - and thereupon catalyses its reduction (to chlorophyllide; reaction 2)-, only after this substrate has absorbed one photon of light (reaction 1). Such an

enzyme should be called a "photoenzyme".

The product of the above cycle consists of free chlorophyllide, i. e. of chlorophyllide detached from the photoenzyme (reaction 3). After phytylisation, the chlorophyll attaches again to a protein carrier (C 685-672; this step is not shown in the above scheme).

The electronic configuration of protochlorophyllide changes in the light-dependent reaction (1). We intend to give some further, shortened, informations upon this step and to correct, at the same time, material errors appearing in a previous summary of our results.

I. The kinetics of the phototransformation of P 657-647 into P 690-676 is first order when 647 nm photons (monochromatic light), or photons of longer wavelength, are given, independently of the light intensity. When 630 nm photon (monochromatic light), or photons of shorter wavelength, are given, the phototransformation does no longer follow a first order kinetics; a "second order" kinetics is found. This also holds true independently of the intensity of the light.

In general, the percentage transformation is given by (see ref. 2):

$$T = 100 - Ae^{-k_1 t} - (100\ A)\ e^{-k_2 t} \qquad (1)$$

When 630 nm photons (or photons of shorter wavelength) are given, A is equal to 50; k_1 and k_2 are not constant with time for any other possible value of A. When 647 nm photons (or photons of longer wavelength) are given, A is either equal to zero or to 100.

These feactures are easily understood if it is assumed that, at 647 nm or at longer wavelength, a single pigment absorbs the

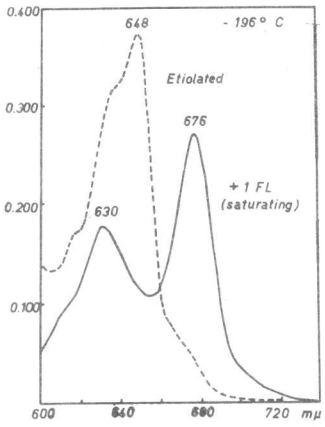

FIG. 1

Low temperature absorption spectrum of an etiolated bean leaf, and of the same leaf after a 1 millisecond flash of saturating polychromatic light. The flashed leaf was frozen (-196°C) within the second following the flash.

photons, while at 630 nm or at shorter wavelength, two pigments with different cross-sections are involved in light absorption. In agreement with this, a 630 nm absorbing pigment is clearly found in the low temperature absorption spectrum of the etiolated leaf (fig. 1). This pigment neither emits a fluorescence, nor changes its absorption in the light. We assume that it transfers excitation energy to P 657-647, or to P 690-676.

The fact that, when the kinetics is "second order", A is equal to 50 indicates that the 630 nm pigment should transfer energy to 50 % of the 657-647 molecules initially found in the etiolated leaf. This can be visualised by assuming that two pigment-lipoprotein complexes are present in equal amount in the etiolated leaf; the first one containing P 657-647 — (complex a) and the second one containing P 657-647 plus the pigment responsible for the 630 nm in vivo absorption (complex b; fig. 2). In complex a, the photons are absorbed by protochlorophyllide only, while in complex b, they are absorbed by protochlorophyllide and/or by the 630 nm absorbing pigment. At wavelengths above 645 nm, the absorption of the 630 nm pigment is negligible, the absorbing pigment being protochlorophyllide in both complexes a and b. As a consequence, the phototransformation follows a first order kinetics (one single K = one single cross-section). On the contrary, at wavelength below 645 nm, the absorption of the 630 nm pigment becomes appreciable, while that of protochlorophyllide decreases; the kinetics of the phototransformation is sum of two first order kinetics with A being equal to 50 (two distinct K's = two different cross-sections).

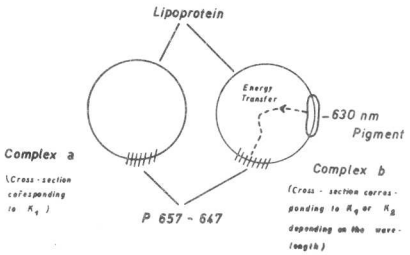

FIG. 2

Schematical representation of the postulated protochlorophyllide-lipoprotein complexes a and b.

II. The rate of the P 657-647 ⟶ P 690-676 phototransformation is temperature dependent. We essentially investigated the

temperature effect with 647 nm photons, - i. e. in a condition in which the kinetics of the phototransformation is first order.

1) The rate of the phototransformation increases from +35 to -18 °C and abruptly decreases below -18 °C; -18 °C can be described as an "inversion point" of the transformation rate.
2) The transformation rate is an exponential function of the reciprocal of the absolute temperature between +35 and -18 °C.
3) The phototransformation becomes very slow at -70 °C and probably stops in the neighbourhood of -75 to -90 °C. Points 1, 2 and 3 are illustrated in fig. 3.

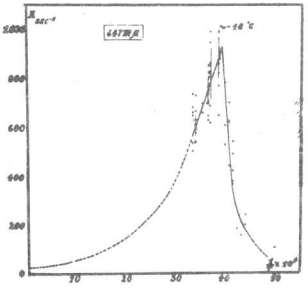

FIG. 3

Temperature dependence of the rate of the photoconversion of P 657-647 into P 690-676; the etiolated bean leaves were irradiated 647 nm photons (first order photoconversion).

4) In general (except in the low temperature range where the photoconversion tends to stop) the transformation rate is higher than would be expected if the photoconversion depended only on the absorption coefficient and on the light intensity.
5) K is equal to:

$$K_{sec^{-1}} = n_T \, (10^3 . \, \varepsilon_{moles^{-1}.cm^2} . I_{einsteins.sec^{-1}.cm^{-2}} \qquad (2)$$

where

$$n_T = e^{m \left(\frac{1}{T}\right)} \qquad (3)$$

T being the absolute temperature and m a constant. Applying (2) and (3) and extrapolating the experimental data for K (as measured between -35 and -18 °C) to infinite temperature leads to a value of ε of the order of the molar absorption coefficient of protochlorophyllide at 647 nm in methanol.

This result indicate:

1) that, when 647 nm photons are given, protochlorophyllide is indeed the photoreceptor for its own transformation;

2) that, at normal temperature, the absorption of a single photon induces the P 657-647 → P 690-676 change in more than one single molecule of the pigment-lipoprotein complexes. We are therefore forced to assume that in the etiolated bean leaf (= in the prolamellar body of the bean leucoplast) some temperature dependent mechanism conveys to neighbouring molecules the excitation produced at a given site by the absorption of a photon.

The temperature dependent nature of the process shows that light energy is not the only energy involved in the conversion of P 657-647 into P 690-676. Some quantity of vibrational energy is also required, probably from the pigment-lipoprotein complex. At sufficiently low temperature, this limited amount of vibrational energy is no longer available, and the photoconversion of P 657-647 stops, - a situation encountered for more and more molecules of the protochlorophyllide-lipoprotein complex when lowering the temperature below -18 °C.

On the other hand, the reorganisation of the interaction between the lipoprotein and the protochlorophyllide molecule, involved in the P 657-647 → P 690-676 transformation, is likely to be initiated by the delocalisation of an electron caused by the absorption of a photon, plus the vibrational, required "surplus" energy. Therefore, the life-time of the delocalisation should essentially depend on the vibrational energy; it should vary inversely to the temperature.

It is still impossible to present a correct theoretical treatment of the dependence of the delocalisation life-time on the temperature. However, it could be tentatively assumed that this life-time determines the value of the n_T factor defined in equations (2) and (3), although we must be aware of the weaknesses and the danger of such speculations. Our goal is only to show how difficult the interpretation of facts is which could appear at first sight as relatively simple. We are dealing with some primary photoact, but this act nevertheless probably involves energy transfer from a pigment molecule to another, as well as from the site of the photon absorption to neighbouring pigment-lipoprotein complexes. This last point seems particularly extraordinary. Furthermore, the photoact does not realise, but

only prepares the reduction of protochlorophyllide, a process which occurs later on in the dark. All of this suggests some definite spatial arrangement of the reactants. It also implies that the protein, especially the binding between pigment and protein, plays an essential role. We find here features which are also found in the complete photochemical apparatus. It therefore appears that the conversion of protochlorophyllide into chlorophyllide provides a good model system for the study of basic physical processes which possibly also underly, at least partly, the primary photoacts involved in photosynthesis.

Details of this preliminary report will be given elsewhere.

1) SIRONVAL, C. and MICHEL, J.-M., European Photobiol. Symposium, Book of Abstracts, Hvar, Jugoslavia, 105 (1967)
2) BOARDMAN, N. K., Biochim. Biophys. Acta 64, 279 (1962).

D I S C U S S I O N

Shlyk: Would you make any comments to the paper of RUBIN, KRASNOVSKY and TUMERMAN (about 5 years ago), where the separation of protochlorophyllide into chlorophyllide in a light step and dark step has been also reported?

Sironval: You refer probably to the paper of A. B. RUBIN, L. E. MINCHENKOVA, A.A. KRASNOVSKY and L. A. TUMERMAN in Biofizika 7, 571 (1962). In this paper the authors described the appearence of a 690 nm fluorescing species as an intermediate step, but they consider it to be "chlorophyll". It is difficult to say, if this step is identical to the P 690-676, that we have found. The experimental conditions of the Russian authors and our conditions are very different. In particular, we irradiate for some seconds, while they irradiate four minutes. However, if the 690 nm species of RUBIN et al. is related with P 690-676, it does still consist of protochlorophyll (a particular type of binding of protochlorophyll to the protein) rather than of "chlorophyll" as stated in the work you mention.

Shlyk: **Have you seen a dependence of the limit of transformation on temperature described by SMITH?**

Sironval: **When irradiating with 647 nm photons, the P 657-647 into P 690-676 transformation rate abruptly decreases when the temperature is lowered under minus 18 °C. Below -40 to -60 °C, it seems that a certain proportion of the pigment molecules are no longer converted. We are studying this point.**

Shlyk: Have you made any experiments in UV-light for which some pecularities are known?

Sironval: I did not experiment with UV-light.

Goodwin: A possible explanation of your observation that the conversion of protochlorophyllide into chlorophyllide is only 50 % efficient is that a dissimilation reaction is taking place: in other words one molecule would be reduced whilst a simultaneous oxidation of a second molecule is taking place. Have you any indication of the formation of an oxidized protochlorophyllide?

Sironval: I do not have any such indication. But your hypothesis has to be considered.

Krasnovsky: What is your idea about the nature of the intermediate "protochlorophyllide" formed after a flash of light?

Sironval: The evidence points to the conclusion that this form (P 690-676) represents a particular electron configuration of the protochlorophyllide molecule, which results from changes in the interaction between pigment and lipoprotein after the absorption of a photon by the P 657-647 pigment.

Sapozhnikow: Wouldn't it be advisable to prevent the dark part of protochlorophyll transformation by means of oxidants?

Sironval: It would be certainly worthwhile and interesting.

Meister: How could you get such large light intensities at very narrow band width to transform all of the protochlorophyllide within one second?

Sironval: I use interference filters transmitting about 70 % of the light intensity at the band maximum, the band width being of the order of 30 Å at half band height.

Meister: The absorption spectrum of etiolated leaves before the illumination shows a small shoulder at 670-680 nm. Is it chlorophyll a?

Sironval: Sometimes it shows this shoulder. It seems to be due to some chlorophyllide probably formed when the etiolated plants are manipulated. It is practically impossible to manipulate the plants without any traces of active photons, especially when water is given or when the etiolated leaves are cut and preparated etc. But the amount of chlorophyllide a formed in that way is general very low.

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    Beispiel:

    1. AUSTIN, J. D. and PIERCE, D. H., J. Amer. chem. Soc. **55,** 661 (1933)
    2. YOFFE, G. T. and NESMEYANOV, A. N., Handbook of Magnesium-Organic Compounds **II**, 239 (1956), Pergamon Press, London
    3. BURLAKOWA, E. W. u. a., in TARUSOW, B. N. (Red.): Praktikum pd obstschei biofisike. Wip. III-IV, (1961) Gos. Isd. „Wysschaja Schkola" Moskwa

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