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Plasmodial Pigmentation of the Acellular Slime Mould *Physarum Polycephalum* in Relation to the Irradiation Period

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Abstract

Yellow Plasmodia of the acellular slime mould *Physarum polycephalum* irradiated with white light of 70 $\mu mol \bullet m^{-2} \bullet s^{-1}$ fluence rate showed two distinct changes in their colour depending on the irradiation period:
1) depigmentation of plasmodia (photobleaching) - within a short-term (hours) exposure, and
2) plasmodia taking on an orange colour as a result of a long period (a few days) of continuous exposure to radiation.

Photobleached plasmodia transferred to the dark resynthetized the pigments and slowly (after about 10 hrs) become yellowish and then regained their yellow colour. General features of these pigments (extraction, solubility, absorption spectra) were comparable to those isolated from non-irradiated organisms.

The pigments isolated from plasmodia subjected to a long period of irradiation differed qualitatively from those produced in the dark grown cultures. The general features of these pigments (e. g. solubility and absorption spectra) point to their being of the carotenoid type.

Keywords: acellular slime mould, *Physarum polycephalum,* photobleaching, carotenoids

Introduction

Acellular slime moulds *(Myxomycetes)* are simple eukaryotes with the Plasmodium as the main trophic stage in their developmental cycle. In nature, plasmodia live in dark and humid places: under the bark of rotten logs, under decaying leaves. Then they migrate to the surface of the substrata where after some time they sporulate. In a standard laboratory culture plasmodia are protected from light and kept in the dark because radiation has an inhibitory effect on their growth. Plasmodia of many species are pigmented, the colour being widely differentiated and ranging from white, yellow, orange, red of various shades, to black. Very frequently taxonomists find in nature the same species of slime mould with different plasmodia colours, and changes in pigmentation have also been observed in plasmodia which stayed for some period on the surface of the substrata. Examples of such phenomena are mentioned by many authors in their papers on taxonomy, and one can read in the book by Martin and Alexopoulos [19] that the observations of *Reticularia splendens* in nature for some period showed the colour changes in Plasmodium of this species from watery white through rose to brown; plasmodium of *Licea variabilis* was yellow or rose or brown-violet; *Dianema depression* was found to possess Plasmodium white or rosy; *Physarella oblonga* - Plasmodium yellow or white; in *Craterium minutum* Plasmodium was white, bright yellow, orange; *Didymium iridis* - yellowish or brown; *Physarum pusillum* was watery-white or light red; in *Physarum polycephalum* Plasmodium was described as yellow, and orange yellow. Based on the observations of the slime moulds growing in their natural habitat it would be difficult to judge which factor is responsible for colour changes in plasmodia when they emerged on the surface of the substratum. Such a possibility is offered by organisms cultured under controlled laboratory conditions. It was found for some species that radiation in the visible range of the spectrum controls many physiological responses in the slime moulds [6, 23, 24, 25, 26] and that there are some reports on the observations, carried out mainly as a by-study, that light is the factor influencing plasmodial pigmentation [1, 6, 10]. Baranetzki [1] observed that yellow plasmodia of *Fuligo septica* became pale-yellow after exposure to sunlight. Gray [10] observed bleaching of the yellow coloured plasmodia in the species: *Physarum polycephalum, Ph. tenerum, Leocarpus fragilis* and in pale yellow *Fuligo septica* exposed directly to artificial radiation emitted from electric bulbs. Depigmentation in *Ph. polyce-*

phalum plasmodia was also described by Daniel [6]. On the other hand, plasmodia of *Ph. gyrosum*, white in standard culture, (i. e. in the dark) take on yellow colour when exposed to white and blue radiation [12]. Yellow plasmodia of *Ph. nudum* change colour to brown in white light and during irradiation with wavelengths in the blue region of the spectrum [24, 25]. There are discrepancies in the papers by various investigators in their opinions on the chemical nature of the pigments, even for the same species. Taking into consideration the above mentioned reports it seemed worthwhile to carry out the investigation of the effect of radiation on plasmodial pigmentation of *Physarum polycephalum*, the species being the preferable and model material for research in the field, especially of photobiological problems. The aim of the present study was the explanation (on the example of *Physarum polycephalum)* of the occurrence plasmodia of various pigmentation in the same species, the study of the nature of changes in the pigments in plasmodia subjected to radiation and the determination of light conditions inducing these changes. It was expected that this might explain the discrepancies reported on the chemical nature of the plasmodial pigments of *Ph. polycephalum*, and might indicate the direction for further study of this subject. Though the acellular slime moulds are simple eukaryotic organisms, they exhibit many structural and functional features homologic with higher *Eucaryota*, and the Plasmodium of *Ph. polycephalum* is recognized as a useful model system for the study of some photobiological problems, among them pigments, photoreception, and photoregulation, which are still waiting for the elucidation which refers not only to the *Myxomycetes*, but is of more general importance.

Materials and Methods

The investigations were carried out on the acellular slime mould *Physarum polycephalum* Schw. Plasmodia were grown on a semi-defined agar medium according to Daniel and Baldwin [7] in 9 cm diameter Petri dishes in the dark at 22°C continuously for various time periods. From the exposed plasmodia and from those kept in the dark, pigments were extracted (see below) and their absorption spectra were determined and calculated per protein contents in the plasmodia sample.

From plasmodia irradiated for a short period and from those kept in the dark pigments were extracted with the solvent mixture methanol/water/borate buffer at the ratio 80:5:15 (by volume) according to Majcherczyk et al. [18]. Plasmodia exposed long-term to radiation were homogenized with 1 mol (NaCl)•dm^{-3} and the pigments were then extracted with methanol. Protein was measured using the method developed by Peterson [21], the principle of which is based on the method of Lowry et al. [17].

Miniature fluorescent tubes "cool day-light", 6 W, emitting mainly the short wavelengths of the visible part of the spectrum were used as light sources; the temperature during exposure to radiation was 22 to 23ºC. Irradiance was measured by means of an LI-Cor spectroradiometer (U.S.A); the absorption spectra were recorded using a Unicam SP 800 recording spectrophotometer.

Chemicals such as glucose, vitamin B, mineral salts and organic solvents were Polish products, haemin was obtained from ICN Pharmaceuticals, Cleveland, U.S.A.; biotin from SERVA; pepton BIO-Lysat from BIO-Merieu, France; Albumine Bovine No A-8022 SIGMA, borate buffer from MERCK, Germany; and bacto-agar from Difco, Detroit, Mich., U.S.A. Distilled water was obtained using a Corbabid - AQUA distiller, Warsaw.

Results

Plasmodia of *Physarum polycephalum*, yellow when cultured in the dark (a standard culture), became milky-white within 3 h of exposure to radiation (white light) at a fluence rate of 70 µmol•m^{-2}•s^{-1}, emitted from the fluorescent tubes (Plate I: A and B). The spectrophotometric measurements revealed a decrease in the absorption (photobleaching) of the crude pigment extract isolated from irradiated plasmodia, showing good correlation between photobleaching and the pigment decreasing in the irradiated plasmodia. The lowest value of the absorption is reached at about 3 h of exposure. Fig. 1 presents the absorption spectra of the crude pigment extract from nonirradiated plasmodia, and from those irradiated for 3 h. In photobleached plasmodia a small amount (about 10%) of the pigment compound was still present, and the situation did not change when the period of irradiation was longer, e. g. 6 h. A distinct relationship between the time of pigment decrease and the fluence rate of radiation manifests itself

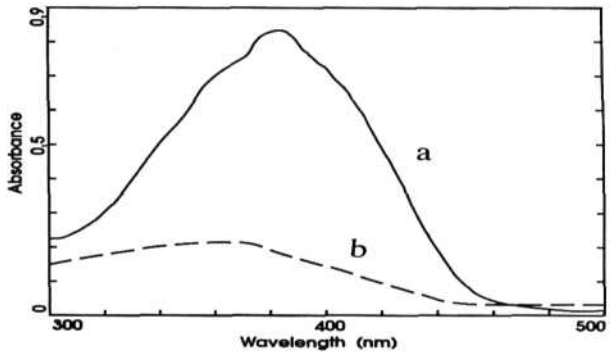

Fig. 1. Absorption spectra of the crude pigment extracts (in methanol) from *Physarum polycephalum* plasmodia kept in the dark (a), irradiated (b). Fluence rate: 70 µmol•m^{-2}•s^{-1}.

(Tab. 1). The photobleached plasmodia are still alive, and when transferred to the dark chamber they slowly regained their pigmentation, becoming yellow again, but not until they had been kept in the dark for a few hours. The pigment from non-exposed plasmodia and that from plasmodia kept in the dark following irradiation was easily extracted with the same solvent mixture (Material and Methods), and the absorption curves were comparable to those

Table 1. Photobleaching of the *Physarum polycephalum* plasmodia depending on the fluence rates of the radiation.

Fluence rates (µmol · m^{-2} · s^{-1})	Time od exposure (h)	Pigment amount (% of initial value)
6.3	20	65
18	10	30
70	3	10
70	6	10
92.5	2	10
159	0.5	10

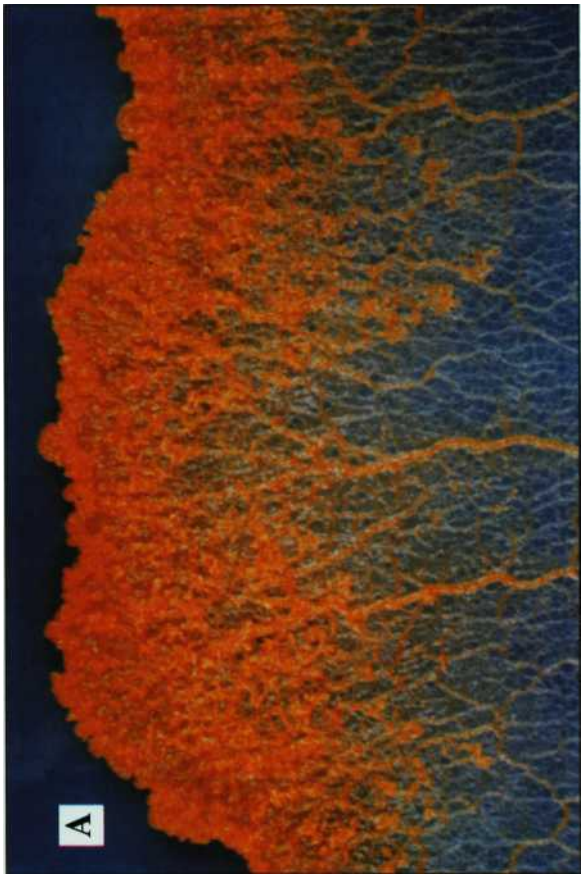

Plate I: Plasmodium of *Physarum polycephalum* from the standard dark culture (A), 3 h irradiated with white light of 70 µmol•m^{-2}•s^{-1}, fluence rate (B), after 3 days of irradiation (C).

obtained from non-irradiated material (Fig. 2). The time course of the repigmentation of the photobleached Plasmodia is presented in Fig. 3. The photobleached Plasmodia were viable even after they had been exposed to the highest (at our disposal) fluence rate of radiation (159 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and were still capable of the basic biological functions, as indicated by their general behaviour, capacity to grow, regeneration after cutting, migration, sporulation and repigmentaion when transferred to the dark. Under prolonged exposure (about 24 h and longer) with the fluence **rate of** 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the photobleached plasmodia of *Physarum polycephalum* became creamy, yellowish, light-orange, and within 3 to 4 days of conninuous irradiation they assumed orange pigmentation (Plate I: C). The pigments could **not** be extracted from plasmodia irradiated for a long period using the same solvent which gives good results when used in the case of non-irradiated cultures. Applying the solvent mixture methanol/water/borate buffer at the ratio 80:5:15 the pigments were easily extracted from plasmodia cultured in the dark or from those repigmented in the dark following bleaching, whereas when using this solvent or even the solvent mixture of different ratios of the components the

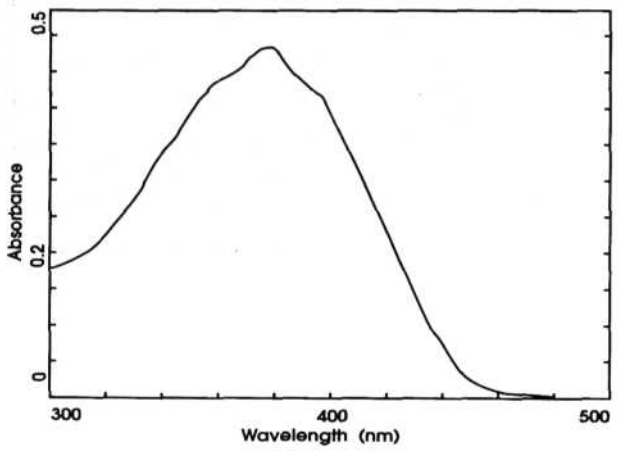

Fig. 2. Absorption spectrum of the crude pigment extract from *Ph. polycephalum* plasmodia kept for 16 h in the dark following 3 h exposure to light. Fluence rate and solvent as in Fig. 1.

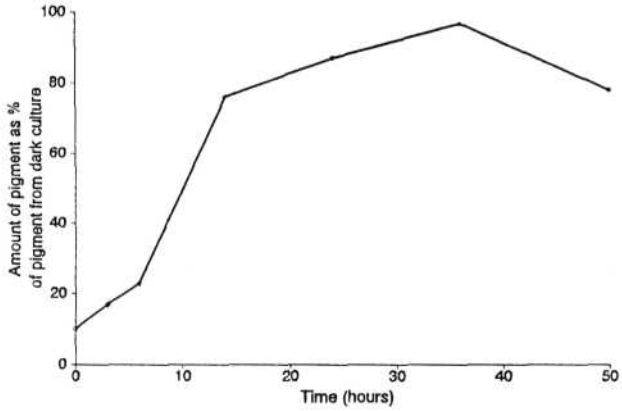

Fig. 3. Time course of pigment resynthesis in photobleached *Ph. polycephalum* plasmodia. X-axis: plasmodia kept in the dark following 3 h irradiation. Y-axis: amount of pigment expressed as the percentage of pigment from non-irradiated plasmodia. Fluence rate as in Fig. 2.

photo pigments could not be extracted. Pigments from *Ph. polycephalum* plasmodia of long-term exposure to radiation could only partially, in very small amounts, be extracted with the use of methanol, acetone, and tetrahydrofurane, and about 90% of the yellow compound remained in the material and was not eluted by repeated extraction. Extraction with dichloromethan, chloroform, ethyl acetate, benzene, hexane, petroleum ether, or other water immissible solvents from fresh and Na_2SO_4 dried material was not successful, or only traces of pigment were extracted. Repeated extraction did not improve recovery. The pigments were, however, eluted from plasmodia even with methanol,

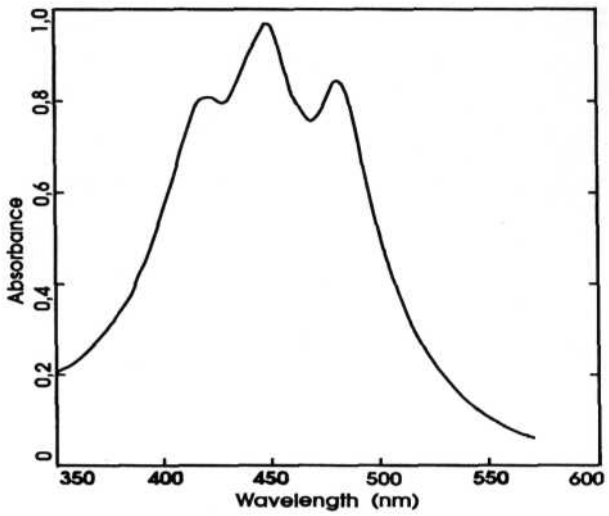

Fig. 4. Absorption spectrum of crude pigment extract of *Ph. polycephalum* plasmodia irradiated continuously during 3 days. Fluence rate and solvent as in Fig. 1.

acetone when the material was pretreated with 10% of trichloric acetic acid (TCA) for some minutes or when it was homogenized with a few ml of 1 mol(NaCl) • dm^{-3}. All these data indicate that pigment synthetized in the plasmodia over a long period of irradiation differs qualitatively from that produced in the dark, or synthetized in photobleached plasmodia after short-term irradiation, and then protected from radiation. The absorption spectrum of a saponified crude pigment extract from plasmodia irradiated for a long period is shown in Fig. 4. Physico--chemical features of the pigments (solubility, absorption spectrum, reactions with $SbCl_3$, and with cone. H_2SO_4) indicate their belonging to the carotenoid-type of compounds. In *Ph. polycephalum* these pigments were produced only during exposure to radiation and the production was stopped when plasmodia were transferred to the dark. Photobleached (short period irradiated) plasmodia transferred to the dark produced pigments characteristic of the dark culture, and also plasmodia irradiated for a long period (3 days) and transferred to the dark synthetized the pigments characteristic for the dark culture, but in such plasmodia a carotenoid type pigments were preserved. Thus, in such plasmodia two different groups of pigment compounds were present, which might be extracted by means of different solvents. When attempting to separate the pigment isolated from irradiated plasmodia into 2 phases, i. e. petroleum ether and methanol, according to Davies [8], two pigmented phases were obtained, the petroleum phase being intensively

pigmented, while the methanol phase was only yellowish. During separation of a crude pigment extract from Plasmodia irradiated for a long period, using thin layer chromatography with silica gel and the solvent benzene/methanol/petroleum ether at the ratio 2:1:2, four spots were found, but solely in one of them (Rf of 0.56) the greatest amount of the pigment was accumulated, in the other three - only traces of the pigment being present. The absorption maxima of the pigment from the main spot were at 425, 451, 480 nm in methanol. Unfortunately, the small amount of material did not permit measurement of the absorption in different solvents or a more precise purification of the material.

Discussion

In plasmodia of the acellular slime moulds a great variety of physiological responses were found to be influenced by light, i.e. growth, migration, protoplasmic streaming, contracting activity of protoplasmic veins, and sporulation [25]. Moreover, radiation has influenced the pigments, which is manifested by the change in the plasmodia colour. The presented results for *Physarum polycephalum* indicate that plasmodia of this species responded to irradiation (white light) by changing the colour, and this can proceed in two different ways depending on the period of their exposure. During a short period of irradiation (hours) at a constant fluence rate of 70 $\mu mol \bullet m^{-2} \bullet s^{-1}$ depigmentation (photobleaching) of plasmodia takes place (Plate I: B, Fig. 1), and under prolonged exposure of 2 to 3 days the plasmodia colour became deep yellow and then orange (Plate I: C).

There are a few papers supplying the information on pigment changes in plasmodia exposed to radiation, which was discovered by chance in the course of the study of other problems [6, 10, 12, 24]. Photobleaching of the *Physarum polycephalum* plasmodia was observed by Gray [10, 6]; unfortunately, no information is available from their papers concerning the radiation under which the plasmodia were exposed, which makes impossible the comparison of the results presented in this paper with previous findings. Daniel [6] mentioned that three crude pigment fractions obtained from plasmodia of *Ph. polycepalum* lost their colour during irradiation *in vitro*. Changes of colour from lemon-yellow to creamy was noticed by Baranetzki [1] in Plasmodium of *Fuligo septica* found in nature on the surface of natural substratum; the author believed it was the effect of the slime mould's exposure to solar radiation. A long period of keeping the photobleached plasmodia in the dark indispensable for their repigmentation indicates that the pigments were synthesized *de novo* in the slime mould. Therefore, one can suppose that during plasmodia irradiation a photodegradation (photodestruction) of the pigments takes place rather than a simple conversion of these compounds into leukoforms. Preliminary investigations carried out using high performance liquid chromatography (HPLC) showed that the kinetics of the individual pigment bleaching were very similar to each other; the composition of the resynthetized pigments was found to be the same as that from control, nonirradiated plasmodia which were kept all time in the dark (Rakoczy, Majcherczyk, Huettermann unpubl.).

The depigmented plasmodia under prolonged, continuous irradiation slowly regain pigmentation and within 2 to 3 days of exposure became yellowish, and then orange due to the production of the carotenoid type pigments. This is the only information for the acellular slime moulds that Plasmodium of *Ph. polycephalum* show two types of colour changes in response to the action of the radiation. In order to find out how common are these phenomena for the acellular slime moulds further study in this field is needed with the use of many individuals of these organisms.

The chemical nature of the pigments responsible for the plasmodia colour is not definitely recognized, though for a long time it has been a subject of interest for various investigators; moreover, there are discrepancies in this question regarding even the same species e.g. for *Ph. polycephalum*. Pigments of this species were isolated from plasmodia growing in the laboratory under different light conditions, and from plasmodia or sporangia of various maturity collected from natural conditions [3, 5, 15, 18, 28 and others]. The present opinion is that the main pigments of this species are polyenes containing nitrogen easily dissolving in the solvents of small polarity, and which absorb radiation in the range of short wavelengths of the visible part of the spectrum (maximum absorption of the crude pigment extract in methanol is at 380-382 nm and a bathochromic shift towards of the longer wavelengths depending on the acidity of the solvent takes place, but not exceeding 400 nm. The pigmentation of *Ph. polycephalum* plasmodia changed significantly with the age of the culture due to the acidification of the medium by growing Plasmodium. The plasmodia were yellow within a few days, however, in prolonged cultures, the colour turned to deep yellow, even orange in old culture, but this change was not accompanied by any alteration in the general absorption of the pigment extracts [26].

The data of this work indicate that the discrepancies found in literature concerning pigments in *Ph. polycephalum* could be due to the materials collected from different light conditions and used for the study. It is necessary to take this into consideration, particularly the radiation conditions, and not only those in which the slime mould was actually grown, but also it is necessary to know its past light conditions.

Carotenoids belong to pigments widely distributed throughout the living world: they occur in plants, animals, and bacterial systems. Pigments of this type have also been found in the acellular slime moulds in the premature fructifications *of Lyco**gala** epidendrum, L. **flavo-fuscum*** [5, 15, 16, 29], and *L. exiguum* (Rakoczy, Majcherczyk, Huettermann unpubl. data), β-carotene is probably the main pigment in *L. epidendrum*. It is difficult to judge whether the carotenoid pigments found in these species collected from natural conditions were synthetized in effect of the action of solar radiation on plasmodia when they emerged on the surface of a substratum from the dark where they lived. Plasmodia of *Lycogala* found on the surface of natural substrata were described as rosy or red in colour [19], but the colour when they lived under bark in the dark is unknown.

Similarly, as in many species of fungi [2, 27], plasmodial carotenoids in the acellular slime moulds can be synthetized also under irradiation. This is true at least for *Physarum nudum* [25] and now for *Physarum polycephalum*. Contrary to some fungi, in which a short term of irradiation was able to induce carotenoid synthesis and the process being continued in the dark, in *Ph. polycephalum* plasmodia the pigments of such a type were synthetized during long-term continuous exposition to radiation. Radiation is a known factor which has a specific, peculiar effect

on the synthesis of the carotenoids. In some plants its action contributes to increasing the quantity of carotenoids, while in the others, e. g in non-photosynthetic plants it triggers or induces *de novo* the synthesis of this compound type [2, 9]. It has been well documented that carotenoids act as the photoprotectants in microorganisms [13, 14, 20]. One cannot exclude that these pigments produced in irradiated plasmodia of *Ph. polycephalum* also play a protective role against the inhibitory effect of radiation on the growth in young plasmodia and a harmful effect on the synthesis of a specific protein necessary for the development of the reproductive forms. Visible radiation inhibits the type of differentiation into spherules and it is needed for the induction of sporulation. It was established by various investigators [11] for *Ph. polycephalum* that during differentiation into reproductive forms the specific proteins are produced. The carotenoid pigments synthesis in the slime mould could be very important from the ecological point of view - it enables Plasmodium to survive, permitting it to remain alive under long-term continuous irradiation. The obtained results are only preliminary ones and further detailed study in this field is necessary.

Obtained results throw new light on the plasmodial pigments and indicate the necessity of paying special attention to the type of material used in the course of the investigations of the chemical nature of these compounds. They also open a new way for investigations on the physiological role of the pigments present in the plasmodia.

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