PN 1215

Effect of light and streptomycin on the incorporation of sulfate into sulfolipids in *Euglena gracilis*

Sulfolipids are present both in animal tissues and plants. Whereas the sulfolipids from animals contain a sulfate ester linkage with carbohydrates of cerebrosides, the plant sulfolipids are shown to be sulfonic acids where there is a direct linkage between carbon and sulfur.

The importance of sulfolipids in photosynthetic organisms has not been thoroughly elucidated. However, the work of BENSON *et al.*[1] indicate that sulfocarbohydrates may undergo a similar type of glycolysis as phosphocarbohydrates. Sulfolipids are also present in various photosynthetic algae like Chlorella. The high concentration of these lipids in algae suggests that they may play an important role in the structural integrity of the organism or its metabolism. Numerous reports from various laboratories suggest that sulfate incorporation in plants and algae is light dependent[2,3].

The present investigation was undertaken to study the following three aspects: (1) uptake of sulfate by normal green cells, cells grown in dark and the streptomycin-bleached cells, (2) the enzymic synthesis of active sulfate under the conditions described above and (3) the incorporation of sulfate into sulfolipids under the same experimental conditions.

Euglena gracilis Z-strain originally obtained from Dr. G. I. M. Ross was used.

TABLE I

EFFECT OF LIGHT AND STREPTOMYCIN ON $^{35}SO_4^{2-}$ UPTAKE

100 ml of medium containing 100 μC of $^{35}SO_4^{2-}$ was cultured with *Euglena gracilis* under the three conditions mentioned in the text. After growing the cells for 5 days, the flasks were taken out, shaken thoroughly and the initial count determined. The cells were washed till the washings were free of $^{35}SO_4^{2-}$ and the sulfate uptake by the cells was determined by counting.

	Initial counts/100 sec/ml of medium $\times 10^{-6}$	$^{35}SO_4^{2-}$ *uptake by the cells (counts/100 sec/mg dry wt.* $\times 10^{-4}$*)*
Green	2.3	6.0
Dark-grown	2.2	6.3
Streptomycin-bleached	2.4	6.1

To study the uptake of sulfate, the cells were cultured in 100 ml of single-strength medium[4] with 100 μC of $^{35}SO_4^{2-}$. One set of cells were grown over fluorescent light, another set in dark and the third set in the medium containing 600 μg/ml of streptomycin sulfate for 3 days in dark and then transferred to light. To correct for the dilution of $^{35}SO_4^{2-}$ by streptomycin sulfate, Na_2SO_4 (at the same concentration with respect to sulfate) was added to the first two sets of flasks. The cells were collected on the 5th day and washed thoroughly with 0.1 M sodium acetate, adjusted to pH 4, till the washings are free of $^{35}SO_4^{2-}$. The cells were suspended in a constant volume of water and the radioactivity of the cells after drying on a planchet counted in a scintillation counter. It was found that the uptake of $^{53}SO_4^{2-}$ was not affected by the mode of culturing of the cells (Table I).

Abbreviation: PAPS, phosphoadenosinephosphosulfate.

The presence of enzyme system synthesizing active sulfate in photosynthetic organisms has been indicated by various investigators[5]. In the present investigation the enzyme system has been identified in Euglena as follows: cells grown in light were collected and disrupted as mentioned earlier[6] and centrifuged at 20000 × *g* for 20 min. The supernatant containing the sulfate-activating enzyme system was dialysed for 3 h against 20 vol. of 0.005 M phosphate buffer (pH 7.2). The assay method used was the same as that of BALASUBRAMANIAN AND BACHHAWAT[7]. The formation of [^{35}S]PAPS by the Euglena enzyme system was demonstrated by paper electrophoresis and it was further confirmed by coupling with phenol sulfokinase of liver and identifying the *p*-nitrophenol-[^{35}S]sulfate formed[8].

The relation between age of the cells and the enzyme activity was next studied. It was found that the specific activity of the PAPS-synthesizing enzyme system increases with age of the cells till the 5th day and then decreases for both light- and the dark-grown cells. The organism has a growth curve with a logarithmic phase of 3–6 days[9]. Cells grown in presence of streptomycin have very little PAPS-synthesizing activity, compared to the other two kinds of cells, and the specific activity of the enzyme remains almost constant throughout the period of growth (Fig. 1).

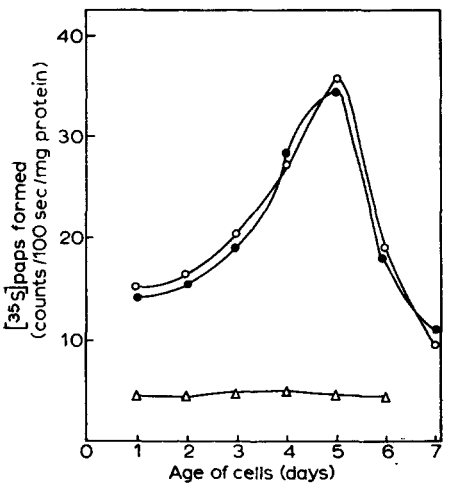

Fig. 1. Relation of age of cells to the specific activity of the PAPS-synthesizing enzyme system. O—O, normal green cells; ●—●, dark-grown cells; △—△, streptomycin-bleached cells.

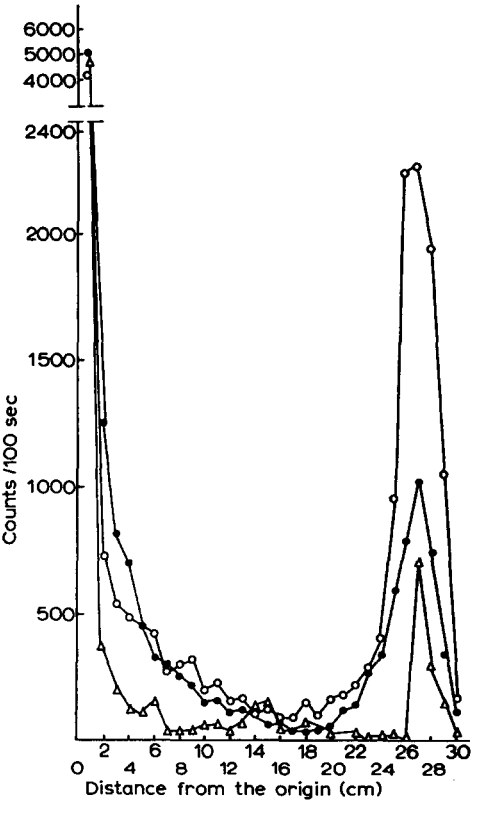

Fig. 2. Distribution of radioactivity on the paper after the sulfolipids are chromatographed in the solvent mentioned in the text. O—O, normal green cells; ●—●, dark-grown cells; △—△, streptomycin-bleached cells.

The studies on the $^{35}SO_4^{2-}$ incorporation into sulfolipids were carried out as follows: cells were grown under the three different experimental conditions with 100 μC of $^{35}SO_4^{2-}$ and the sulfolipids were isolated under identical conditions by the method of BLOCK[10]. The sulfolipids extractable in chloroform–methanol (2:1, v/v)

were concentrated to a small volume and chromatographed in ethanol–*tert.*-butyl alcohol–conc. NH_4OH–water (60:20:5:15) solvent[10]. The distribution of radioactivity on the paper for the sulfolipids isolated from the three different kinds of cells is shown in Fig. 2. Inorganic $^{35}SO_4^{2-}$ stays at the origin but the sulfolipid isolated under the conditions described above has an R_F value of 0.9.

These results indicate that light has no role so far as the sulfate uptake and activation are concerned. One of the most important steps in the utilization of sulfate in plant and bacterial systems is the reduction of sulfate to sulfite and the formation of PAPS is an obligatory step in the reduction of sulfate to sulfite by microorganisms[11]. The role of streptomycin in the inhibition of the active-sulfate synthesis and subsequent inhibition of incorporation of sulfate into sulfolipid indicate that PAPS may be the precursor for the sulfate incorporation into sulfolipid. Work by various authors indicate that sulfate reduction may be dependent on light[12]. The above studies show that the synthesis of sulfolipid is dependent on light. The sulfate needs to be activated to PAPS before it can be reduced to sulfite and these two steps precede its incorporation into sulfolipid. Diminished photosynthetic formation of ATP and reduced nucleotide may be responsible for the impairment of the sulfolipid synthesis.

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