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Carotenoids as Protectors against Photodynamic Inactivation of the Adenosine Triphosphatase of *Mycoplasma laidlawii* Membranes

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Carotenoids are common components of the bacterial plasma membrane. Recent reports indicate that they act as protectors against photodynamic destruction, which is of particular importance to photosynthesizing micro-organisms and to bacteria exposed to solar radiation (Jensen, 1965). Thus highly pigmented bacteria resisted the lytic effect induced by light far better than did colourless mutants (Burchard & Dworkin, 1966). More specifically, membrane carotenoids were shown to protect membrane enzymes such as NADH oxidase and succinate dehydrogenase against inactivation by light in the presence of photosensitizing dyes (Mathews & Sistrom, 1960).

The adenosine triphosphatase activity of the *Mycoplasma* organisms has been localized in their cell membrane (Pollack, Razin & Cleverdon, 1965). In view of the vital roles that have been attributed to this enzymic activity in biological membranes (Skou, 1965) it seemed worth while to test whether it is sensitive to photodynamic inactivation, and whether carotenoids can protect against it. *Mycoplasma laidlawii* is particularly suitable for this type of experiment, since it synthesizes carotenoid pigments localized in the membrane and the degree of membrane pigmentation can be controlled by regulating the amount of acetate and propionate in the growth medium (Razin & Rottem, 1967).

Methods. M. laidlawii (oral strain) was grown in a modified Edward medium (Razin, 1963). To obtain highly pigmented cells sodium acetate was added to the growth medium to a final concentration of 20 mM, and colourless cells were obtained by addition of 50mM-sodium propionate to the medium. The organisms were harvested after 16 hr. of incubation at 37° by centrifugation at 13000*g* for 15 min. The sedimented cells were washed once in 0·25 M-NaCl and resuspended in the same solution. Portions (1ml.) of pigmented or colourless *M. laidlawii* cell suspensions containing 8 mg. of cell protein were placed in serological test tubes. Various concentrations of toluidine blue (British Drug Houses Ltd., Poole, Dorset) were added. The test tubes were tightly closed with rubber caps and placed horizontally in an ice bath. The tubes were exposed for 3 hr. to the light of a 300 w tungsten lamp placed at a distance of 7 cm., when the lamp supplied approx. 2000 ft.-candles. Cell suspensions placed in test tubes wrapped with dark paper and aluminium foil were simultaneously incubated in the ice bath and served as dark controls. At the end of the incubation period the cell membranes were isolated by osmotic lysis of the organisms (Razin, 1963) and their adenosine triphosphatase activity was measured by the release of P_i from ATP. Results were expressed as μmoles of P_i released/mg. of membrane protein in 30 min. (Rottem & Razin, 1966).

Results and discussion. M. laidlawii cells grown in the presence of acetate were highly pigmented, whereas those grown with propionate were essentially colourless. Extraction of a pellet of acetate-grown cells (containing 10 mg. of cell protein) with 5 ml. of boiling ethanol resulted in a yellow extract having E_{440} 0·48–0·56. The ethanol extract obtained from propionate-grown cells was colourless and had E_{440} 0·01–0·03. Exposure of a colourless *M. laidlawii* cell suspension to visible light in the presence of 2·5μM-toluidine blue resulted in the loss of 78% of the membrane-associated adenosine triphosphatase activity. Pigmented *M. laidlawii* cells exposed to light under the same conditions lost only 13% of this enzymic activity (Table 1).

Table 1. *Photodynamic inactivation of the adenosine triphosphatase activity of pigmented and colourless* M. laidlawii *membranes*

The illumination of the cells and the determination of the adenosine triphosphatase activity in the membranes were carried out as described under 'Methods'. Where indicated, an ethanolic solution of 20 mg. of β-carotene (0·02 ml.)/ml. was added to 1 ml. of cell suspension before illumination. The enzymic activity is expressed as μmoles of P_i released/mg. of membrane protein in 30 min.

Concn. of toluidine blue (μM)	Adenosine triphosphatase activity: Colourless cells: No carotenoids added	Colourless cells: With β-carotene	Pigmented cells
0	4·25	4·40	4·65
2·5	1·04	3·48	4·15
10	0·60	1·64	2·74

The difference between pigmented and colourless cells was less pronounced at higher toluidine blue concentrations. The dye did not affect the adenosine triphosphatase activity of cells kept in the dark. No photoreaction occurred in the absence of toluidine blue. β-Carotene as well as carotenoids extracted from pigmented *M. laidlawii* cells protected the adenosine triphosphatase activity of colourless cells against inactivation by light (Table 1). The adenosine triphosphatase activity of the membranes was not affected by light in the presence of toluidine blue when the experiment was carried out under nitrogen. This indicates that the photoinactivation process is of an oxidative nature, as was also shown for photosynthetic bacteria (Griffiths, Sistrom, Cohen-Bazire & Stanier, 1955), *Sarcina lutea* (Mathews, 1963) and *Myxococcus xanthus* (Burchard & Dworkin, 1966).

To the best of our knowledge, photodynamic inactivation of adenosine triphosphatase has not been described so far. Inactivation of the membrane adenosine triphosphatase may be associated with the photokilling effect of photosensitizing dyes in view of the vital roles attributed to this enzymic activity. Our results are in line with the suggestion by Mathews & Sistrom (1960) that the plasma membrane, and not the cell nucleoid, is the cellular site for the lethal action of light.

The biological significance of the carotenoids in *M. laidlawii* is still not clear. Our results supply indirect evidence for the thesis that carotenoids may protect *M. laidlawii* cells against solar radiation. *M. laidlawii* has been so far the only *Mycoplasma* isolated from sources other than the animal body, such as sewage (Laidlaw & Elford, 1936), soil, compost and withered leaves (Seiffert, 1937). Perhaps the presence of carotenoids helps this *Mycoplasma* to survive or multiply in these environments where there is a danger of solar radiation.

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