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BIOCHEMISTRY

Cytochrome c – Cytochrome Oxidase in Bioluminescent Fungi

REFERENCES have been made to the presence of cytochrome oxidase in the luminescent bacteria[1–3], but none to the presence of cytochrome oxidase in luminescent fungi. Therefore a survey investigation was made of five genera of bioluminescent fungi using a modified '*G*-Nadi' reagent. It should be noted that the '*G*-Nadi' reaction is not a conclusive test for cytochrome *c*–cytochrome oxidase, but can be considered a good presumptive test when suitable controls are utilized[4,5].

Cultures of *Panus stipticus*, *Armillaria mellea*, *Armillaria fusipes*, *Mycenna polygramma*, *Clitocybe illudens*, and *Collybia velutipes* were grown on 10 per cent bread crumb agar at room temperature until an adequate light output-level of approximately 120–600 mμ lumens was reached. Culture and photometric techniques have already been described[6]. Squares, 1·5 cm × 1·5 cm, were cut from the mycelial mats of each organism, and the lower surface was scraped clean of adhering agar. The squares were crushed with a stirring rod in a test tube and 0·5 ml. phosphate buffer (M/15) *p*H 5·8, 1·25 ml. 10 per cent α-naphthol in ethanol, and 1·25 ml. of a 0·12 per cent aqueous solution of dimethyl-P-phenylene diamine hydrochloride[7] were added and mixed. Four controls were run for each experiment. The first control consisted of the '*G*-Nadi' reagents without the organisms, the second consisted of the '*G*-Nadi' reagents with the various organisms plus 1 drop of a 0·1 per cent solution of potassium cyanide. The third control consisted of heating the square of mycelium in distilled water for 15 min at 60° C. The water was decanted, the squares crushed, and the '*G*-Nadi' reagents were then added. The last control was a crushed square (1·5 cm by 1·5 cm) of bread crumb agar to which the '*G*-Nadi' reagents were added. Development of a blue colour (indophenol blue) within 3 min was considered a positive test. All the organisms gave a positive '*G*-Nadi' reaction (Table 1).

Table 1

Organism	'*G*-Nadi' plus organism	Organisms heated to 60° C for 15 min and then '*G*-Nadi'	'*G*-Nadi' plus organisms plus KCN	'*G*-Nadi' reagent without organism or KCN
P. stipticus	+	–	–	–
A. fusipes	+	–	–	–
A. mellea	+	–	–	–
C. illudens	+	–	–	–
C. velutipes	+	–	–	–
M. polygramma	+	–	–	–

The negative '*G*-Nadi' reactions obtained on the addition of potassium cyanide and the heat inactivation of the specimen help to strengthen the supposition that cytochrome *c*–cytochrome oxidase was responsible for the positive '*G*-Nadi' reaction.

The presumptive demonstration of cytochrome *c*–cytochrome oxidase (and the possible presence of the other cytochromes) in the bioluminescent fungi is quite attractive, for it fits into the evolutionary scheme proposed by McElroy and Seliger[8]. In this scheme bioluminescence was a suggested technique whereby anaerobic organisms dealt with the oxygen which gradually accumulated in the primitive reducing atmosphere before the development of cytochrome and cytochrome oxidase systems. The luminescent bacteria at the present time have cytochrome–cytochrome oxidase systems, plus the luminescent system which McElroy and Seliger call 'vestigial'. In a similar manner the presumptive cytochrome *c*–cytochrome oxidase system and the 'vestigial' luminescent system were present in the five genera of luminescent fungi (Basidiomycetes) examined. Thus, further support has been given to McElroy and Seliger's proposition by the presumptive presence of a cytochrome *c*–cytochrome oxidase and bioluminescent system in the more highly developed and evolutionarily advanced bioluminescent fungi.

Further work is being carried out to confirm the presence and types of the cytochrome–cytochrome oxidase systems spectrometrically.

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Ribonucleic Acid-primed Synthesis of RNA following Viral Infection

THERE are several possible mechanisms for the replication of RNA in RNA viruses. Viral RNA may induce synthesis of DNA followed by a DNA directed synthesis of viral RNA or, alternatively, there may be direct synthesis of RNA on an RNA template from the infecting virus. In order to distinguish between these two alternatives, the activities of the polymerase enzymes, DNA nucleotidyltransferase and the DNA-primed and RNA-primed RNA nucleotidyltransferases of normal Krebs II mouse ascites tumour cells and of cells infected with encephalomyocarditis virus[1] have been investigated.

No consistent differences could be demonstrated in the DNA nucleotidyltransferase and the DNA-primed RNA nucleotidyltransferase activities of infected and uninfected cells but significant increases in the activity of RNA-primed RNA nucleotidyltransferase were regularly observed in the infected cells.

Washed Krebs II cells were suspended in Earle's medium in duplicate stoppered flasks at a concentration of 1×10^7 cells per ml. To one flask a preparation of virus (3 plaque-forming units per cell) was added. Samples of infected and uninfected cells were withdrawn at zero time and at 1- or 2-h intervals after infection and centrifuged at 750*g* for 5 min at 0°. The packed cells were resuspended in 0·01 M *tris* buffer *p*H 7·5, homogenized in a Potter type homogenizer and submitted to sonic vibration (50 W; 20 kc/s) for 1–2 min at 0°. Complete disruption of cells and nuclei by this treatment was confirmed microscopically. The suspension was centrifuged at 2,250*g* for 15 min at 0° and the supernatant fraction was removed and its protein concentration adjusted to 1·5–2·0 mg/ml. with 0·01 M *tris* buffer *p*H 7·5. Protein was estimated by the method of Lowry, Rosebrough, Farr and Randall[2].

DNA nucleotidyltransferase was assayed by the method of Keir, Binnie and Smellie[3], DNA-primed RNA nucleo-