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Light sensitivity of the *n*-alkane-induced fatty alcohol oxidase from *Candida tropicalis* and *Yarrowia lipolytica*

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Summary. Fatty alcohol oxidase (FAOD) activities in microsomal membrane fractions from *n*-alkane-grown *Candida tropicalis* and *Yarrowia lipolytica* rapidly decreased on exposure to light. The rate of inactivation of the enzyme depended upon the intensity and wavelength of the incident light, but was diminished under anaerobic conditions. Partially purified and solubilised FAOD preparations remained photosensitive: FAOD from *Y. lipolytica* was more photo-labile than that from *C. tropicalis*.

Introduction

The utilisation of *n*-alkanes by various yeasts proceeds via the production of a fatty alcohol. Since the early work of Lebeault et al. (1970) the enzyme responsible for the oxidation of this fatty alcohol to the corresponding fatty aldehyde was assumed to be an nicotine adenine dinucleotide (NAD^+)-dependent dehydrogenase. Recently, the presence of fatty alcohol oxidases (FAOD) in a variety of *n*-alkane-utilising yeasts has been suggested by Krauzova (1985, 1986), Il'Chenko et al. (1984) and Blasig et al. (1988). In a previous communication (Kemp et al. 1988) we provided firm evidence for the presence of such an FAOD in the yeast *Candida tropicalis* (ATCC 20336) when grown on *n*-alkanes. We have also observed an induced FAOD in alkane-grown *Yarrowia lipolytica*.

In this paper we report the photo-labile nature of these FAOD and discuss the implications this has for the nature of these enzymes and for the procedures required to protect their activity.

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Materials and methods

Membrane preparations and enzyme assays. Candida tropicalis ATCC 20336 and *Yarrowia lipolytica* ATCC 2076 were grown as previously described (Kemp et al. 1988). Cells were harvested, microsomal membrane preparations were made and FAOD and fatty aldehyde dehydrogenase (FALDH) activities were assayed by the methods previously reported (Kemp et al. 1988). Specific activities were computed as nmol product formed/min per milligram of protein. All unnecessary exposure to light was avoided and the enzyme fractions were stored in the dark until the time of assay, unless otherwise indicated.

Photo-inactivation studies. Membrane fractions from alkane-grown yeasts were suspended in 10 m*M* *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulphonic acid) (HEPES) buffer (pH 7.3) containing 0.2 m*M* ethylenediaminetetraacetic acid (EDTA) and 0.1 m*M* dithiothreitol (DTT), to give 3–5 mg protein/ml. A sample of this suspension was placed in a plastic cuvette, with a 1-cm light path, held at the side of a glass-walled ice bath. The ice bath was then placed in a closed box with a single aperture opposite the sample cuvette (Fig. 1). A xenon lamp was used as a source of intense white light. The intensity of the light was controlled by placing neutral density filters (Eastman Kodak, Rochester, New York, USA) over the aperture of the box.

The wavelength of the incident light was controlled by using a selection of optical filters placed over the aperture of the box. The optical characteristics of the filters were assessed using a Pye Unicam SP8100 (Cambridge, England) scanning spectrophotometer.

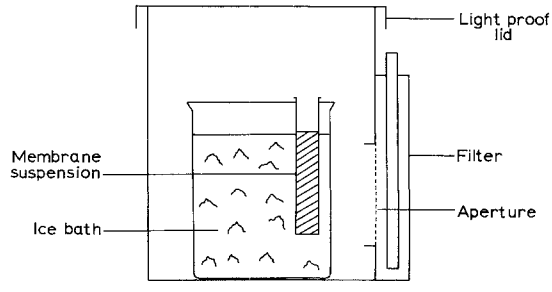

Fig. 1. Apparatus used to study the effects of light intensity and wavelength on photo-inactivation

Anaerobic conditions were achieved by passing O_2-free N_2 through the membrane sample in a 1-ml cuvette sealed with a Subaseal rubber cap, vented with a syringe needle. Stringent anaerobic conditions were achieved by the injection of decanol (100 μ*M*) and peroxidase (50 μg) into a membrane sample which had previously been gassed with nitrogen. The oxidation of the substrate by FAOD then effectively removed all traces of O_2.

Protein concentration. This was assayed using the bicinchoninic acid method of Smith et al. (1985).

Materials. The EDTA was obtained from BDH (Poole, Dorset, UK) and HEPES, peroxidase and sodium cholate were obtained from Sigma (St. Louis, Mo, USA); neutral density filters were obtained from Eastman Kodak; bicinchoninic acid reagent was obtained from Pierce UK (Chester, England); DTT was obtained from Boehringer (Mannheim, FRG); and all other chemicals used were of analytical grade.

Results and discussion

Photo-inactivation

Exposure of microsomal membranes from *n*-alkane grown yeasts to light resulted in the inactivation of FAOD activity. No loss of activity was observed in a control sample of membrane stored at 0° C in the dark, nor was the activity of FALDH affected by exposure to light (Fig. 2).

The loss of FAOD activity was exponential and a logarithmic plot of the activity against the time of exposure to light produced a straight line indicating a first order reaction. The intensity of the incident light was varied using a series of neutral density filters. The rate of inactivation decreased as the intensity of the incident light decreased (Fig. 3). The rate of photo-inactivation could also be shown to be dependent upon the optical density of the neutral filters (Fig. 4). There was thus a strict proportionality between the amount of light reaching the FAOD and the rate of its inactivation.

The loss of activity was highly dependent upon the wavelength of the incident light. By

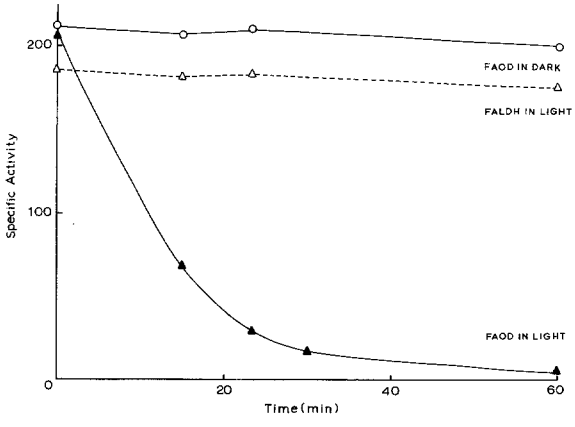

Fig. 2. Effect of exposure to light on the activity of fatty alcohol oxidase (FAOD) and fatty aldehyde dehydrogenase (FALDH). The FAOD activities were measured using dodecanol as substrate; FALDH activities were measured using decanal as substrate. All samples were maintained at 0° C

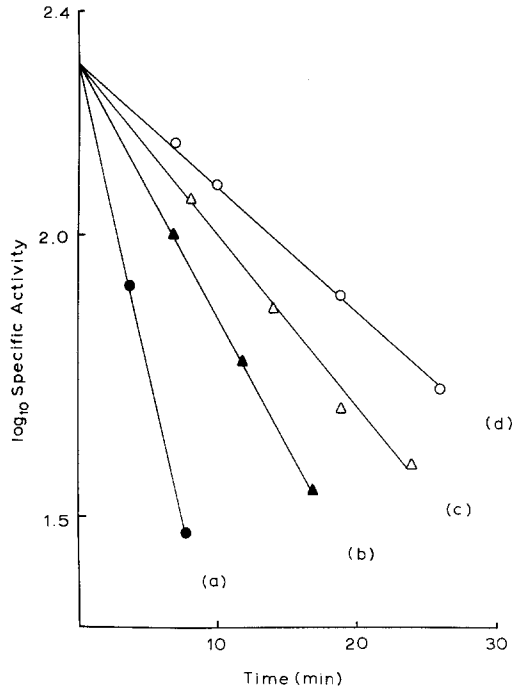

Fig. 3. Effect of neutral density filters on the rate of photo-inactivation of FAOD: *(a)* no filter; *(b)* neutral density filter OD 0.232; *(c)* neutral density filter OD 0.474; *(d)* neutral density filter OD 0.777

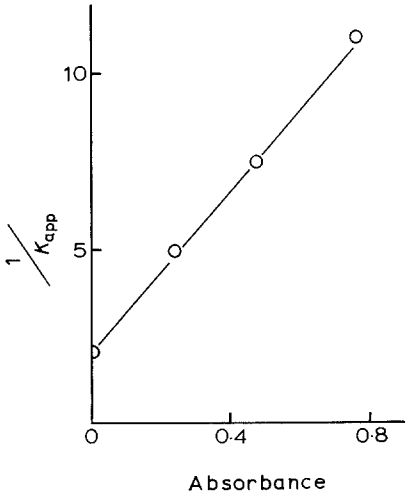

Fig. 4. Inverse proportionality of the apparent first-order rate constant of photo-inactivation (K_{app}) to the absorbance of the neutral density filters used to regulate the intensity of the incident light falling on the microsomal membrane preparations. Values of K_{app} were obtained from Fig. 3