SPIN-LABELED YEAST CELLS

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Received December 8, 1971

SUMMARY: The facultative yeast cells have been spin-labeled biosynthetically with the 12-nitroxide stearic acid label under aerobic as well as anaerobic conditions. ESR spectra of the aerobic cells were different from those of the anaerobic cells. The signal intensity of the aerobic cells decreased upon removal of oxygen from the culture. KCN also caused decay of the signal to a lesser extent. The ESR peak height of some spin-labeled anaerobic cells increased for several hours and then decreased upon aeration. The increase and decrease of the ESR signal of the spin-labels buried in the yeast cell membranes are related, at least partly, to the respiratory activity of the mitochondria.

Increasing numbers of papers have recently appeared on the spin-label studies of the biological membranes.[1-7] The spin-labels used in these studies are mostly synthetic fatty acid derivatives, which were able to detect the local environments of the membranes. The biosynthetically incorporated labels, rather than the synthetic ones, would be more desirable to study biological membranes in more intact states. Keith et al. were the first to label the membranes biosynthetically; they fed Neurospora crassa with the fatty acid label and found the labels incorporated into the membrane lipids.[1]

In the present study, we have chosen the facultative yeast cells for the biosynthetic spin-labeling of the membranes. The cells require unsaturated fatty acid and ergosterol for anaerobic growth and the spin-labeled anaerobic yeast would provide a unique system for investigation of structural aspects of mitochondriogenesis. Moreover, there are useful mutants for studies of membrane structure and function. We here report briefly our results on the spin-labeling of yeast cells under aerobic as well as anaerobic conditions.

EXPERIMENTAL

Culture Conditions: The cells of Saccharomyces cerevisiae (ATCC 12341) were grown at 30°C in a medium described by Schatz and Klima[8] in Erlenmeyer flasks. For aerobic growth, glucose (1% or 10%) and the 12-nitroxide stearic acid label (< 0.1mg/ml) were added to the medium and the flasks were shaken on a reciprocal shaker. The label, N-oxyl-4'-4'-dimethyloxazolidine derivative of 12-ketostearic acid, was synthesized according to the procedure described by Waggoner et al.[9] For anaerobic growth, the stearic label (0.1 ~ 0.2mg/ml) or mixture of oleic acid and the label at various ratios was supplemented to the medium, in addition to ergosterol (12 ppm) and glucose (10%). After inoculation, the medium was flushed with oxygen-free nitrogen for 3 hrs and the cells were grown with constant stirring. The gas evolved during the growth was allowed to escape through a mercury trap.

ESR Measurements: A fixed volume (~5ml) of the culture was taken and centrifuged at low speeds. The supernatant was discarded and the sedimented pellet was taken in a cylindrical aqueous sample tube (inside diameter ~0.8mm) for ESR measurements. For washing of the anaerobic cells, a phosphate buffer solution (pH 6.5) was previously flushed with nitrogen. ESR spectra were recorded at 23°C on an X-band commercial spectrometer (JEOLCO Model ME-1X).

RESULTS

Growth Curve: The yeast cells precultured to their early stationary phase were inoculated in a 10^4 dilution into the medium containing glucose (1% or 10%) and the stearic label (0.025mg/ml) for aerobic growth, and into the medium containing glucose, ergosterol, and the stearic label (0.2mg/ml) for anaerobic growth. The growth of the cells was followed turbidimetrically. The ESR spectra of the culture were also measured during the growth. The signal consists mainly of sharp three lines, which measures amount of the label dissolved in the culture. The obtained growth curves are shown in Fig. 1, together with the control aerobic and anaerobic growth data. The growth curves indicate that the stearic label does not affect both aerobic and anaerobic

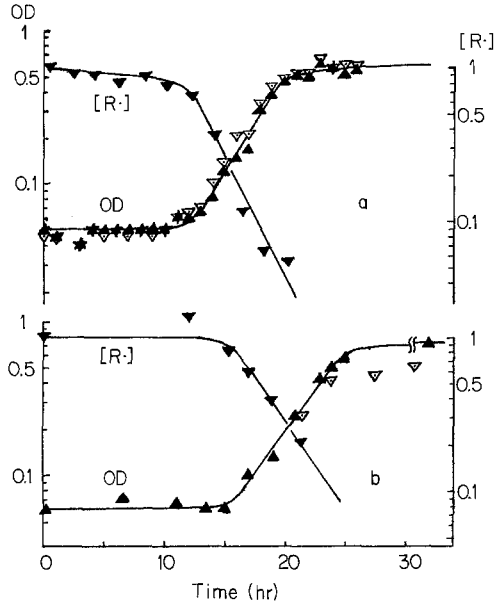

Fig. 1. Growth curves of the yeast cells in presence of the stearic label. [R·] represents the relative amount of the spin-labels dissolved in the culture. a) Aerobic growth with 10% glucose and 0.025mg/ml stearic label. The open triangles represent the growth with no added labels. b) Anaerobic growth with 10% glucose, 12 ppm ergosterol, and 0.2mg/ml stearic label. The open triangles represent the control growth with 0.2mg/ml oleic acid in place of the label.

growth. The label in the culture starts to decrease with the increase of the cells and, in the early stationary phase, it decreases below the sensitivity of the spectrometer.

The lipids of the spin-labeled aerobic and anaerobic cells were extracted with chloroform : methanol (2:1) and separated on silica gel H thin-layer chromatogram with various eluents. The spin-label signals were found in fatty acids, neutral lipids, phosphatidylcholine, phosphatidylethanolamine, and other phospholipid fractions. Preliminary quantitative analysis showed that more signals were found in the fractions of fatty acids and neutral lipids than in the phospholipids fraction and the signal intensity per inorganic P was not evenly distributed among the phospholipids.

ESR Spectra of Spin-Labeled Aerobic Cells: Fig. 2a shows a typical ESR spectrum of the yeast cell pellet centrifuged down from the culture grown to the early stationary phase. Fig. 2b is the spectrum of the pellet washed once with the buffer solution, which is a little narrower than that before

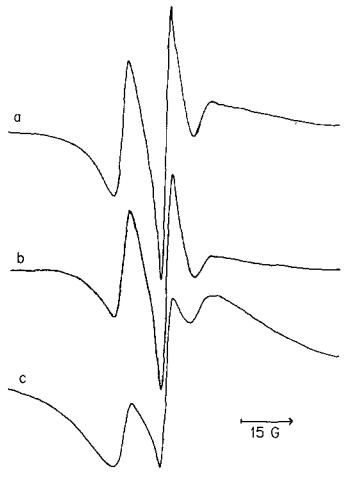

Fig. 2. ESR spectra of the yeast cells grown aerobically for 24 hrs with 1% glucose and 0.05mg/ml stearic label. a) Pellet collected by centrifugation from the culture. b) The pellet after washing with the buffer. c) Pellet collected by centrifugation from the culture.

washing. Somewhat broader spectra were also frequently observed. Sometimes, quite broad spectra were obtained, which were not affected by washing with the buffer. An example of such spectra is shown in Fig. 2c. The broadness is perhaps due to the spin-spin exchange interaction.

The ESR signal intensity of the yeast cell pellets kept filled in the capillary sample tube was found to decrease with time, the half-life being 1 hour. When the culture was flushed with nitrogen for various times, the signal intensity of the yeast cell pellet collected by centrifugation decreased with the nitrogen bubbling time. The decay is shown in Fig. 3, together with that of the control aerobic yeast cells. It is evident that the lack of oxygen in the culture caused decrease of the ESR signal of the spin-labeled aerobic cells. Some regeneration of the ESR signal was observed, as marked in Fig. 3, when the culture flushed with nitrogen was again aerated on the shaker. Addition of 1mM KCN to the aerobic culture also caused decay of the signal, but to a lesser extent (ca. 50% of the initial control after 3 hr incubation). On the other hand, incubation for 20min with 10mM ascorbate gave no effect on the ESR spectra. Washing of the aerobic cells with the buffer solution made the signal intensity decrease to approximately one half. Nitrogen flush of

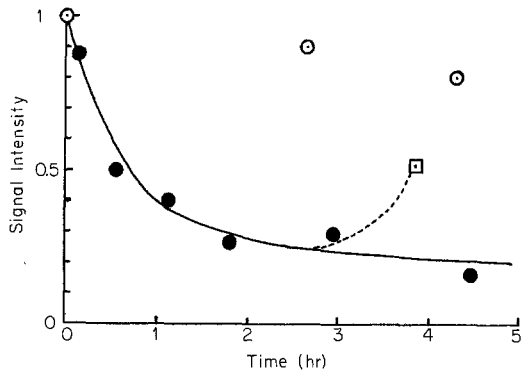

Fig. 3. Effect of nitrogen flush on the ESR signal intensity of the spin-labeled aerobic yeast cells. The culture grown for 24 hrs with 0.05mg/ml label was divided into two portions. One portion was flushed with nitrogen at 30°C (-●-) and the other, a control, was further cultured aerobically at 30°C (-⊙-). The point marked ⊡ shows the signal intensity obtained by re-aeration of the yeast cells flushed with nitrogen for 2.7 hrs.

the buffer suspension of the yeast cells resulted in further rapid decay of the signal, while the control under aerobic conditon showed only slow decay.

When the stearic label was added to the unlabeled aerobic yeast cell culture grown to the early stationary phase and the cells were centrifuged immediately after the addition, the pellet showed an ESR spectrum not very different from that given in Fig. 2a. The signal showed somewhat different decay behavior from that of the cells cultured with the label. The stearic labels are supposed to be dissolved in the cell membranes.

ESR Spectra of Spin-Labeled Anaerobic Cells: Fig. 4 shows the spectra of the anaerobically grown yeast cells. These spectra are different from those of the aerobic cells. The spectrum a is obtained from the cells cultured with 0.1mg/ml of the stearic label after anaerobic preculture with no lipid supplements. The exchange-broadened spectrum b is that of the cells cultured with a higher content of the label (0.2mg/ml). The cells grown with oleic acid as well as the label showed much narrower spectra depending on the ratios of the oleic acid to the label. An example is the spectrum c obtained with 0.1mg each of the fatty acids per ml. A still narrower spectrum was observed from the cells grown with a higher content of oleic acid (0.16mg oleic acid and 0.04mg label per ml). These results indicate that the rigidity of the

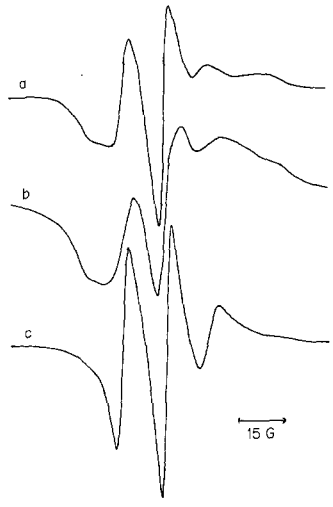

Fig. 4. ESR spectra of the yeast cells grown anaerobically with 10% glucose, 12 ppm ergosterol, and a) 0.1mg/ml stearic label for 24 hrs after anaerobic preculture with no lipid supplements, b) 0.2mg/ml stearic label for 40 hrs, and c) 0.1mg oleic acid and 0.1mg stearic label per ml for 40 hrs.

membrane lipids depends on the presence of the unsaturated acid; more fluid with higher contents of the unsaturated acid.

To follow the adaptation to oxygen, the spin-labeled anaerobic yeast cell suspension in the buffer containing 5% ethanol was flushed with air and the ESR spectra of the pellets were measured at times. The lightly labeled cells ($\ll$0.1mg/ml) and the cells labeled with the higher content of oleic acid responded to aeration. The peak height of the ESR spectra increased for several hours and then decreased upon aeration. The anaerobic yeast cells labeled more heavily and those labeled with 0.1mg each of oleic acid and label failed to respond to the aeration. This result seems reasonable since the membrane lipids require proper ratio of unsaturated to saturated fatty chains for the normal functioning and the 12-nitroxide stearic acid is not the same as the unsaturated acid.

DISCUSSION

It has been shown that the yeast cells can be spin-labeled biosynthetically under aerobic as well as anaerobic conditions. The ESR signal of the spin-labeled aerobic cells was stable under aerobic conditions with nutrients.

The signal decayed, however, upon removal of oxygen from the culture. KCN also caused decay of the signal to a lesser extent. The ESR signal of the spin-label disappears on reduction, the label thus acting as an electron acceptor. The ESR signal of some anaerobic cells showed increase for several hours and the following decrease upon aeration. This phenomenon may be related to the oxygen-induced appearance of the respiratory chain in the mitochondria. The results obtained with the aerobic and anaerobic cells suggest that the decrease and increase of the ESR signal of the spin-labels buried in the hydrophobic media of the membranes are related, at least partly, to the respiratory activity of the mitochondria of the yeast cells.

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