STABILIZATION OF THE YEAST DESATURASE SYSTEM BY LOW LEVELS OF OXYGEN

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Abstract. The stability of particulate *palmitoyl-CoA desaturase* preparations from anaerobically grown yeast cells was increased by exposure to low levels of oxygen. The stabilizing effect of oxygen may be based upon the increased amounts of palmitoleic acid and ergosterol that become available to the cells. These results suggest the evolutionary appearance of this system at a time when atmospheric oxygen was at a low level.

The eukaryote, *Saccharomyces cerevisiae*, is unable to sustain cell division under strictly anaerobic conditions unless supplied with an exogenous source of sterols and unsaturated fatty acids during growth (Andreasen and Stier, 1954). The metabolic pathways, leading to either the synthesis of unsaturated fatty acids or sterols, contain enzymes that have an absolute oxygen rquirement for activity (Bloomfield and Bloch, 1960). Thus, in anaerobic culture, yeast cells are lipid-depleted and viability decreases after a few generations (Jollow *et al.*, 1968). This oxygen-dependency of the sterol and unsaturated fatty acid systems would imply a late emergence of the yeast in evolutionary time (Bloch, 1968). However, the level of oxygen necessary for the desaturation of long-chain fatty acids and for sterol synthesis is considerably lower than that present in the current atmosphere. In 1973, Rogers and Stewart reported that, in growing cells, the half-maximal oxygen concentration for a full complement of sterols and unsaturated fatty acids was of the order of 0.002 PAL (present atmospheric level). The oxygen required for sterol synthesis in cell-free yeast systems has not yet been determined; however, when *in vitro* assays of the *palmitoyl-CoA desaturase* of this organism were performed over a wide range of oxygen concentrations, it was found that this enzyme became saturated at low levels of oxygen, the enzyme reaching half-maximal activity in an atmosphere containing only 0.04% oxygen (0.002 PAL) (Whitaker and Klein, 1977).

During continuing studies of the yeast desaturase system, it became clear that oxygen, in addition to being a necessary component of the enzymatic reaction, may play an additional role: that of stabilizing the enzyme complex. We found that cellular particulates, containing the desaturase, rapidly lost activity when subjected to washing or density gradient centrifugation, but only if the yeast cells had been grown anaerobically. By contrast, similar preparations, from aerobic cells, were completely stable under these conditions (Klein and Volkmann, 1975). It was also shown that growth in the presence of palmitoleic acid and ergosterol yielded stable enzyme preparations from anaerobic cells, suggesting that these lipids might be involved in

keeping the various elements of this enzyme complex in proper association.

For the present study, cells of *S. cerevisiae* strain LK2G12, were grown anaerobically (Klein and Jahnke, 1968, 1979), and then exposed to various levels of oxygen in order to determine the amount necessary to confer stability on this enzyme complex. For the enzyme assays, cells were harvested, resuspended in a buffer containing $2\,mM$ tris (hydroxymethyl) aminomethane and $2\,mM$ $MgCl_2$, at pH 7.5, after which the cells were disrupted in a Braun homogenizer (Klein and Jahnke, 1971). The resultant crude homogenates were freed of unbroken cells and large particulates, and the supernatants were then centrifuged at $100\,000 \times g$ for 60 minutes to sediment all the remaining cell particulates. The pellet from this centrifugation, which contains the crude *palmitoyl-CoA desaturase*, was used for the enzyme assays (Klein and Volkmann, 1975). To test the stability of the desaturase, crude particulates obtained from cells subjected to different oxygen atmospheres, were resuspended in buffer using a Potter–Elvejem homogenizer. The suspensions were centrifuged for 60 minutes at $100\,000 \times g$, repelleting the particulates which were then resuspended in fresh buffer. The percent of original *palmitoyl-CoA desaturase* activity remaining after such a single washing was used as a measure of enzyme stability.

The ergosterol content of the cells was determined by the method of Shaw and Jefferies (1953). Palmitoleic acid was determined from gas chromatograms (Klein, 1955) and protein by the method described by Lowry *et al.*, (1951).

TABLE I

The effect of oxygen on desaturase stability in *S. cerevisiae*

Atmosphere[a]	Stability[b] (Percent of remaining activity)	Palmitoleic acid (mg/g dry weight)	Ergosterol
Controls			
Unaerated cells	32	0.5	0.6
Cells grown with palmitoleic acid			
and ergosterol	95	11.4	2.9
Treated cells			
0.01 % (V/V) oxygen	76	1.1	0.8
0.03 % (V/V) oxygen	86	1.4	1.0
0.10 % (V/V) oxygen	93	2.8	1.3
0.30 % (V/V) oxygen	96	5.1	2.2
1.00 % (V/V) oxygen	99	6.2	2.6

[a] Anaerobic cells were harvested, resuspended in 0.1 M phosphate buffer, pH 7.0, with 5 % glucose and stirred for 2.5 hrs under the designated atmosphere. The gases used were oxygen/helium mixtures from analyzed premixed tanks (Air Products Co.). The aeration procedures have been described by Klein and Jahnke (1968, 1979). Where indicated, Palmitoleic acid (in absolute ethyl alcohol) was added to yield a final concentration of 30 mg/l; ergosterol (in absolute ethyl alcohol) was added to yield a final concentration of 110 mg/l.
[b] Stability = Enzyme activity after washing/Enzyme activity before washing $\times$ 100.

As is seen in Table I, the stability of *palmitoyl-CoA desaturase* preparations is markedly affected by the presence or absence of oxygen. Cells grown anaerobically yield enzyme preparations that readily lose activity after a single washing in buffer. However, exposure of anaerobically grown cells to levels of oxygen, considerably lower than ambient, yields stable desaturase preparations. The exposure of anaerobically grown cells to as little as 0.01 % oxygen substantially increased the stability of this enzyme complex, and above 0.03 % oxygen, the enzyme is completely stable to disruption by washing. Furthermore, there is a strong correlation between the stability of the enzyme and the cellular content of palmitoleic acid and ergosterol, both of which require atmospheric oxygen for their formation. As is seen in the Table, anaerobic cells, grown in the presence of ergosterol and palmitoleic acid yield stable desaturase preparations. These experiments also indicate that extremely low levels of oxygen stimulate the synthesis of these lipids, and are adequate to provide the necessary complement of unsaturated fatty acid and sterol to stabilize the desaturase complex. Oxygen, therefore, seems to be indirectly involved in the stabilization of the enzyme. The principal effect appears to be on the reactions of the oxygen-requiring enzymes of the sterol and unsaturated fatty acid systems, making their products available for the stabilization of the *palmitoyl-CoA desaturase.* The possible role of such products in stabilizing this enzyme complex has been discussed by Klein and Volkmann (1975).

The formation of palmitoleic acid and ergosterol is essential for yeast survival. Because oxygen is required for this, it has often been suggested that these eukaryotes emerged after the Earth's atmosphere became highly oxidizing. However, our studies indicate that the stability of the desaturase system of this yeast is fully achieved at remarkably low levels of oxygen. This eukaryote could well have flourished before the atmospheric oxygen reached a level of 1 %. At the very least, our data indicate the difficulty of assigning an evolutionary niche to organisms based upon the presence or absence of oxygen-requiring metabolic pathways. Taken at face value, the data might also indicate that the desaturase system in *S. cerevisiae* is of ancient heritage, arising early in the period during which the atmosphere was becoming oxygenated.

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