# OXYGEN AS A FACTOR IN EUKARYOTE EVOLUTION: SOME EFFECTS OF LOW LEVELS OF OXYGEN ON *SACCHAROMYCES CEREVISIAE*

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**Abstract.** A comparative study of the effects of varying levels of oxygen on some of the metabolic functions of the primitive eukaryote, *Saccharomyces cerevisiae*, has shown that these cells are responsive to very low levels of oxygen: the level of palmitoyl-Co A desaturase was greatly enhanced by only 0.03% (v/v) oxygen. Similarly, an acetyl-CoA synthetase associated predominantly with anaerobic growth, was stimulated by as little as 0.1% oxygen, while an isoenzyme correlated with aerobic growth, was maximally active at much higher oxygen levels (> 1%). Closely following this latter pattern were three mitochondrial enzymes that attained maximal activity only under atmospheric levels of oxygen.

## 1. Introduction

Eukaryotic organisms are generally believed to have arisen approximately one billion years ago, long after the Earth's atmosphere had accumulated appreciable quantities of free oxygen through biological photosynthesis (Margulis, 1970). Evidence for estimating the age of the eukaryotic era is derived mainly from various lines of fossil evidence, such as measurements of the size distribution of microfossils (Schopf and Oehler, 1976; Schopf, 1977) and from techniques for demonstrating the presence of 'eukaryotic' organelles within these fossils (Barghoorn and Schopf, 1965; Schopf and Blacic, 1971; Schopf and Oehler, 1976). In addition, inferences have sometimes been drawn from the presence or absence of oxygen-dependent biochemical processes in extant organisms in attempts to place them into phylogenetic and evolutionary relationships (Goldfine and Bloch, 1963; Margulis, 1970).

In the present work, the primitive eukaryote *Saccharomyces cerevisiae*, was grown in the presence of a wide range of oxygen concentrations to determine the effect of oxygen on the appearance of three enzymes that have been under investigation in our laboratory and which are known to be influenced by oxygen. These enzymes are: (1) two acetyl-coenzyme A synthetases, one found only in aerobically-grown cells and the other in nonaerobically-grown cells (Satyanarayana *et al.*, 1974; Klein and Jahnke, 1979), and (2) palmitoyl-coenzyme A desaturase, an enzyme known to require atmospheric oxygen for its activity (Bloomfield and Bloch, 1960; Whitaker and Klein, 1977).

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## 2. Materials and Methods

Cells of *S. cerevisiae*, strain LK2G12, were first grown for 48 hr at 30°C in 2-l flasks containing 1.7-l of medium under strictly anaerobic ($N_2$-flushed) conditions (Klein and Jahnke, 1968, 1979). After this initial period of incubation, oxygen/helium mixtures were introduced into individual flasks from previously analyzed, premixed gas tanks (Air Products Co.) by vigorous bubbling through the partially-grown cultures. Gas mixtures used in this study contained oxygen at the following volume percentages of the total gas: 0.03 or, 0.0014 of the present atmospheric level (PAL)), 0.1 (0.0048 PAL), 0.3 (0.0144 PAL), and 1.0% (0.0479 PAL). These percentages were verified by gas chromatography of both the input and output gas streams during oxygenation of each flask. Because the values of these streams were essentially identical, we can assume that each suspension was in equilibrium with its atmosphere during incubation. In addition to the flasks receiving various mixtures of oxygen, filtered air (20.9% $O_2$) was also used to aerate some flasks. The oxygenation was continued for an additional incubation period of 24 hr; after this second period of growth, the flasks were quickly refrigerated and cycloheximide (100 μg/ml) added to each flask to prevent any further enzymatic changes. The refrigerated cells were quickly harvested by centrifugation and the crude cell homogenates were obtained by homogenization in a Braun apparatus (Klein and Jahnke, 1971).

Samples of homogenates were removed and used for dry weight and total protein determinations (Lowry *et al.*, 1951). Total sterols and fatty acids (saturated and unsaturated) were determined as previously described (Klein, 1955; White and Klein, 1966). Other samples were assayed for the following enzyme activities. The two acetyl-CoA synthetases (ACS's) were assayed by taking advantage of their respective affinities for acetate (Klein and Jahnke, 1979). The desaturation of palmitoyl-CoA was estimated by gas chromatography (Klein and Volkmann, 1975). Three mitochondrial enzymes, succinic dehydrogenase (Arrigoni and Singer, 1962), succinate-cytochrome *c* reductase (Tisdale, 1967), and cytochrome oxidase (Smith, 1955) were also estimated in these homogenates.

## 3. Results

As is evident from Table I, after 48 hr of incubation under the conditions of this experiment, anaerobic growth was sparse. The resultant cells contained only traces of sterols and unsaturated fatty acids, about 0.04 and 0.18%, respectively, of the dry weight of the cells. By contrast, cells grown in air in this medium contain more than ten times these concentrations. Exposure of cells to as little as 0.03% $O_2$ increased both the sterols and unsaturated fatty acids, while even greater increases in these constituents resulted from further elevation of the oxygen level. Similar effects of oxygen on these cellular compounds had already been noted in yeasts by Goldfine and Bloch (1963) and by Rogers and Stewart (1973). Of interest, however, was the possible effects of oxygen on the content of the yeast enzymes under investigation in this laboratory.

TABLE I

Effect of varying levels of oxygenation on the enzyme activities and cellular constituents of *S. cerevisiae*[1]

| Oxygen %(v/v) | Dry weight mg | Total protein mg | Total sterols mg | Fatty acids[2] | | | | Total enzymatic activities[3] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total | Unsaturated | Saturated | Unsaturated / Saturated ratio | PCD | Nonaerobic ACS | Aerobic ACS | Mitochondrial enzymes | | |
| | | | | | | | | | | | SDH | SCR | COX |
| 0[4] | 435 | 307 | 0.17 | 3.16 | 0.78 | 2.38 | 0.3 | 0.07 | 3.4 | 0.4 | 1.5 | 0.2 | 1.6 |
| 0.03 | 732 | 474 | 0.36 | 7.45 | 2.58 | 4.87 | 0.5 | 1.12 | 7.7 | 1.8 | 11.5 | 3.9 | 11.1 |
| 0.1 | 1040 | 755 | 0.80 | 10.80 | 6.28 | 4.98 | 1.3 | 1.61 | 25.3 | 0 | 9.0 | 2.5 | 15.9 |
| 0.3 | 1010 | 726 | 0.97 | 12.00 | 6.70 | 5.32 | 1.3 | 1.62 | 37.1 | 0 | 8.4 | 3.0 | 17.1 |
| 1.0 | 1330 | 952 | 2.33 | 19.50 | 13.80 | 5.68 | 2.4 | 1.71 | 52.3 | 0 | 9.7 | 1.0 | 20.9 |
| 20.9 | 1990 | 1318 | 12.30 | 50.20 | 42.40 | 7.80 | 5.4 | 0.76 | 36.7 | 29.7 | 133.3 | 46.4 | 115.9 |

[1] Cells were incubated for 48 hr under strictly anaerobic conditions, and then oxygenated for an additional 24 hr with $O_2$ : He mixtures. Assay of input and output $O_2$ streams by gas chromatography demonstrated $O_2$ was in excess under these conditions. Cells were harvested and crude cellular homogenates prepared for analysis.

[2] All fatty acids expressed as mg of total homogenate. Unsaturated fatty acid represents sum of palmitoleic and oleic acids; saturated sum of palmitic and stearic acids.

[3] PCD, palmitoyl-coA desaturase; ACS, acetyl-coA synthetase; SDH, succinate dehydrogenase; SCR, succinate-cytochrome *c* reductase; COX, cytochrome *c* oxidase. Total activities expressed as μmol/min.

[4] Control, i.e. initial culture at start of oxygenation period.

As a point of reference (Table I), only traces of the mitochondrial marker enzymes were found in cells grown under strictly anaerobic conditions. Incubation in atmospheres containing oxygen stimulated the appearance of these enzymes even at low concentrations, but maximal mitochondrial enzyme activity was evidenced only at 20.9% $O_2$.

Of the two ACS's, formation of the *aerobic* enzyme followed a course very similar to that of the mitochondrial enzymes with respect to the concentration of available oxygen, being virtually absent except at 20.9% $O_2$ (Table I). Surprisingly, the content of *non-aerobic* ACS also appeared to be a function of the oxygen concentration, reaching a maximum at 1% $O_2$. Of the three enzymes of interest here, palmitoyl-coA desaturase appeared to be extremely sensitive to oxygen. With cells cultivated in 0.03% $O_2$, the total desaturase activity increased 16-fold. The resultant cells exhibited the highest specific activity for this enzyme, while further increases in the $O_2$ level beyond 0.03% only yielded higher total concentrations of this enzyme. The specific activity of the desaturase was essentially unaffected by additional $O_2$ in the atmosphere up to a concentration of 1% $O_2$; beyond that level, the activity declined.

## 4. Discussion

From the data presented here, it is evident that different enzyme systems within the eukaryote *S. cerevisiae* are formed at different levels of atmospheric oxygen. The mitochondrial enzymes, while subject to some stimulation at low concentrations of oxygen, become maximally active beyond 1% $O_2$, showing their greatest activity under normal oxygen tensions. Closely following this pattern is the aerobic ACS, which has no known role in respiration. In this connection, it is interesting to note that in previous studies, this enzyme was shown not to be a mitochondrial enzyme, despite the fact that it sediments in crude mitochondrial preparations. (Density gradient studies show it to be bound to membranes that sediment in a denser range than the mitochondria (Klein and Jahnke, 1971)). While a specific physiological role for the enzyme has not been determined, it is known to be present only in cells capable of respiring, thus suggesting a close coupling between it and functioning mitochondria.

The nonaerobic enzyme does not appear to be correlated with the mitochondria in any way, but it is nevertheless also stimulated by the presence of oxygen. However, considerably lower quantities of oxygen are required for full activity of this form of ACS. For example, at 0.1% $O_2$, the total amount of this enzyme increased about eight-fold and the specific activity was 70% of maximum. At concentrations of oxygen greater than 0.3% no further increase in specific activity of the nonaerobic ACS was observed. (The reduced content of this enzyme in cells grown in air is probably due to the preferred synthesis of the aerobic isoenzyme under these conditions (Klein and Jahnke, 1979)). Why oxygen should be involved in the formation of the nonaerobic ACS is not readily obvious. As with the palmitoyl-coA desaturase, discussed below, it is possible that certain lipids, the synthesis of which requires $O_2$, are necessary for structural integrity of the enzyme.

Most sensitive to the presence of oxygen was the enzyme, palmitoyl-coA desaturase. At 0.03% $O_2$, the total amount of this enzyme increased 16-fold over that found in

anaerobic cells, and the specific activity of the enzyme rose from 0.23 to 2.34. Furthermore, while higher concentrations of oxygen resulted in more growth and hence more total desaturase, the specific activity of this enzyme reached its maximum already at 0.03% $O_2$ and remained at this level until the $O_2$ level reached 1.0% beyond which there was a decrease in the amount of enzyme. While $O_2$ is known to be required for the activity of this enzyme, the reason for the stimulation of desaturase *formation* by oxygen is not certain. It is known that palmitoleic acid and ergosterol stabilize this enzyme in homogenates (Klein and Volkmann, 1975) and both of these substances require oxygen for their synthesis in this organism. For the formation of palmitoleic acid at least, it has been shown (Whitaker and Klein, 1977) that very low concentrations of oxygen are necessary to saturate the enzyme catalyzing its synthesis. Thus, if a similar situation existed in the synthesis of ergosterol, the availability of even small amounts of oxygen could result in the provision of those lipids necessary for stabilizing the palmitoyl-coA desaturase. (The reduced levels of desaturase seen at high oxygen levels are presumably the result of feedback inhibition caused by the high concentration of lipids found in these cells (Bloomfield and Bloch, 1960).

Taking this data as a whole, it seems important to emphasize that caution be used in ascribing evolutionary and phylogenetic significance to biological systems that interact with molecular oxygen. In the eukaryote studied here, there was wide variation in the influence of oxygen on the presence and amount of different enzymes; several systems required high concentrations of oxygen for full activity while one, the desaturase responded maximally at an oxygen concentration almost 3 orders of magnitude lower, and the nonaerobic ACS at concentrations about 2 orders lower, than current atmospheric levels. The oxygen content of the primitive atmosphere was considerably lower than that of the present atmosphere. Berkner and Marshall (1965), for example, have estimated this to be of the order of 0.001 PAL, although even lower estimates have been proposed by Margulis *et al.* (1976). Our data suggest that biological systems interacting with oxygen could have been operational very early in biological evolution, and significant aerobic metabolism could have occurred. As is evident from these studies, the primitive eukaryote, *S. cerevisiae* can sense, and is affected by, low levels of oxygen, and at least some of its responses to oxygen occurred at levels compatible with the atmospheric model for the primitive Earth proposed by Berkner and Marshall. Since the early work of Pasteur (1861), *S. cerevisiae* has been known to have an absolute requirement for oxygen for growth. Because the growth of this organism has been found to be dependent on unsaturated fatty acids and sterols (Andreasen and Stier, 1954), which require oxygen for their synthesis, it has generally been regarded that the systems for the synthesis of these substances developed in an aerobic environment. However, traces of oxygen in the range of that postulated for the primitive Earth atmosphere, apparently are sufficient to permit the synthesis of those lipids that limit the growth of these organisms.

## References

Andreasen, A. A. and Stier, R. J.: 1954, *J. Cell Comp. Physiol.* **43**, 271.
Arrigoni, O. and Singer, T. P.: 1962, *Nature* (London) **193**, 1256.
Barghoorn, E. S. and Schopf, J. W.: 1965, *Science* **150**, 337.
Berkner, L. V. and Marshall, L.: 1965, *J. Atmosph. Sci.* **22**, 225.
Bloomfield, D. K. and Bloch, K.: 1960, *J. Biol. Chem.* **235**, 337.
Goldfine, H. and Bloch, K.: 1963, *Control Mechanisms in Respiration and Fermentation*, B. Wright (ed.), The Ronald Press Co., New York, pp. 81–103.
Klein, H. P.: 1955, *J. Bacteriol.* **69**, 620.
Klein, H. P. and Jahnke, L.: 1968, *J. Bacteriol.* **96**, *1632.*
Klein, H. P. and Jahnke, L.: 1971, *J. Bacteriol.* **106**, 596.
Klein, H. P. and Jahnke, L.: 1979, *J. Bacteriol.* **137**, 179.
Klein, H. P. and Volkmann, C. M.: 1975, *J. Bacteriol.* **124**, 718.
Lowry, O. H., Rosenbrough, N. J. and Farr, A. L.: 1951, *J. Biol. Chem.* **193**, 265.
Margulis, L.: 1970, *Origin of Eukaryotic Cells*, Yale University Press, New Haven and London, 102–142.
Margulis, L., Walker, J. C. G. and Rambler, M.: 1976, *Nature* **264**, 620.
Pasteur, L.: 1861, *Compt. Rend.* **52**, 1260.
Rogers, P. J. and Stewart, P. R.: 1973, *J. Bacteriol.* **115**, 88.
Satyanarayana, T., Mandel, A. D. and Klein, H. P.: 1974, *Biochimica et Biophysica Acta* **341**, 396.
Schopf, J. W. and Blacic, J. M.: 1971, *J. Paleontol.* **45**, 925.
Schopf, J. W. and Oehler, D. Z.: 1976, *Science* **193**, 47.
Schopf, J. W.: 1977, *Chemical Evolution of the Early Precambrian*, C. Ponnamperuma (ed.), Academic Press, New York, San Francisco and London, pp. 101–109.
Smith, L.: 1955, *Methods of Biochemical Analysis*, D. Glick (ed.), Vol. II. Wiley (Interscience), New York, pp. 427–434. Tisdale, H.: 1967, *Methods in Enzymology*, R. W. Estabrook and M. E. Pullman (eds.), Academic Press, New York, Vol. X, p. 213.
Whitaker, N. S. and Klein, H. P.: 1977, *Chemical Evolution of the Early Precambrian*, C. Ponnamperuma (ed.), Academic Press, New York, San Francisco and London, pp. 211–214.
White, D. and Klein, H. P.: 1966, *J. Bacteriol.* **91**, 1218.