ON THE NECESSITY OF MOLECULAR OXYGEN FOR THE SYNTHESIS OF RESPIRATORY ENZYMES IN YEAST

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It is known that, in the presence of O_2, the synthesis of respiratory enzymes in wild type S. cerevisiae is repressed by the fermentation of sugars, e.g. glucose (Slonimski, 1955; Ephrussi et al., 1956 ; Galzy and Slonimski, 1957). This repression may be quite considerable, depending on the enzyme studied (up to 90-99 % in the case of D- and L-LCR[++]) (Somlo, 1965). It is known furthermore that, when yeast is grown on glucose under presumably anaerobic conditions, the synthesis of respiratory enzymes is even more repressed (more than 99.9 %) (Slonimski, 1953 ; Somlo, 1965). The absence of respiratory enzymes in anaerobically glucose-grown yeast can be interpreted in either of two ways : a) it is simply due to a quantitatively more severe repression by fermentative metabolism, and the presence of O_2 is not a compulsory condition for the synthesis of these enzymes ; b) the presence of O_2 is necessary for the synthesis of respiratory enzymes.

According to Tustanoff and Bartley (1964), when yeast is grown on galactose (instead of glucose), under continuous flushing with N_2, the harvested cells have considerable respiratory capacity, as well as cytochrome oxidase activity. The authors therefore favour the interpretation that O_2 is not necessary for the synthesis of respiratory enzymes. This interpretation is open to question, since these authors did not exclude the possibilities that either a) the traces of O_2 contaminating the N_2, or b) the contact with air during the handling of yeast (harvesting by centrifugation, washing of cells, etc.) prior to enzyme assay, were sufficient to induce the apparently "anaerobic" synthesis of respiratory enzymes. It is the purpose of the present paper to examine these possibilities.

We have grown several parallel cultures on glucose and on galactose using commercial N_2 of the highest available purity (Azote-R, Société

++ Abreviations used : D-LCR, L-LCR = D- and L-lactic-cytochrome c-reductases ; DPNH-CR, S-CR = DPNH- and succinate-cytochrome c-reductases.

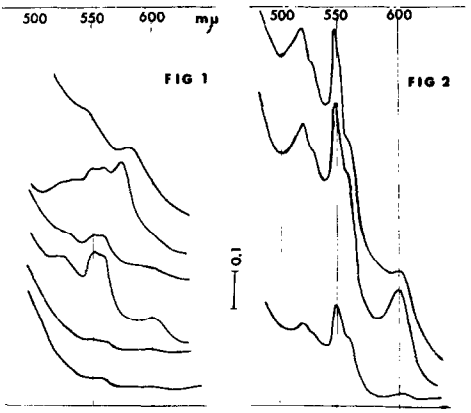
Air Liquide, O_2 content $\leq$ 0.001 %, according to the furnisher), without any precautions to eliminate contaminating traces of O_2. Under these conditions, we found that "anaerobic" cultures grown on galactose are indeed always of a much more aerobic type, though to variable degrees, than those grown on glucose (see fig. 1 and table 1.).

Starting from this condition, more strictly controlled anaerobiosis can be reached. If, at the same time, the characteristics of galactose-grown cultures were to change to the characteristics observed in glucose-grown anaerobic cultures, it would be possible to extrapolate to the condition of complete absence of O_2, which is difficult to achieve experimentally. In this case, we should be entitled to conclude that the synthesis of respiratory enzymes does not take place in the absence of O_2.

Fig. 1 shows absorption spectra of yeast in a series of cultures under progressively more anaerobic conditions. It will be seen that glucose-grown anaerobic yeast lacks the characteristic bands of cytochrome c (α 549 mμ), b (α 560 mμ), a-a_3 (α 603 mμ), which are clearly visible in the corresponding aerobically grown culture (fig. 2, III). This result confirms previous observations (Slonimski, 1953). In experiments in which the same commercial N_2 was used, three independent galactose-grown, "anaerobic" cultures gave an absorption spectrum which clearly showed the bands of cytochromes c, b and a-a_3, although to a variable extent (spectra N° II-IV). When the culture on galactose was carried out after flushing with Azote-R purified by passage through pyrogallol (spectrum N° V), the bands of the respiratory pigments were very much weaker. When actidione was added before the culture flask was opened, in order to inhibit protein synthesis during aerobic harvesting and handling (Fukuhara, 1965), the spectrum was even more of the anaerobic type (spectrum N° VI).

Figure 2, for comparison, shows the absorption spectra of aerobic cultures grown on glucose, galactose and ethanol.

Table 1 shows the activities of various respiratory enzymes, determined in the extracts obtained from the same yeast cultures which have been used for the measurement of absorption spectra. As in the case of the latter, it is seen that there is a clear decrease in the specific activity of all respiratory enzymes, as experimental conditions were progressively more anaerobic. D- and L-LCR activities from galactose-grown cultures under maximally anaerobic conditions (N° VI) were equal to or lower than those from glucose-grown anaerobic cultures (N° I). Total activity per flask of each respiratory enzyme, in the case of



Legend for figure 1 : Absorption spectra of yeast grown under progressively more anaerobic conditions. S. cerevisiae strain "Yeast Foam". Aerobic precultures (on Difco Yeast Extract 1 %, ammonium sulfate 0.12 %, KH_2PO_4 0.1 %, and glucose or galactose 5 %) were grown until the stationary phase (5mg dry weight/ml), cf. fig. 2., cultures N°I and III. These served to inoculate "anaerobic" cultures, in media of the same composition containing in addition ergosterol, 50 mg/l, and Tween 80, 0.5 ml/l, dissolved in ethanol (final concentration 5 g/l). The inoculum of "anaerobic" cultures corresponded to 15 μg dry weight/ml. Anaerobic culture flasks were in all cases provided with a mercury-filled gas valve, and N_2 was bubbled through for 45 min (150 ml N_2/min) accompanied by vigorous magnetic stirring. The volume of N_2 gas bubbled through was at least 30 times the gas phase volume of the culture flask. After bubbling, the stopcock was closed, and the flask transferred to a shaker at 25° C. Cultures were grown for 24 hours (= 7 generations), then cooled, opened, immediately poured onto crushed ice and quickly centrifuged at 0° C. Absorption spectra were read on the unwashed pellet, within 10 min after opening of flasks, using solid yeast pellets, 2 mm thick, and a Cary 15 recording spectrophotometer, according to the technique of Péré, Clavilier and Slonimski (to be published). I : glucose-grown, commercial N_2 used for anaerobiosis (Azote-R, O_2 content $\leqslant$ 0.001 %). II-III-IV : galactose-grown, same commercial N_2. Three independent cultures, yielding variable results. V : galactose-grown, Azote-R further purified by bubbling through pyrogallol. VI : galactose-grown, same as V, but actidione (50 mg/l) added from special, air-tight, side arm, 1 hour before opening the flask. Abscissa : wave-length. Ordinate : absorbancy. Spectra have been spaced for convenience of reading.

Legend of figure 2 : Absorption spectra of aerobic yeast cultures. I and III are spectra of aerobic precultures (respectively galactose- and glucose-grown), used to inoculate "anaerobic" cultures. II : culture on ethanol (10 g/l).

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experiment N° VI, was equal or inferior to the total activity originally present in the aerobic inoculum and diluted by 7 generations of anaerobic growth (see legend in Table 1).

Our results indicate that the synthesis of respiratory enzyme does nor occur when yeast is grown on galactose under conditions where contamination by molecular O_2 is minimal. We have calculated that solutions

TABLE I

Respiratory enzyme activities in yeast grown under progressively more anaerobic conditions

Condition of culture	D-LCR	L LCR	DPNH-CR	S-CR	cytochrome oxidase
I	0.44 (0.4)	0.2 (0.4)			
II	2.6 (0.7)	2.2 (0.7)			
III	1.8 (0.4)	1.3 (0.4)			
IV	5.0 (0.2)	8.7 (0.2)			
V	0.6 (1.0)	0.35 (1.0)	1.1 (0.3)	0.07 (0.3)	0
VI	0.1 (0.9)	0.2 (0.9)	1.1 (0.2)	0.04 (0.2)	0

Conditions of cultures were those indicated in the legend of fig. 1. Enzyme determinations were made on aliquots of suspensions used for absorption spectra. After one washing in ice-cold water, yeast cells were broken (within 20 min after opening of culture flasks) in a Nossal shaker for 2 x 30 sec, according to Somlo (1965). Homogenates were centrifuged for 10 min at 1 200 x g, and enzyme activities were determined on the supernatant, after 1 hour dialysis against 1 000 vol. of Tris buffer, 0.01 M, pH 7.4. Enzyme activities were measured as described by Somlo (1965). The activities shown in the table are expressed in mμmoles of substrate oxidized/min/mg protein of extract. Blanks have been subtracted. In brackets : blank activities (without added substrates).

Enzyme activities in the aerobic precultures, used for inoculating "anaerobic" cultures, were the following :

glucose-grown : D-LCR 6.0, L-LCR 9.0, DPNH-CR 16.6, S-CR 2.1, cytochrome oxidase 18.6.

galactose-grown : D-LCR 9.0, L-LCR 52.0, DPNH-CR 30.0, S-CR 16.5, cytochrome oxidase 20.0.

equilibrated with the unpurified commercial N_2 employed here contained 10^{-8} to 10^{-7} M O_2. Under these conditions the synthesis of respiratory enzymes in galactose-grown cultures can then be accounted for in either (or both) of two ways :

1) The biosynthesis of respiratory enzymes was induced by these very

low concentrations of O_2. This implies an extremely high affinity of the enzyme forming systems for molecular O_2.

2) The rate of respiratory adaptation in yeast previously grown under anaerobiosis, with galactose as a carbon source, was so high that contact with air during handling, prior to enzyme assay, was sufficient to elicit a rapid synthesis of respiratory enzymes.

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References

Ephrussi, B., Slonimski, P., Yotsuyanagi, Y. and Tavlitski, J., Compt. Rend. Lab. Carlsberg, 26, 87 (1956).
Fukuhara, H., Biochem. Biophys. Res. Comm., 18, 297 (1965).
Galzy, P. and Slonimski, P., Compt. Rend., 245, 2556 (1957).
Slonimski, P., Formation des Enzymes Respiratoires chez la Levure, Masson, Paris (1953).
Slonimski, P. P., Proc. Intern. Congr. Biochem., 3rd, Brussels, 1955, Academic Press, New York, (1956) p. 242.
Somlo, M., Biochim. Biophys. Acta, 97, 183 (1965).
Tustanoff, E. R. and Bartley, W., Biochem. J., 91, 595 (1964).