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FORMATION INDUITE DE CYTOCHROME PEROXYDASE CHEZ LA LEVURE

par

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EPHRUSSI ET SLONIMSKI[1,2] ont montré que la levure cultivée en anaérobiose est dépourvue d'un certain nombre d'enzymes d'oxydoréduction, notamment du système WARBURG-KEILIN, et qu'il suffit d'aérer la levure en milieu glucosé pour qu'elle acquière tous les enzymes et transporteurs d'électrons de ce système.

A la liste des enzymes dont la formation est induite par l'oxygène dans la levure, nous avons ajouté récemment la catalase[3]; nous pouvons y joindre maintenant la cytochrome peroxydase.

Cet enzyme, que nous avons dosé selon ABRAMS *et al.*[4] dans les autolysats de levure, n'existe qu'à l'état de traces ($Q = 0.02$) dans la levure cultivée en anaérobiose, et il apparaît en quantités considérables au cours de l'aération. ($Q = 1.63$ après 6 heures d'aération en milieu glucosé sans source d'azote assimilable.)

Le mutant "petite colonie" d'EPHRUSSI[5] forme la cytochrome peroxydase dans les mêmes conditions*.

On se souviendra que l'aération fait apparaître le cytochrome c[1,2] et la catalase[3] chez ce mutant, sans qu'il se forme jamais de cytochrome oxydase. La formation de cytochrome c sous l'action de l'oxygène dans ces cellules dépourvues de cytochrome oxydase semblait paradoxale[2]; elle s'explique peut-être par la formation de cytochrome peroxydase qui établirait le lien manquant entre le cytochrome c et l'oxygène, par l'intermédiaire de l'eau oxygénée qui se forme toujours dans des cellules exposées à l'air.

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THE EFFECT OF DRYING AT 110° ON SODIUM DEOXYRIBONUCLEATE

by

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It was established some years ago[1] that drying of sodium deoxyribonucleate (DNA) over phosphoric oxide caused irreversible changes which brought about a decrease in its solution viscosity. These changes were generally regarded as physical in nature and elemental analyses[2-4] of DNA have usually been made on samples which have previously been dried over phosphoric oxide at 110° *in vacuo* for periods long enough to obtain constant weight. That changes other than physical were possibly involved in such drying might have been indicated by the phosphorus contents which were almost always less than the theoretical value of 9.3% and rarely greater than 8.9%.

In this laboratory further indications that intensive drying at 110° might have chemical effects were obtained in studies of the electrometric titration curves of DNA from various sources[5]. In tht continuous titration of DNA not previously dried, the back titration curves after acid treatmene (i.e. from *ca.* pH 2 to 11) and alkali treatment (i.e. from *ca.* pH 12 to 3) were not coincident and from a study of the curves it was concluded that a slow hydrolysis of internucleotide linkages was occurring in the more alkaline solutions (cf.[6]). In agreement with this the number of secondary phosphoryl groups titrating after alkali treatment was always greater than after acid treatment, which has no such effect (e.g., for thymus DNA the no. of secondary phosphoryl groups per 4 g atoms of P are 0.54 and 0.33 after alkali and acid treatment, respectively; for herring sperm DNA the corresponding figures are 0.28 and 0.18). This view gains further support from the observation that, under conditions where the time of contact with alkali is never greater than 30 sec[7], the two back titration curves are, in fact, exactly coincident[8] and this eliminates other possible explanations that could be advanced[5].

However, when DNA was dried over phosphoric oxide at 110° (0.01 mm Hg) for 30 minutes before it was dissolved and titrated by the continuous method (i.e., under conditions where alkaline hydrolysis can occur owing to the greater time of contact with alkali) the discrepancy between the two back titration curves was significantly less than with DNA not previously dried. This implied that drying at 110° rendered the DNA less susceptible to hydrolysis by alkali and could be explained by hydrolysis during the drying process of the same type of linkage as is attacked by alkali. The number of secondary phosphoryl groups revealed in the back titration after *acid* treatment is indeed slightly greater for DNA which has been previously dried at 110° (Ref. 5, Table I), although this difference cannot be reliably deduced.

Other evidence for such an effect of drying at 110° *in vacuo* has been adduced by SIMMONDS, CHAVOS AND ORBACH[9] who have shown that if thymus DNA is heated at 110° over phosphoric oxide, with no prior removal of the 30% or so of moisture usually present in "air-dry" samples, then the percentage of hydrogen in the solid when free from water is greater than in samples dried by an alternative technique. In this latter method the bulk of the moisture is removed by storage over phosphoric oxide *in vacuo* at room temperature. The authors consider that a hydrolytic reaction has occurred during the drying at 110° with the uptake of the elements of water. Moreover, the milder drying technique gives samples containing 9.2–9.4% P, in accordance with the accepted formulae.

These experiments and the titration data already described strongly suggest that heating DNA at 110° *in vacuo* in the presence of a considerable amount of moisture leads to hydrolysis of internucleotide linkages with release of secondary phosphoryl groups. DNA which has been dried at 110° is, on this view, less susceptible to alkaline hydrolysis because the weaker internucleotide linkages which would be the first to be attacked by alkali have already been ruptured during the drying process.

In addition to this hydrolytic effect, drying at 110° for 30 minutes[5] or one day[10] reduces the characteristic difference between forward and back titration curves and eliminates it entirely after 4 days[10]. This feature of the titration curves is associated with hydrogen bonding of titratable groups[11] so that the hydrogen bonds of the double helical structure, regarded as present in the moist fibre[12], must gradually be destroyed by drying at 110°. A similar effect of heat has been observed with DNA solutions[13,14] and optical[15] and X-ray studies[16] also show that intensive drying produces a less ordered structure.

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