Two methods of measuring rate of deoxyribonucleic acid synthesis

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Abstract
Measurement of DNA synthesis

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Two methods of estimating the DNA level have been developed. Spectrophotometric measurements of the DNA content of an approximated number of conidio by the diphenylamine color reaction was used, as was estimation of DNA level by sensitivity to photoreactivating light at varying time intervals after UV irradiation. Strong correlation was found between the two methods.

Photoreversal of UV-induced lesions in the DNA cannot take place if doubling of the DNA following irradiation has taken place. The mutation rate from adenine deficient to "wild-type" at each delay period was used to indicate inversely the amount of DNA already doubled, and thus protected from the mutation-blocking effect of photoreactivation. A high mutation rate indicates little photoreactivation and vice versa.

In both methods, conidio of an \textit{ad-4} mutant strain (F54) of \textit{Neurospora crassa} were suspended in sterile water and adjusted to a concentration of \(6.6 \times 10^6\) conidia/ml. by dilution. The conidia were exposed to ultraviolet light for five minutes, using a constant volume of solution, which was continually agitated. The ultraviolet source was a 15-watt Sylvania germicidal lamp at a distance of 50 cm. from the suspension. This dosage of ultraviolet was previously found to be lethal to more than 95% of the conidia of this strain.

The major obstacle to spectrophotometric studies of the conidia was the hard case surrounding the conidium. This was overcome by immediately sonifying the refrigerated suspension of irradiated conidia. Sonification for one minute at a high intensity resulted in destruction of the conidial case on all spores. The naked conidia were hydrolysed with cold dilute (10%) trichloroacetic acid. Two ml. of diphenylamine reagent (1.5 gm. of diphenylamine in 100 ml. glacial acetic acid and 1.5 ml. of concentrated sulfuric acid with 0.5 ml. acetaldehyde added just before use) was added to each 1 ml. sample and the mixture was placed in a 30°C water bath for 16-18 hours. After incubation, the sample was read on the spectrophotometer at 600 ml. Aliquots of the conidial suspension were sonified and treated at 0, 5, 15, 30, 60, and 90 minutes following irradiation.

In the photoreactivation study, the conidia were suspended and irradiated as indicated above. The irradiated conidia were allowed to incubate for various delay periods and were then exposed for five minutes to white light.

Vomvoyianni 1965 Can. Jour. Bot. 43:765), Five such strains were shown to have resulted from a single-gene mutation. There may be more than one mutational site for resistance to these hydrocarbons as has been shown for another ascomycete (Georgopoulos and Panopoulos 1966 Can. Jour. Genet. Cytol, 8:347). At least one of these sites is linked to the mating type locus and to patch (see also NN949:44), On control medium hydrocarbon resistant strains tend to sporeulate less abundantly than the respective wild types.

Although patch confers no tolerance to the hydrocarbons all hydrocarbon resistant mutants "escape" the effect of L-sorbose at least as effectively as patch. On media containing sucrose and L-sorbose same of these mutants grow much better than Patch. Whether different levels of inhibition by sorbose are associated with different genes for resistance to aromatic hydrocarbons is now been investigated.  –  –  –  Department of Biology, Nuclear Research Center "Democritus", Athens, Greece.


3-Deoxy-D-arabino-heptulosonic acid 7-phosphate synthetase (DAHP synthetase) is the first enzyme of aromatic biosynthesis in micro-organisms and in \textit{E. coli} has been shown to be a regulatory system of at least 3 isoenzymes (Doy and Brown 1965 Biochim. Biophys. Acta 104:377). Control is by feedback inhibition (phenylalanine and tyrosine) and repression (phenylalanine, tyrosine and tryptophan (Brown and Day 1966 Biochim. Biophys. Acta 118:157).

DAHP synthetase has now been examined in dialysed crude extracts of wild type \textit{N. crassa} 74A, grown an Vogel's minimal medium at 25° for 48 hrs. Under the conditions stationary phase had not been reached. Extracts were made by grinding with glass and KH\textsubscript{2}PO\textsubscript{4} - NaOH buffer 0.1M pH 6.4 and dialysing against 0.025M of the same buffer. The supernatant was used after centrifuging the debris. DAHP synthetase was estimated essentially as described by Day and Brown.

The substrates are erythrose 4-phosphate and phosphoenolpyruvate and initial velocity measurements were determined by varying one substrate (10\textsuperscript{-5}M - 2 x 10\textsuperscript{-3}M) in the presence of excess of the other (2 x 10\textsuperscript{-3}M). By plotting v against s, sigmoid curves were obtained which, within experimental error, had a positive initial slope. Reciprocal Plots of 1/v against 1/s show the characteristics more clearly. Parts of these data replotted as 1/v against 1/s yield a straight line as required if 1/v against 1/s is a parabola. However, it appears likely that this is fortuitous and that the present data are more consistent with the characteristics of a non-rectangular hyperbola. It is important to make this distinction.

A parabolic 1/v against 1/s curve is consistent with a model: 

\[ \frac{K_1}{v} = \frac{K_2}{\frac{s}{1+s^2}} + ES + ESS - ES + \text{product.} \]