PHOTOREACTIVATION OF TRANSFORMING DNA BY CYTOCHROME b_2 FROM YEAST.

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Photoreactivation is the restoration of ultraviolet light induced lesions in biological systems by light with a longer wavelength (Jagger, 1958). Although the phenomenon had been described earlier, it aroused great interest after Kelner (1949) published an investigation on the photoreactivation of microorganisms. Rupert (1960) demonstrated photoreactivation of a transforming principle from Haemophilus influenzae by a yeast extract in combination with visible light. Several attempts to isolate the photoreactivating enzyme have been described (Rupert, 1964 and Muhammed, 1966). Dimerization of the pyrimidine bases in DNA is generally accepted as the result of UV-irradiation (Beukers and Berends, 1961). It has been found that photoreactivation runs parallel to a splitting of the thymine dimers in DNA (Wulff et al., 1962). No release of dimer containing fractions by enzymic degradation could be observed after photoreactivation (Setlow et al., 1964, 1965). The elucidation of the nature of a photoreactivating enzyme is a fascinating problem because an interesting energy transfer from a photoexcited acceptor to a cyclobutane containing dimer must be involved.

Screening of a relatively large number of microorganisms on photoreactivating activity concomitant with minimal DNA-se content showed that Saccharomyces cerevisiae and Streptomyces griseus are the most suitable starting materials for an isolation of the enzyme.

Initially our attempts to concentrate the enzyme were based on the idea that UV-irradiated DNA must form a complex with the enzyme. UV-irradiated salmon sperm DNA resp. DNA was adsorbed on a Millipore filter (Gillespie et al., 1965) or immobilized on an agarose column (Mc.Carthy et al., 1963). After incubation in the dark with a centrifugated homogenate of the organisms mentioned, the immobilized DNA resp. UV-irradiated DNA were removed. From the two eluates a difference spectrum could be measured because the UV-irradiated DNA had complexed the enzyme from the homogenate. For the extracts of S.griseus a peak was found at 430 mμ, a flat maximum at 555 mμ and two shoulders (at 350 and 370 mμ). In the case of Sacch. cerevisiae there was a small peak at 415 mμ and three shoulders (at 325, 355 and 380 mμ). The difference spectrum for the S.griseus extracts closely resembled the spectrum of cytochrome b in its reduced form (Inoue et al., 1965) and also the action spectrum of photoreactivation of this micro-organism (Kelner, 1949). The difference spectrum shown by the yeast homogenates also suggested a removal of cytochrome b. Elution of the UV-DNA complex with 0,3 M.$(NH_4)_2SO_4$ or exposure of the complex to visible light gave often irreproducible results and we therefore isolated cytochrome b_2 from baker's yeast (Rippa, 1961) and tested this for photoreactivating activity using the restoration of UV-damaged Haemophilus influenzae transforming DNA as assay

(Rupert, 1960). Our cytochrome b_2 preparations appeared to contain still a weak DNA-se activity and this lowered the degree of photoreactivation. We therefore also investigated a commercial (Sigma Chemical Company) cytochrome b_2 isolated from yeast that proved to be DNA-se free.

The results are summarized in the following table:

TABLE.

Preparation	Photoreactivating light	Number of transformants
UV-DNA + cyt. b_2 (according to Rippa, 1961)	-	20
"	+	80
UV-DNA + cyt. b_2 (Sigma Chemical Comp.)	-	40
"	+	560
UV-DNA	+	37
DNA	+	800

Transforming DNA and cytochrome b_2 were in 0.1 M NaCl, 0.01 M K-phosphate pH=7.0. The transforming DNA was mixed with an equal volume of cytochrome b_2 (1 unit). Photoreactivating light was applied for 20 minutes by 2 Sylvania cool-white fluorescence lamps at 37° C.

The remarkable conclusion can be drawn that cytochrome b_2 shows a significant photoreactivating activity.

This conclusion could be confirmed in a chemical assay (Duine and Berends) using the degradation of ^{14}-C-labeled DNA by snake venom phosphodiesterase as test. This assay is more or less similar to that described by Setlow et al. (1964) and depends on inhibition of the enzymic degradation by

pyrimidine dimers. First results show that the action spectrum for photoreactivation of transforming DNA resembles the absorbtion spectrum of cytochrome b_2 (Duine and Berends).

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