FURTHER STUDIES ON THE UV-INDUCED PHOTOREVERSIBLE AND
NON-PHOTOREVERSIBLE MUTATION THROUGH THE
ACTION SPECTRA IN YEAST CELLS\textsuperscript{11}

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The possibility that a certain molecular alteration in the constituents of DNA by
UV can be directly related to the mutational event has haunted many investigators (see
Freese 1963 and Setlow 1964, for reviews). It has recently been possible to correlate
the lethal action of UV with thymine dimer (TT) production in some systems (Setlow and
Setlow 1962, 1963), but it is not yet possible to make a definite correlation for mutagenesis.

In the meantime, clear evidence for TT was provided in the case of streptomycin
resistance in E. coli by Witkin et al. (1963) that mutational events were photoreversible
in normal strains but not in strains that lack photoreactivating enzyme. However, this
was not the case for the mutation represented by reversions to prototrophy, indicating
there are lesions other than TT that contribute to mutation (Kaplan 1963; Witkin 1963).

In the previously reported experiments (Ito et al. 1964), a more physical analysis for
the induced forward mutation, adenine independent to adenine requiring, was attempted,
penetrating into the molecular level, by combining UV action spectrum for the mutation
induction with photoreversion experiments. The shape of the action spectrum generally
resembled that of the absorption spectrum of thymine in the experiments. It was also
found that the total cross section of the mutation at 2700Å was phenomenologically
separable into two components, photoreversible (major) and non-photoreversible (minor),
indicating bases other than thymine may also be involved in this particular mutation.

In this paper are presented further studies on the two components of the cross
sections extended to several other wavelengths. The results may provide the comple-
mentary bases to the biochemical approaches for the interpretation of mutagenesis by
UV.

MATERIALS AND METHODS

Preparation of cells: The yeast cells used in the experiments, a diploid strain of
Saccharomyces cerevisiae heterozygous for adenine locus (ad\textsuperscript{+}/ad\textsuperscript{−}his\textsuperscript{−}/his\textsuperscript{+}arg\textsuperscript{−}/arg\textsuperscript{−}),

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were prepared according to the previously described procedures (Ito et al. 1962a). Small non-budding cells from the logarithmic phase of culture were gathered and suspended in distilled water (about $10^8$ cells/ml) for UV irradiation and VL (visible light) illumination.

**UV irradiation and VL illumination**: Monochromatic UV (bandwidth 24 Å) from a grating monochromator with Ushio 2-KW xenon arc lamp (Ito 1964) was used for all UV irradiation. The absolute intensities were determined by both actinometric means and a vacuum thermocouple calibrated with a standard lamp. This was further checked through intercomparison of the cross sections of the monomerization reaction of T4T (Nakai et al. 1965). The intensities, at the experimental position were $4.25 \times 10^{12}$ quanta/cm²/sec for 2310 Å; $4.21 \times 10^{12}$ quanta/cm²/sec for 2350 Å; $4.94 \times 10^{12}$ quanta/cm²/sec for 2390 Å; $11.7 \times 10^{12}$ quanta/cm²/sec for 2537 Å; $16.8 \times 10^{12}$ quanta/cm²/sec for 2600 Å; $24.1 \times 10^{12}$ quanta/cm²/sec for 2650 Å; $29.5 \times 10^{12}$ quanta/cm²/sec for 2700 Å; $34.9 \times 10^{12}$ quanta/cm²/sec for 2800 Å. Techniques of post-illumination by VL have also described previously (Ito et al. 1964). To minimize the heating effect during the illumination suitable precautions were taken. Duration of illumination was, throughout the experiments, 1 hour, which was chosen for obtaining saturation in photoreversing effect, yet keeping other disturbing effects to a minimum under the experimental conditions.

"Illumination" was used to avoid unnecessary confusion in referring to the exposure to all radiation from the photoreversing lamps (Matsuda 250-W reflector lamp and Hitachi FL 20-W fluorescent lamp), even though they emit also near ultraviolet to a lesser extent. The measurement showed that light of wavelength below 3850 Å was at most a few percent of the total energy emitted.

**Estimation of incident intensity inside the cell ($I_i$)**: The incident flux ($I_o$) to the cell is ordinarily taken as flux that reaches the cell surface. However the incident beam is subjected to complicated scatterings at the cell boundary. The scattered radiation out of the cell does nothing to the cell as far as the effect on the genetic material is concerned. Thus, true incident flux ($I_i$) with respect to the genetic structures is a part of $I_o$ and may be expressed as $I_i = t \cdot I_o$, where $t$ is a constant that depends, for instance, on the molecular arrays in the cell membrane. The cross section of the physical change in the genetic material should be evaluated based on $I_i$.

We have assumed that 0.4 is a very reasonable value for $t$ (for details, see Ishizaka and Ito, 1965). The absorption data by Wilkie (1963) in yeast cells is in general accord with this value. For VL illumination such a correction was not made, as the purpose of this treatment is limited to getting maximum photoreversion only.

**Detection of mutational change**: Mutational changes were detected, with respect to adenine locus, by observing the color of the colonies on nutrient agar after four days of incubation. Pink color has genetically been identified mostly as mutation, either lethal or viable, at the locus although recombinational processes may be involved to some extent. (Ito et al. 1962a, 1962b; Yamasaki et al. 1963, 1964).
RESULTS

A typical result of the photoreversion experiments is shown in Figure 1 for the induced genetic change by 2650Å and 2800Å. The upper straight line shows the dependence of the induced frequencies on the doses, and the lower one is a similar curve except that the photoreversing light for maximum reversion has subsequently been applied. In both cases the straight lines were fitted by least squares. Therefore, the ratio of the two slopes may be taken as a fraction of the non-photoreversible component, $\alpha$, for the induced change by respective wavelengths. Similarly, for the other five wavelengths five fractions were obtained, and these are listed in Table 1 as $1-\alpha$, that is, photoreversible fraction. Over the range of the wavelengths employed the fractions are not very different although there is some

Fig. 1. Induced forward mutation at adenine locus by monochromatic UV and post-illumination effect of VL at saturation dose in diploid heterozygous $(ad^+/ad^-)$ yeast cells. Straight lines have been fitted by least squares.

Fig. 2. Action spectra for the forward mutation $(--\bullet--)$ at adenine locus and its non-photoreversible component $(\cdots\square\cdots)$ by UV. Calculations of the latter have been based on the figures in Table 1. The cross section has been corrected for scattering of UV at the cell boundary.
tendency of increase around 2537 Å~2600Å. The average value over the wavelengths used is somewhat less if compared with those by Kaplan (1956) in S. marcescens and Zelle et al. (1958), in E. coli but the general features of wavelength-dependence among these works are in good conformity.

Based on these fractions and the newly measured scattering factor, 0.6 (1−t, see METHODS), the cross sections of the total induced genetic change and of the non-photoreversible change were calculated for several wavelengths in the UV range (Fig. 2). Unfortunately it is not possible to state that the action spectrum for non-photoreversible change definitely resembles the absorption spectrum of cytosine or cytidylic acid. The results only show clearly that the majority of the overall genetic change is photoreversible, and that non-photoreversible change, leading to seemingly the same genetic change, is accompanied proportionately by the photoreversible change for each wavelength in the dose range used.

An incidental observation revealed that the unusually small cross section at 2390Å described in the previous paper (Ito et al. 1964) might, at least partly, be due to the inadequacy of the approximation, which was based on the mutation frequencies only in the lower levels (up to 0.2×10⁻²). This was caused by the practical limitation, for the shorter wavelengths, in the handling of relatively long exposures in a limited time. The recent improvement of the apparatus made it possible to conduct in much shorter time a series of experiments at even higher doses, yielding frequencies comparable to those obtained previously in the other wavelengths, and it turned out that the previously obtained cross section at this wavelength has to be corrected by a factor of almost 2 if calculated on the same level of the frequencies as the other. (The value shown in Figure 2 has not been corrected in this way.) This means that the dose-frequency curve considerably deviates from the linear relation in the shorter wavelengths. The significance of this phenomenon has to be investigated.

DISCUSSION

Mutation cross section of UV: Cross section of mutation as a reaction probability may be expressed in the most general form as the sensitivity per unit incident radiation dose (or relative efficiency). Hollaender and Emmons (1941), for example, obtained such a mutational efficiency for spores of Trichophyton mentagrophytes. However, cross section expressed in absolute units, as seen in certain in vitro experiments recently being
reported (Johns el al. 1962; Deering and Setlow 1963), is more desirable in order that the mutation in the microorganism can be discussed in terms of physical change in DNA molecule (Ito et al. 1964).

Thus if one is able to have a reliable scattering factor, then the cross section derived in absolute units would be of great significance; and the comparison with those of the absorption of hypothetical targets in DNA molecule (thymine base, for example) or some of the known cross sections of elementary photochemical changes of the bases may be justified.

Since the newly obtained scattering factor is substantially different from our previous estimation (Ito et al. 1964), the previous figures of the cross sections of the mutation have been recalculated (Fig. 2). They are of the order of magnitude of $10^{-18}$ cm$^2$/quantum depending on the wavelengths. This is roughly one several-hundredths of the lethal cross section obtained in haploid yeast cells (unpublished data). No comparable data are available in the literature for this type of representation of specific mutation cross section in absolute units so far as the authors are aware, except for the inactivation of the phages (for example, Rauth 1965).

Photoreversible and non-photoreversible components of UV induced mutation: The present data show that the photoreversible fraction of the mutagenic effects of UV in yeast cells is not very different for seven wavelengths ranging from 2310~2800Å, and the average value is about 70%. More or less similar figures have been reported in different systems (Kaplan 1956; Zelle et al. 1958).

Kaplan (1963), Witkin et al. (1963), and Witkin (1964) reported that certain mutations to prototroph are photoreversible in E. coli phr$^-$ strain, and concluded that thymine dimer is not the sole mutational damage in DNA. Existence of such a fraction in the mutation might mean that the photoreversible component can be further divided into the two components, thymine-dimer damage and the presumed non-thymine-dimer damage instead of only thymine dimer as envisaged in earlier work (Ito et al. 1964).

Unfortunately the accuracies of the results do not permit a conclusion as to whether or not the spectrum of the non-photoreversible component (about 30%) more closely resembles that of the absorption of cytosine than that of thymine. However, two points should be mentioned. Firstly, as Setlow (1963) pointed out in a similar experiment of the inactivation of transforming DNA, the bases in native DNA may not act as independent absorbers of UV. Secondly, various recovery mechanisms (Jagger and Stafford 1965) may well be operating, being differentially overlapped, in cells so that the spectrum for non-photoreversible fractions exhibits a complicated pattern as was noted also in the early work in E. coli by Zelle et al (1957). Different possible phases of those mechanisms have been discussed recently by many workers in E. coli systems (Kaplan 1963; Witkin 1964; Jagger 1964; Jagger and Stafford 1965).

In this connection, recent results by Setlow et al. (1965), in homopolymer complex of polydeoxyinosinic acid: polydeoxy-cytidylic acid, very interestingly indicate that cytosine
dimers (CC) are formed by UV irradiation and destroyed by visible light in the presence of photoreactivating enzyme. Such a possibility had been speculated by us in vivo system (Ito et al. 1964), when an unexpectedly high photoreversion rate was found in mutation. However, because the relative yield of CC, and hence conversion to uracil dimer, appears to be considerably less if compared with TT (Smith 1963; Setlow et al. 1965), the sole contribution of CC to mutation production, consisting of all of the photoreversible component, is hard to reconcile with the observed mutation cross section unless the size of the gene becomes much more expanded in terms of, especially, C,C sequence.

As to a non-photoreversible component we would merely point out that there is a possibility that one or some of the photoproducts of cytosine is responsible because UV action on cytosine shows a complicated pattern of reactions of which cytosine is quite different from that of thymine.

Genetical implications: Now we can deduce tentatively three types of molecular lesions that might be related to the mutational effects in yeast cells, namely, thymine-dimer damage, and non-thymine-dimer damage of either photoreversible or non-photoreversible of which the nature is yet unknown. On the other hand, from the genetic point of view, we know operationally three types of mechanisms that phenotypically lead to the same change, ad*→ad−, as judged by colony color in the diploid heterozygous system (ad+/ad−). They are mitotic crossing-over, allelic recombination, and point mutation (direct base change). Previously it was reported (Ito et al. 1962a) that at most 20~30% of UV-induced variants in the system may be attributable to recombinational processes. Nevertherless, the majority were presumably true point mutations, although the experimental system was not appropriate to assess what fraction was allelic recombination or the like. Thus, this question may now be raised: in what way are the three types of DNA lesions and of the genetic effects discussed in the above interrelated. Since that the major DNA lesion which is photoreversible by the exposure to light of longer wavelength (longer than 3850Å) represents enzymatically reparable pyrimidine dimer, i.e. direct base change, the non-photoreversible component in this experiment might correspond to either mitotic crossing-over or allelic recombination or both. Further study is needed to assign each genetic process to the specific non-photoreversible molecular damages.

SUMMARY

UV induced adenine-requiring mutation in yeast cells was investigated quantitatively at several wavelengths of monochromatic UV and at maximum photoreversion conditions in the hope of elucidating the nature of mutagenesis. The results may be summarized as follows:

1. In general, mutant production by UV and the remaining mutant, after subsequent
treatment by VL at maximum photoreversion, both increased almost linearly against the UV dose, irrespective of the wavelengths used except for some deviation in the shorter wavelengths.

(2) Photoreversible and non-photoreversible fractions of the induced mutations were determined by taking the ratios of the above two slopes.

(3) Using a scattering factor of UV at the cell boundary, reliable absolute cross sections of the mutation were obtained for the several wavelengths.

(4) Some implications of the above findings were discussed with special reference to the relation between mutation production and the inducible photochemical changes in DNA bases by UV.

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LITERATURE CITED


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