Photodynamic inactivation of transforming principle

Photodynamic action is the dye-sensitized autoxidation of a biological substrate accelerated by the action of visible light[1]. Using an aromatic amine as the substrate we have demonstrated that the dye-sensitized photo-oxidation in this case involves the reaction of oxygen with the dye molecules in the long-lived (triplet) excited state[2]. Many types of dyes, such as azo dyes or triphenylmethane dyes (in the free state) do not undergo transitions to the triplet state, and hence are not photosensitizers. It is of interest to determine whether those dyes, which are sensitizers for the synthetic substrate, are also effective for a biological substrate.

We have investigated the dye-sensitized photo-inactivation of transforming principle since it is a relatively pure substance which loses its biological activity when no physical changes in its structure are evident. DNA* was prepared from a rough strain of *Diplococcus pneumoniae* which was resistant to streptomycin[3].

Assays for the transforming activity of the DNA were carried out in a manner identical with that described by ROSENBERG *et al.*[4]. It was found that at DNA concentrations greater than 2 μg/ml the transforming activity was independent of concentration. All subsequent determinations were made at three different DNA concentrations greater than this value. The average percentage of cells transformed was about 5.6 %.

For the determination of the photodynamic inactivation of the transforming activity of DNA, part of a solution containing 23 μg DNA/ml was used as a control, and dye was added to the remainder to give a final dye concentration of $5 \cdot 10^{-6}$ *M*. Aliquots of these solutions were irradiated for varying lengths of time, using a 500-W tungsten-lamp projector with a u.v.-cut-off filter (Corning No. 3–74) to eliminate radiation below 400 mμ. The samples were illuminated at a distance of 15 cm from the front surface of the projector lens so as to eliminate heating effects. In each case a sample not containing dye was also illuminated, and served as a second control sample: in no case did those samples differ in transforming activity from samples which did not contain dye, and which had not been irradiated. All samples were then tested for transforming activity, each inoculum tube containing 2.3, 3.5, and 4.6 μg DNA/ml, with $5 \cdot 10^{-7}$, $7.5 \cdot 10^{-7}$ and 10^{-6} *M* dye, respectively. Where photodynamic inactivation occurred the remaining transforming activity was in all cases still independent of DNA concentration in the concentration range chosen. The transforming activities given in Table I represent the average of triplicate assays at the three different DNA concentrations and are in error by no more than 10 %.

In Fig. 1 it is seen that about half the original activity (5399 cells transformed per ml of suspension) is lost after 15-min irradiation in the presence of methylene blue. There is no photodynamic effect in the absence of dye, and the dye has no effect in the dark. In Table I are listed the results for 15-min irradiation with various dyes. The dyes were used in $5 \cdot 10^{-6}$ *M* concentration in the original solutions so that upon addition to the inoculum the viability of the cells was unimpaired. This was checked by performing total counts on inocula with and without dye present. Although

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Abbreviations: DNA, deoxyribonucleic acids.

* The preparations of DNA having transforming properties and its assay were carried out in the laboratory of Dr. D. L. HUTCHINSON at the Sloan Kettering Institute in cooperation with Dr. F. M. SIROTNAK, whose many helpful suggestions are hereby gratefully acknowledged.

none of the dyes are bactericidal at the concentrations employed, both Thioflavin TG and Proflavine decrease the transforming activity, even in the absence of light, and any photo-inactivation phenomena are thus masked. These observations may possibly be correlated with the known mutagenic activity of acriflavine, of which

TABLE I

Dye	*Control (no dye) Transforming activity**	*Activity and control*	
		Dye alone % of	*Dye and light*
Rose bengal	1212	100.5	59.8
Methylene blue	5368	95.6	44.0
Proflavine	1682	68.2	64.2
Neutral red	1682	110.1	40.6
Crystal violet	1682	101.4	72.1
Thioflavine TG	1682	71.0	63.2
Cresyl violet	1055	105.3	93.0
Methyl orange	1433	100.5	98.9
Alizarin red	1641	96.2	97.9
Riboflavin	9120	89.9	51.9

* "Transforming activity", the number of cells transformed per ml of suspension under the conditions of assay as outlined in the text.

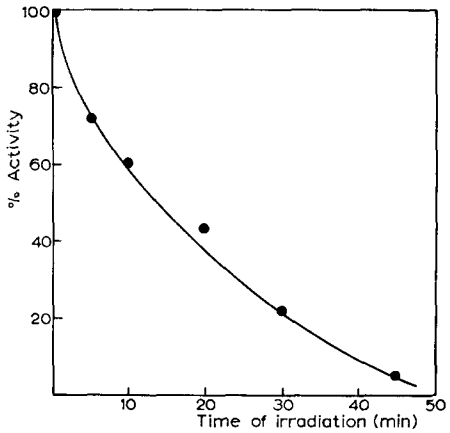

Fig. 1. Rate of photodynamic inactivation of Transforming Principle. $3.5 \cdot 10^{-6}$ *M* methylene blue was used as sensitizer. The oridinate indicates the remaining activity as a percentage of the original activity.

proflavine is the principal constituent. Our failure to demonstrate photodynamic inactivation with proflavine is in agreement with the observation that acriflavine failed to photosensitize the inactivation of the transforming principle of *Hemophilus influenzae*[5], although here no dark effect was observed.

The anthroquinone dye alizarin red, the azo dye methyl orange, and the oxazine dye cresyl violet are without photodynamic effect on the transforming principle, as they are when *p*-toluenediamine is employed as a substrate. The triphenylmethane dye crystal violet acts as a sensitizer because it is bound to the nucleic acid, and, again in conformity with prior observations using the aromatic amine as substrate, it can act as a photosensitizer in the bound state. Rose Bengal (a xanthene dye), methylene

blue (a thiazine dye), and riboflavine, are as efficient sensitizers for the inactivation of transforming principle as they are for the photosensitized oxidation of *p*-toluenediamine. Contrary to our previous report[2], we have since found that in the strict absence of oxygen, and using ethylenediaminetetraacetic acid as electron donor, neutral red (an azine dye) can be photoreduced. In the presence of no more than a trace of oxygen it can sensitize the photopolymerization of acrylamide. Our failure to control the oxygen level accounts for the difference between the present results and those obtained earlier. Spectrophotometric studies also show that neutral red is a sensitizer for the photodynamic oxidation of *p*-toluenediamine. In conformity with these findings the present study shows that neutral red is also able to act as sensitizer for the photodynamic inactivation of transforming principle.

Thus, in every case the ability of the dyes to photosensitize the autoxidation of the organic substrate is paralelled by their ability or inability to sensitize the inactivation of transforming principle.

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Received March 18th, 1960

Biochim. Biophys. Acta, 42 (1960) 533–535

The isolation of bacterial mutants defective in amino acid transport

Many bacterial strains accumulate amino acids from the surrounding medium[1–4]. The mechanism is obscure, but the phenomenon clear. SCHWARTZ, MAAS AND SIMON[5] have reported that mutants of *Escherichia coli* W with an impaired concentrating mechanism for arginine can be isolated by selection on canavanine-supplemented plates, and for glycine by selection on D-serine-supplemented plates.

We have successfully used another method for isolation, and have obtained three mutant types of *E. coli* W which are defective in the ability to accumulate either histidine, proline or glycine. The first two are new; the last one presumably similar to that isolated by SCHWARTZ *et al.*[5].

For simplicity, these mutants are referred to below as transport-negative (Tr^-). These mutants may be analogous to the permease mutants of the β-galactoside system[4,6], but we prefer the general term "transport" because the process is not understood.

The isolation procedure starts with the use of an auxotroph, *e.g.*, His^-, deficient in some step in histidine biosynthesis, but capable of accumulating histidine from the medium. The wild type (with respect to the transport character), hence $His^-Tr^+_{his}$,