Induction of Bacteriophage and Colicin by Means of Acridine Orange

Acridine derivatives and similar tricyclic dyes can influence the biological properties of microorganisms; they can in particular inactivate them and induce mutations etc.[1]. It has been proved for some dyes that these biological effects are conditioned by the participation of visible light. The photodynamic mechanism for inactivation and mutagenic effect on bacteria was demonstrated by WACKER et al.[2] for methylene blue and thiopyronine, and by KOUDELKA et al.[3] for acridine orange (AO). This mechanism can be explained by the energy transfer from the excited dye molecule attached to important cellular structures, especially to nucleic acids, with the result that these structures are destroyed[4,5]. According to PEACOCKE and SKERRETT[1], the bactericidal and bacteriostatic effects of acridine dyes are explained by the same mechanism.

In the present work we used the photodynamic effect of AO on the induction of phage and colicin production in lysogenic and colicinogenic *E. coli* cells.

The following strains of *E. coli*, first described by ŠMARDA[6], were used: strain 18 (both lysogenic and colicinogenic), II (sensitive to phage produced by strain 18), and B_1 (sensitive to its colicin).

The cells of strain 18 were harvested from a broth culture towards the end of the logarithmic phase of growth. They were resuspended in synthetic medium M-9[7] and diluted with the same medium to obtain about 10^7 cells per ml. To 5 ml of suspension a $10^{-4}M$ solution of acridine orange hydrochloride was added, to a final concentration of $2 \cdot 10^{-6}$–$2 \cdot 10^{-5}M$. The same suspension, to which the corresponding volume of M-9 was added, served as control. All samples were incubated 1 h at 37°C. From each pair of samples with the same dye concentration one portion was transferred into a Petri dish and irradiated for 30 min with a 200-W lamp at a distance of 20 cm. The control was also irradiated at the same time. The other portion of samples with AO was incubated in the dark. All samples, both irradiated and non-irradiated, were carefully centrifuged. The cells from the pellet were transferred to suitable volumes of broth and incubated at 37°C.

2-ml samples were removed at intervals varying from 0 to 6 h and the number of living cells (colony formers) in them counted. The titre of phage and colicin was then estimated in the ultrafiltrates or supernatant fluids obtained after centrifugation.

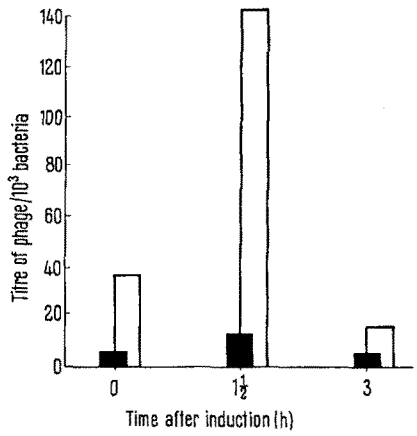
In these experiments we observed an increase of free phage titre to 4 times the control value (titre in the suspension without the dye) at intervals ranging from 1.5–5 h after irradiation. The effective concentration of AO was $1.6 \cdot 10^{-5}M$. The colicin titre was increased to twice the control value by means of $2 \cdot 10^{-6}M$ AO. The result of this experiment was not, however, fully convincing. We did not detect any titre increase in the suspensions incubated in the dark.

In all experiments we observed a drop in the number of living cells in irradiated suspensions (with AO) in comparison with the control. This was not demonstrated in non-irradiated cultures. The doses of light used exerted the photodynamic bactericidal effect on *E. coli* cells. The drop in the number of living cells causes, of course, a drop in phage and colicin production as well. For this reason we determined the relative titre of phage and colicin per living cell.

On comparing these relative titre values with those in a control culture without dye, we found an 11-fold (maximum) increase in the production of phage in the cultures irradiated with AO. This increase was detectable up to 5 h after induction (Figure). As far as colicin is concerned, we again observed an increase of its titre through the photodynamic action of $2 \cdot 10^{-6}M$ AO to only twice the control value.

For the induction of phage in lysogenic and of colicin in colicinogenic bacteria many physical and chemical agents have been used (for review see [8]). Most of them are simultaneously mutagens. Of the acridine dyes only proflavine[9] and acriflavine[10] have been used for the induction of phage, but no positive results were achieved. Phage production due to the simultaneous effect of AO and visible light was successfully performed in our experiments. The induction effect in the non-irradiated control group was not significant; here the minute changes in phage titre (max. 2-fold increase) did not exceed the range of experimental deviations.

The induction effect thus obtained is relatively low in comparison with that of UV-irradiation or other inducing agents. However, it is possible that phage induction by the photodynamic action of AO could be increased by varying the experimental conditions. We suppose that the negative result in proflavine and acriflavine induction[9,10] was due to neglect of the photodynamic action of the dye. As nucleic acids are the site of reaction of AO, proflavine and other similar dyes in the cell, we suggest that regulative mechanisms of the cell metabolism,



Titre of free phage per 10^3 living bacteria in *E. coli* 18 culture irradiated without AO (the black column) and in the presence of $1.6 \cdot 10^{-5}M$ AO.

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directed by nucleic acids, are influenced by the photodynamic action of dyes. Phage induction can then be explained in the same way.

After the experiments described above had been finished, the paper of GEISSLER and WACKER[11] appeared. These authors observed induction of bacteriophage λ in *E. coli* K12 by means of thiopyronine only after visible light irradiation. In contrast to AO, it is well known that thiopyronine photooxidizes the guanine residues in the DNA molecule.

Zusammenfassung. Es wird über Ergebnisse bei der Induktion der Phage- und Colicinsynthese in *E. coli* (Stamm 18) durch photodynamische Wirkung des Acridinorange berichtet. Der Phagentiter wurde 11mal, der Colicintiter kaum 2mal erhöht. Acridinorange zeigte ohne Belichtung keinen deutlichen Effekt.

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Charge-Transfer Complexations among Biochemically Reactive Compounds[1]

The theory of charge-transfer spectra was developed by MULLIKEN[2] and a review published by ORGEL[3]. As pointed out by KOSOWER[4], a necessary consequence of the existence of electron donor-acceptor complexes stabilized by charge transfer is the ability of the ground state to absorb light, undergoing an electronic transition to an excited state for which the major contributing form is the complex.

Studies on the complexation of flavins have been reviewed by BEINERT[5]. Complexing reagents cause a decrease of intensity and a slight band shift toward longer wavelengths in both absorption and fluorescence spectra of flavins. Indeed, charge-transfer interactants in biochemistry include purine and pyrimidine derivatives, aromatic amino acids, hormones, vitamins and coenzymes, and agents which are known to uncouple electron transport from oxidative phosphorylation[6].

The present work was done to extend generally our recognition of the phenomenon of charge-transfer complexations among biochemically significant compounds, especially in the present case with complexers of the vitamin, riboflavin, which participates as coenzyme forms in oxidations catalyzed by flavoproteins, and with complexers of a recently recognized uncoupler of oxidative phosphorylation, 1,1,3-tricyano-2-amino-1-propene[7].

Experimental. Spectrophotometric measurements were made with a Beckman Model D.U. with photomultiplier tube using fused silica cuvettes at room temperature. The wavelengths selected for noting changes in optical density of any given compound were those near a characteristic chromophore where maximal decreases were seen upon complex formation.

The relative magnitude of complexation of several representative compounds with riboflavin is shown by the data in the Table. Most of the complexing reagents shown are presumed to undergo π bond interactions. Though π, π complexes probably predominate, n, π interaction is also possible in certain instances. As is true with charge-transfer complexes in general, the degree of association of these complexes is temperature dependent.

The spectral changes which accompany complexation of riboflavin with 1,1,3-tricyano-2-amino-1-propene are shown in Figure 1. As seen in part A of the Figure, ab-

[1] This work was supported by Public Health Service Grants HE-04138 and AM-04585.

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Complexation of various compounds with D-riboflavin[a]

Complexing compound	64 μM compound alone $\lambda m\mu$	O.D.	130 μM riboflavin added O.D.	Δ O.D.	% decrease
L-Tryptophan	275	0.36	0.03	0.33	92
p-Aminobenzoic acid	265	0.90	0.22	0.68	76
Triethylenemelamine	225	2.70	0.64	2.06	76
Flavianic acid	225	1.27	0.43	0.84	67
Picric acid	220	0.89	0.30	0.59	66
m-Dinitrobenzene	240	0.97	0.52	0.45	46
Caffeine	275	0.60	0.33	0.27	45
o-Phenylenediamine	290	0.20	0.14	0.06	31
3,5-Dinitrosalicylic acid	215	0.76	0.61	0.15	20
Anthranilic acid	210	1.45	1.16	0.29	20
Carbonylcyanide *p*-chlorophenylhydrazone	230	0.50	0.41	0.09	18
L-Phenylalanine	205	0.55	0.52	0.03	5

[a] Values for $\lambda m\mu$ and O.D. of complexing compounds were measured in 0.1 M sodium phosphate buffer, pH 7, against a buffer blank. Values for O.D. of complexing compounds in the presence of riboflavin were measured in the buffer against a buffer plus riboflavin blank.