Vol. 89, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 27, 1979 Pages 729-734

CONVERSION OF ENVIRONMENTAL POLLUTANT TO MUTAGENS BY VISIBLE LIGHT

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Received June 13, 1979

SUMMARY

Illumination of DMBA, 3-methylcholanthrene, 2-aminoanthracene and chrysene with visible light resulted in the formation of direct-acting chemicals endowed with genotoxic and frameshift mutagenic activities. These findings may be of relevance in assessing the potential health hazards inherent in the planned conversion to diesel fuels which is expected to result in increased atmospheric levels of polycyclic aromatic hydrocarbons.

Most organic atmospheric carcinogens (e.g. polycyclic aromatic hydrocarbons) depend upon metabolic conversion to ultimate carcinogens to exert their neoplastic and/or genetic actions. Cytochrome P450-dependent monooxygenases play a significant role in this biotransformation and the hepatic tissues are believed to be the primary sites of this enzymic conversion. This metabolic activation of precarcinogens to active intermediates can be studied conveniently in vitro using a combination of microsomal preparations coupled to bacterial indicator systems (1).

In the present study it is shown that exposure of environmental carcinogens to visible light results in the formation of "stable" products endowed with mutagenic and DNA-modifying activities. It is thus conceivable that direct-acting carcinogens may result from the photodynamic activation of atmospheric pollutants. These newly formed photo-induced chemicals which are not dependent upon enzymic activation may thus act directly at the portal of entry (e.g. skin, lungs).

Abbreviations Used: DMBA, 7,12-dimethylbenz(a)anthracene; RBF, riboflavin.

0006-291X/79/140729-06$01.00/0

Table 1

Mutagenicity of DMBA for Salmonella typhimurium TA98 Following Illumination with White Light

Additions	Amount (μg per plate)	Revertants per Plate	
		Dark Controls	Photoactivated
DMBA	0	15	19
	10		173
	50		419
	100		514
	250	22	424
	500	20	380
	1000	28	272
2-Nitrofluorene	100	687	

A freshly prepared solution of DMBA (Stark Associates, Inc. Buffalo, New York, 10 mg per ml of dimethylsulfoxide) was exposed to fluorescent white light (Dura-Test Vita lights, North Bergen, New Jersey, the emission spectrum of this light source has been described (20,21). The temperature was maintained at 23°C and the total light dose was 130 kJ/m^2 (measured at 450 nm). Immediately following illumination, dilutions of the irradiated DMBA as well as of a dark control were tested in the absence of a metabolic activation mixture, for mutagenicity in the Salmonella tester strain TA98 (which is an indicator strain for frameshift mutations (1). The plates were incubated in the dark (22) for 48 hours and revertants to histidine-independence were enumerated. Colonies from each plate were tested further for true mutation by subculturing on histidine-free medium. Each dilution was tested in triplicate and the experiment was repeated on several independent occasions.

It should be noted that although the photoproduct, in dimethylsulfoxide, is stable at -20°C for 1 day, it gradually loses mutagenic activity if storage, even at -20°C, is prolonged.

2-Nitrofluorene is included as a positive control for strain TA98.

DMBA, a widely studied potent carcinogen, presumably derives its carcinogenic potential from its metabolism to the corresponding trans -3,4-diol 1,2-epoxide (2). Indeed, for a number of polycyclic hydrocarbons the 3,4-diol-epoxides are more potent carcinogens and mutagens than the parent compounds and unlike the latter they do not require metabolic activation by microsomal enzymes to express their genetic activity (2-10).

It was found, however that illumination of DMBA with white light resulted in the formation of a preparation possessing direct-acting frameshift mutagenic activity (i.e. ability to induce genetic effects in Salmonella tester strain TA98, Table 1). This photo-induced activity did not appear to be dependent upon the generation of singlet oxygen as it was not enhanced in the presence of

Table 2

Photo-Induced Mutagenicity for Salmonella typhimurium TA98 of Several Polycyclic Aromatic Chemicals

Expt.	Additions	Revertants per Plate	
		Dark Controls	Photoactivated
I	None (Solvent)	12	6
	DMBA	27	255
	DMBA + NaN_3	26	341
	DMBA + RBF	21	97
	DMBA + NaN_3 +RBF	16	190
	NaN_3	11	9
	RBF	12	8
	NaN_3 + RBF	14	17
	2-Nitrofluorene (100 µg)	766	
II	None (Solvent)	9	12
	Chrysene	6	71
	2-Aminoanthracene	18	82
	3-Methylcholanthrene	7	85
	2-Nitrofluorene (100 µg)	616	

Freshly prepared solutions in dimethylsulfoxide and mixtures made therefrom [DMBA (final concentration: 5 mg/ml, see Table 1), NaN_3 (final conc.: 0.65 mg/ml, Fisher Scientific Co.), RBF (final conc.: 5 mg/ml, Sigma Chemica Co.), chrysene (final conc.: 4.3 mg/ml, Stark Associates, Inc. Buffalo, New York), 2-aminoanthracene (final conc.: 5.7 mg/ml, Aldrich Chemical Co., and 3-methylcholanthrene (final conc.: 5.6 mg/ml, National Cancer Institute, Chemical Repository, IIT Research Institute, Chicago, Ill.] were exposed to white light (see Table 1, total dose 130 kJ/m^2, measured at 450 nm) whereupon triplicate 100 µl amounts of the specimens were tested at once in the Salmonella mutagenicity assay (1). The plates were handled as described in Table 1. As noted previously, there was a loss of mutagenicity of the photoproducts upon prolonged storage at -20^{0}C.

riboflavin, (Table 2, Expt. I) an efficient photogenerator of singlet oxygen (11-13) nor was it decreased by the presence of sodium azide, a quencher of this species of oxygen (14,15). As a matter of fact, paradoxically, illumination in the presence of riboflavin decreased the mutagenic activity, while sodium azide increased it (Table 2). This suggests the possible presence in the preparation of at least two photoproducts, one of which, the result of an interaction of singlet oxygen with DMBA, is not endowed with mutagenic activity. When the singlet-oxygen-mediated reaction is blocked, as by sodium azide, more of the mutagenic photoproduct is generated. Previous studies had indeed indicated that illumination of DMBA in the presence of a singlet oxygen generator resulted in

Table 3

DNA-Modifying Activity of Photoproducts
As Determined by the Preferential Killing
of DNA Polymerase I-Deficient E. coli

Additions	Amount (μg per tube)	Relative Survival		Dark Control		PhotoProducts			
		Pol A+	Pol A_1^-	Survival Index	Intepretation	Relative Survival Pol A^+	Relative Survival Pol A_1^-	Survival Index	Interpretation
Solvent Control		100%	100%	1.00	-				
Ethyl methanesulfonate (Positive Control)	140	52%	1.8%	0.03	+				
Chloramphenicol (Negative Control)	10	100%	107%	1.07	-				
2-Aminoanthracene	57	100%	100%	1.00	-	67.6%	11.7%	0.17	+
Chrysene	43	100%	100%	1.00	-	100%	42.3%	0.42	+
DMBA	50	100%	91.9%	0.92	±	100%	63.4%	0.63	+
3-Methylcholanthrene	56	80.5%	80.0%	0.99	-	72.9%	56.3%	0.77	+

Bacteria (E. coli pol A^+ and its DNA-polymerase I-deficient derivative E. coli pol A_1^-) were grown in medium HA+T (23). The cultures were diluted in medium HA+T to an approximate density of 1500 cells per ml and 100 μl amounts distributed into tubes which also received 10 μl of the test agent. The mixtures were incubated at 37°C in the dark for 2 hours whereupon the tubes were cooled and each received 2 ml of molten agar (0.75% HA+T agar at 43°C), the tubes were mixed, their contents poured onto agar plates (25 ml of 1.5% HA+T agar) and upon solidification of the top agar, the plates were incubated at 37°C in the dark for 2 days and surviving bacteria were enumerated. Results were expressed as per cent survival compared to that of the respective solvent controls. The survival index (S.I.) is the ratio of per cent survival of pol A_1^-/per cent survival of pol A^+. An S.I. value of 1.00 (0.96-1.0) indicates a lack of preferential killing of the pol A_1^- strain (i.e. a negative result), values in the range of 0.86-0.95 are taken as borderline or weakly positive and values below 0.85 are taken as evidence of a preferential killing of the pol A_1^- strain (i.e. positive result). All determinations were made in triplicate; every assay included a positive (ethyl methanesulfonate) as well as a negative (chloramphenicol) control.

The conditions of illumination and the concentrations of the solutions exposed to light are given in Tables 1 and 2, respectively.

the formation of a photoproduct (16), presumably 7,12-epodioxy-DMBA (17). If the above conclusion is correct, this oxidation product of DMBA is not involved in the mutagenic action of the DMBA photoproduct.

In addition to its mutagenic activity, the photoproduct of DMBA is also capable, in the absence of metabolic activation, of selectively inhibiting the growth of DNA repair-deficient bacteria (DNA polymerase I-deficient E. coli, Table 3) which is taken as an indication of a selective modification of the cellular DNA (18).

Illumination of other polycyclic aromatic chemicals (e.g. 2-aminoanthracene, chrysene, 3-methylcholanthrene) also resulted in the formation of direct-acting frameshift mutagens (Table 2, Expt II) and DNA-modifiers (Table 3).

The present findings which indicate that atmospheric pollutants may be phototransformed to direct-acting mutagens and DNA-modifiers suggest that such a situation may indeed exist in the urban atmosphere. The results are especially timely as the currently planned conversion to diesel fuels will result in increased atmospheric levels of polycyclic hydrocarbons (19). The possibility that this phenomenon is not restricted to precarcinogen but extends to the photoconversion of "non-carcinogen" to genotoxic chemicals as well, introduces a new dimension in the estimation of the health risk to our population and deserve consideration and evaluation.

The present results also have relevance to the routine testing of chemicals for mutagenic activity. Obviously, test chemicals must be prepared freshly, shielded from light, manipulation carried out in subdued light, and plates incubated in the dark.

This investigation was supported by the National Cancer Institute (CP 65855).

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