Arch. Pharm. Res. **11**(1), 1-6(1988)

Environmental Mutagens—Detection, and Modulation of Their Activities

Hikoya Hayatsu
Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700, Japan
(Received October 24, 1987)

Abstract □The use of blue cotton for detecting polycyclic aromatic mutagens in environmental samples (foods, human excretions, river water, *etc*) is reviewed. Since the invention of blue cotton has its origin in studies of mutagen modulators, these studies are also briefly reviewed.

Mutagenic components in the environment, either natural or man-made, have been suspected as the major causes for human cancers. Studies on these mutagens often encounter difficulty because they are usually present in only tiny amounts in a given sample.

Many of these mutagens are polycyclic aromatic compounds. For example, polycyclic aromatic hydrocarbons and heteropolycyclic aromatic amines are among them. We have recently discovered an adsorbent selective for such polycyclic compounds. A blue dye, copper phthalocyanine, covalently linked to cotton (blue cotton) was found to adsorb these compounds very efficiently from their aqueous solutions[1]. The polycyclic compounds thus adsorbed can be recovered easily by eluting the cotton with ammoniacal methanol. The blue cotton procedure has been used for isolating heteropolycyclic amines from cooked foods, for detecting urinary and fecal mutagenicities, and for measuring mutagenicities in the river water. These uses of blue cotton are described in more detail in the following sections.

The finding of this unique property in copper phthalocyanine molecule has originated from our earlier studies on the mechanism of modulation of heteropolycyclic amine mutagenicities by hemin and its derivatives. We found that hemin was able to suppress the mutagenic activity of the heteropolycyclic amines by forming complexes with them[2,3]. The similarity in the chemical structures between hemin and copper phthalocyanine immediately suggested the possibility of the latter compound forming similar complexes. This expectation turned out to be really the case, and the blue cotton method has been developed.

Regarding with the hemin acting as a suppressor for the mutagenicity of heterocyclic amines, we have recently observed that hemoglobin can efficiently degrade the metabolically-activated forms of mutagenic heterocyclic amines *in vitro*. Investigation has shown that this process is an oxidative one. The modulating activity of these heme derivatives on the environmental mutagens is further discussed below.

1. Use of Blue Cotton for Detection of Mutagens

1-1. Nature of blue cotton

Blue cotton bears copper phthalocyanine trisulfonate as a ligand[1] and the chemical formula of the ligand-cotton structure is shown in Figure 1. The dye content in the cotton is usually set at 10 mol/g. This material is now commercially available from Funakoshi Chemicals (Tokyo). The ligand, being a large planar molecule, can form a complex with compounds having planar structures. The affinity of several three-ring compounds, including 9-aminoacridine and 2-acetylaminofluorene, to blue cotton was studied in a quan-

Abbreviations used: MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; N-OH-Trp-P-2, 3-hydroxyamino-1-methyl-5*H*-pyrido[4,3-*b*]indole; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Glu-P-1, 2-amino-6-methyldipyrido[1,2-*a*: 3′,2′-*d*]imidazole; Glu-P-2, 2-aminodipyrido[1,2-*a*: 3′,2′-*d*]imidazole; A C, 2-amino-9*H*-pyrido[2,3-*b*]indole; MeA C; 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; Acetyl Trp-P-1, 3-acetylamino-1,4-dimethyl-5*H*-Pyrido[4,3-*b*]indole.

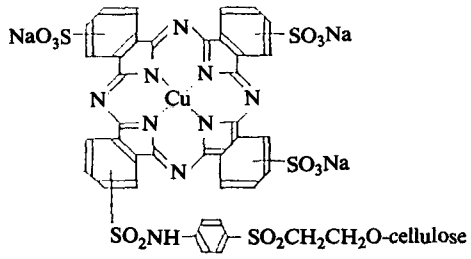

Fig. 1. Structure of blue cotton (1).

titative manner[4]. The results indicate that all of the compounds tested can form 1:1 complexes with this ligand, with the affinity constants in neutral aqueous solutions ranging 10^{-5} -10^{-6}M. Little adsorption to blue cotton was noted for compounds having two or smaller numbers of rings[1]. The elution of adsorbed compounds can best be done with the use of methanol-concentrated ammonia (50:1, v/v), although methanol alone can also be used with less efficiency[1]. Recoveries for mutagens in saline solutions by use of blue cotton are shown in Table I.

1-2. Isolation of heteropolycyclic amines from foods

Since blue cotton is an adsorbent specific for polycyclic planar compounds, it can be used for separating such compounds from complex mixtures. Cooked proteinous foods contain mutagenic heteropolycyclic amines[5]. Blue cotton was used to isolate MeIQx from Difco beef extract[6,7] and from katsuobushi[8], the smoke-dried bonito. DiMeIQx was also isolated from heated fish by this method[9]. Blue cotton was used for isolating MeIQx produced in a model cooking system[10].

1-3. Mutagenicity in urine and feces

Cigarette smokers' urines are mutagenic[11]. By treatment of urine with blue cotton, detection of mutagenicity can be easily carried out[12]. It was found that the appearance of mutagenicity after smoking is a rapid process: within several hrs, the excretion is mostly completed[12].

Since heated meat contains mutagens[5], it may be expected that human urines and feces become mutagenic after ingesting cooked meat. Figure 2 shows the increase of urinary mutagenicity after ingesting fried ground beef, as detected by the blue cotton extraction followed by the Ames mutagenicity test using *S. typhimurium* TA98 with metabolic activation[13].

Similarly, the fecal mutagenicity rises after eating cooked meat (Figure 3)[14]. The disappearance of mutagenicity from the feces takes two to three days, in contrast to that from the urine which takes only half a day.

Table I. Adsorption and recoveries of compounds by the blue cotton method

Compound	Concentration in saline ($\times 10^{-6}$M)	Overall recovery (%)	Assay method
Trp-P-1	20	98	a
[^{3}H]-Trp-P-2	0.2 (10^4 dpm/m*l*)	93	r
Glu-P-1	40	79	a
Glu-P-2	80	54	a
A C	100	79	a
MeA C	100	88	a
IQ	20	71	a
MeIQx	4	89	a
Acetyl Trp-P-1	20	88	a
1-Nitropyrene	2	77	m
2-Aminoanthracene	2	75	m
Benzo[a]pyrene	0.2	60	f
4-Nitroquinoline-1-oxide	50	-	a
[^{14}C]-Nitrosodimethylamine	0.1 (10^4 dpm/m*l*)	-	r
[^{3}H]-Histidine	10 (10^4 dpm/m*l*)	0.6	r

Assay method: (a) absorption spectrum, (r) radioactivity, (m) mutagenicity, (f) fluorescence spectrum.

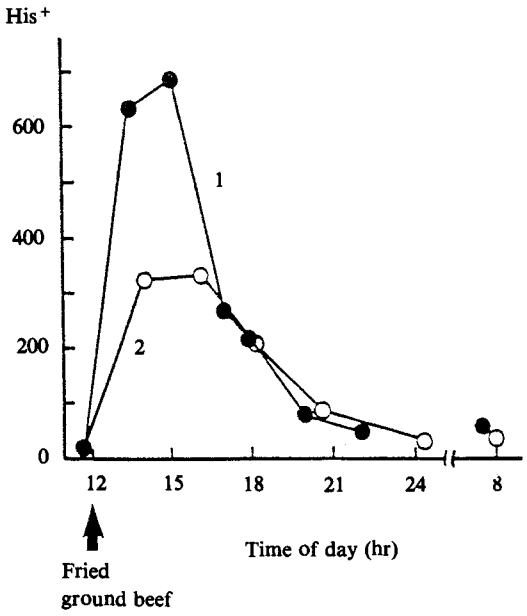

Fig. 2. Urinary mutagenicity after ingestion of fried ground beef (13).
The mutagenicity on *S. typhimurium* TA98 (+ S9) was examined for urines of two healthy, non-smoking male adults. The amount of beef eaten was 150g (for the raw meat).

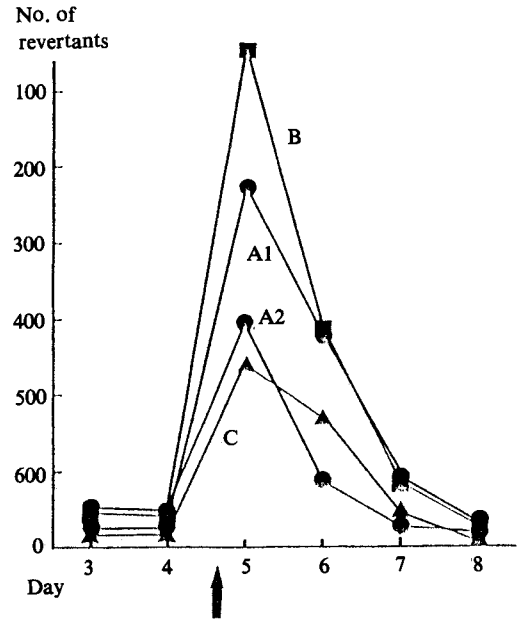

Fig. 3. Fecal mutagenicity arising from ingestion of fried beef (14).
The mutagenicity on TA98 (+ S9) of morning feces was examined. 150g raw-meat equivalent cooked beef was eaten in the evening of the fourth day of the 8-day experiment. Meals containing no cooked meat were taken during the rest of the time of the experiment.

The mutagenic components in both the urine and the feces have been shown to be different from those present in the cooked meat, suggesting that they are metabolites of the food mutagens[13,14]. An animal study was done to identify urinary metabolites of MeIQx, and three mutagenic metabolites were isolated, again by the blue cotton method, and their structures were established (Figure 4)[15].

An oxidative metabolite of IQ was found on incubation of IQ with human fecal flora[16]. The isolation of this metabolite was performed by the blue cotton procedure.

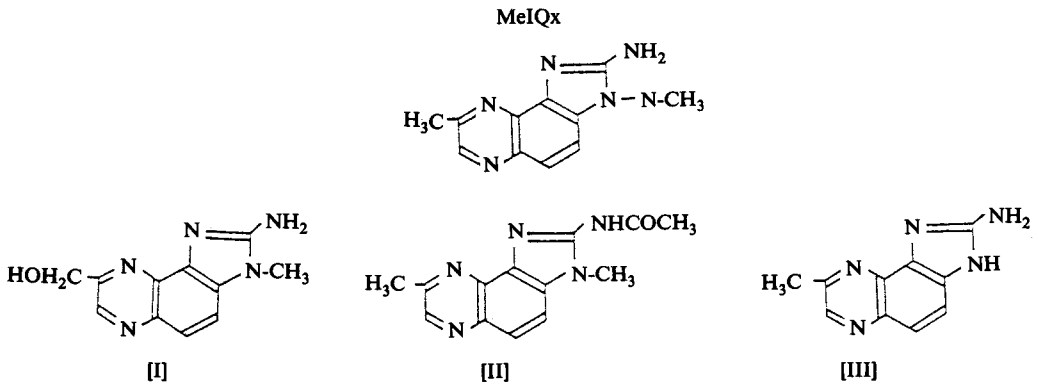

Fig. 4. Structures of MeIQx and its metabolites in rats (15).

1-4 Monitoring mutagenicity of environmental waters

Mutagenicity due to polycyclic compounds can thus be monitored for complex mixtures by use of the blue cotton method. Blue cotton may be allowed to stand in the river to adsorb those polycyclic compounds that by this technique, the mutagenicity of a river in Okayama has been measured, mutagenic (Table II)[1].

1-5 Mutagens found in other sources by use of blue cotton

IQ was detected in cigarette smoke condensate[17]. The high incidence of oesophageal cancer in north-east Iran has been ascribed to ingestion of have been isolated from the pyrolysates, and recently a variety of novel hydroxypheranthrenes have been isolated from the pyrolysates as mutagenic components[18]. Use of blue cotton was very effective as a step to separate these polycyclic aromatics from morphine.

Table II. Detection of mutagenicity in river water

Water of River Asahi	Number of revertants
At an upstream site,	(His$^+$)
outside the urban area	23, 64
At a site near the sea,	
inside the urban area	2054, 2395

2. Modulating Factors for Environmental Mutagens

2-1. Hemin

We have found that hemin (Figure 5), a chemically stable form of heme which is a biological oxygen carrier, can suppress the activity of many mutagens (Figure 6 and Table III)[2,3]. Complex formation takes place between hemin and many of the polycyclic compounds, as detected by spectroscopic measurements (unpublished results). This observation suggests that such complex formation inhibits

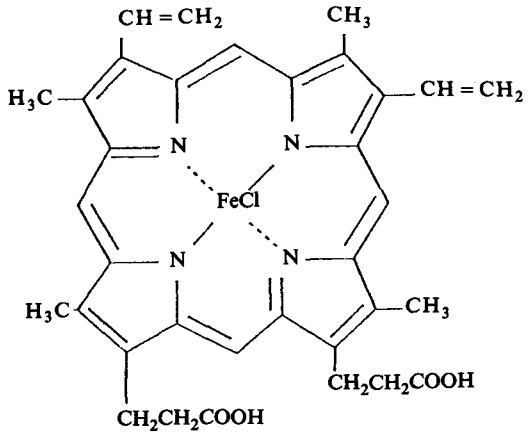

Fig. 5. Structure of hemin.

Table III. Inhibitory effect of hemin for mutagenicity

Mutagen	Amount of mutagen (nmole)	Inhibitory dose of hemin I_{50}	I_{95}[a]	Amount of S9 (*l*)	His$^+$ revertants in the absence of hemin[b]
Trp-P-1	1.8	3	75	10	7800-16900
Trp-P-2	0.1	20	50	10	975-2170
Glu-P-1	1.7	75	200	10	18200-33000
Glu-P-2	9.1	40	100	10	3380-8640
A C	80	30	100	10	3400-9750
MeA C	250	25	100	10	1370-6500
Benzo[a]pyrene	8	10	50	25	738-1022
2-Acetylaminofluorene	100	100-200	500	50	1402-2109
2-Nitrofluorene	100	200	1000	-	3861-5330
Aflatoxin B_1	0.5	10	500	10	636-1293

a) I_{50} is the dose at which 50% decrease in the his$^+$ revertants was observed, and I_{95} is that at which the decrease was more than 95%.

b) Numbers of *S. typhimurium* TA98 revertants given for each mutagen are those obtained in two independent inhibition-experiments.

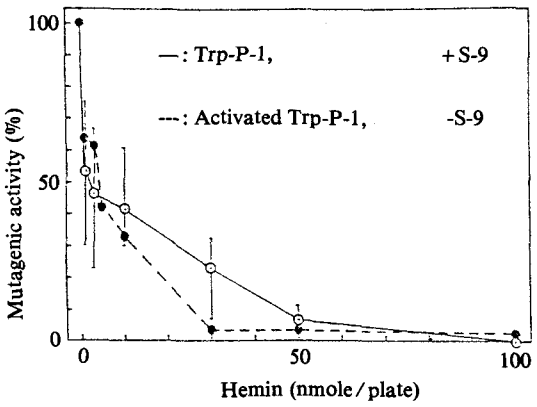

Fig. 6. Inhibition of mutagenic activities of Trp-P-1 and its S9-activated form by hemin (2). The mutagenicity of Trp-P-1 (1.8 nmole) and the activated Trp-P-1 (2 nmole eq.) was assayed on *S. typhimurium* TA98.

the attack of the mutagens on cellular DNA.

Other porphyrin derivatives, including chlorophyllin, are also inhibitory for the polycyclic mutagens[2,3].

2-2. Hemoglobin and myoglobin

These hemoproteins, but not the globin, have been shown to inhibit the mutagenicity of N-OH-Trp-P-2 and many other activated heterocyclic amines[19]. The inhibition is due to the oxidative degradation of the mutagens by catalysis of the hemoproteins[19]. If a reducing agent is present in the system, no degradation of N-OH-Trp-P-2, and therefore no inhibition of the activity, can be detected.

Whether these heme components in the organisms can serve as a defence mechanism against environmental mutagens is an interesting subject to be investigated.

ACKNOWLEDGEMENTS

This work was supported by Grant-in-aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture (626145 25), and by one for Cancer Research from the Ministry of Health and Welfare (61-shi).

LITERATURE CITED

1. Hayatsu, H., Oka, T., Wakata, A., Ohara, Y., Hayatsu, T., Kobayashi, H. and Arimoto, S.: Adsorption of mutagens to cotton bearing covalently bound trisulfo-copper-phthalocyanine. *Mutation Res.* **119**, 233-238 (1983).
2. Arimoto, S., Ohara, Y., Namba, T., Negishi, T. and Hayatsu, H.: Inhibition of the mutagenicity of amino acid pyrolysis products by hemin and other biological pyrrole pigments. *Biochem. Biophys. Res. Comm.* **92**, 662-668 (1980).
3. Arimoto, S., Negishi, T. and Hayatsu, H.: Inhibitory effect of hemin on the mutagenic activities of carcinogens. *Cancer Letters* **11**, 29-33 (1980).
4. Hayatsu, H., Kobayashi, H., Michi-ue, A. and Arimoto, S.: Affinity of aromatic compounds having three fused rings to copper phthalocyanine trisulfonate. *Chem. Pharm. Bull.* **34**, 944-947 (1986).
5. Sugimura, T.: Carcinogenicity of mutagenic heterocyclic amines formed during the cooking process. *Mutation Res.* **150**, 33-41 (1985).
6. Hayatsu, H., Matsui, Y., Ohara, Y., Oka, T. and Hayatsu, T.: Characterization of mutagenic fractions in beef extract and in cooked ground beef. Use of blue-cotton for efficient extraction. *Gann* **74**, 472-482 (1983).
7. Takahashi, M., Wakabayashi, K., Nagao, M., Yamamoto, M., Masui, T., Goto, T., Kinae, N., Tomita, I. and Sugimura, T.: Quantification of 2-amino-3-methylimidazo[4,5-*f*] quinoline (IQ) and 2-amino-3,8-dimethyl imidazo[4, 5-*f*]quinoxaline (MeIQx) in beef extracts by liquid chromatography with electrochemical detection (LCEC). *Carcinogenesis* **6**, 1195-1199 (1985).
8. Kikugawa, K., Kato, T. and Hayatsu, H.: The presence of 2-amino-3,8-dimethylimidazo [4, 5-*f*] quinoxaline in smoked dry bonito (katsuobushi). *Jpn. J. Cancer Res. (Gann)* **77**, 99-102 (1986).
9. Kikugawa, K. and Kato, K.: Formation of mutagens, 2-amino-3,8-dimethylimidazo [4,5-*f*] quinoxaline (MeIQx) and 2-amino-3,4,8-trimethylimidazo [4,5-*f*] quinoxaline (4,8-DiMeIQx), in heated fish meats. *Mutation* **179**, 5-14 (1987).
10. Jagerstad, M., Olsson, K., Grivas, S., Negishi, C., Wakabayashi, K., Tsuda, M., Sato, S. and Sugimura, T.: Formation of 2-amino-3,8-dimethylimidazo [4,5-*f*] quinoxaline in a model system by heating creatinine, glycine and glucose. *Mutation Res.* **126**, 239-244 (1984).
11. Yamasaki, E. and Ames, B.N.: Concentration of mutagens from urine by adsorption with nonpolar resin XAD-2. Cigarette smokers have

mutagenic urine. *Proc. Natl. Acad. Sci. USA* **74,** 3555-3559 (1977).
12. Kobayashi, H. and Hayatsu, H.: A time-course study on the mutagenicity of smoker's urine. *Gann* **75,** 489-493 (1984).
13. Hayatsu, H., Hayatsu, T. and Ohara, Y.: Mutagenicity of human urine caused by ingestion of fried ground beef. *Jpn. J. Cancer Res. (Gann)* **76,** 445-448 (1985).
14. Hayatsu, H., Hayatsu, T., Wataya, Y. and Mower, H.F.: Fecal mutagenicity arising from ingestion of fried ground beef in the human. *Mutation Res.* **143,** 207-211 (1985).
15. Hayatsu, H., Kasai, H., Yokoyama, S., Miyasawa, T., Yamaizumi, Z., Sato, S., Nishimura, S., Arimoto, S., Hayatsu, T. and Ohara, Y.: Mutagenic metabolites in urine and feces of rats fed with 2-amino-3,8-dimethylimidazo [4,5-*f*] quinoxaline, a carcinogenic mutagen present in cooked meat. *Cancer Res.* **47,** 791-794 (1987).
16. Bashir, M., Kingston, D.G.I., Carman, R.J., van Tassel, R.L. and Wilkins, T.D.: Anaerobic metabolism of 2-amino-3-methyl-3*H*-imidazo [4,5-*f*] quinoline (IQ) by human fecal flora. *Mutation Res.* **190,** 187-190 (1987).
17. Yamashita, M., Wakabayashi, K., Nagao, M., Sato, S., Yamaizumi, Z., Takahashi, M., Kinae, N., Tomita, I. and Sugimura, T.: Detection of 2-amino-3-methylimidazo [4,5-*f*] quinoline in cigarette smoke condensate. *Jpn. J. Cancer Res. (Gann)* **77,** 419-422 (1986).
18. Friesen, M., O'Neill, I.K., Malaveille, C., Garren, L. and Hautefeuille, A.: Substituted cancer in Iran: structures and *in-vitro* metabolic activation of novel class of mutagens. *Carcinogenesis* **8,** 1423-1432 (1987).
19. Arimoto, S., Ohara, Y., Hiramoto, K. and Hayatsu, H.: Inhibitory effect of myoglobin and hemoglobin on the direct acting mutagenicity of protein pyrolysate heterocyclic amine derivatives. *Mutation Res.* in press.