DNA STRAND SCISSION OF METHANOL-EXTRACTED CHROMOPHORES OF MACROMOMYCIN AND AUROMOMYCIN

Sir:

Macromomycin (MCR)[1], a polypeptide antitumor antibiotic obtained from culture filtrates of *Streptomyces macromomyceticus* exhibited antitumor activity against Ehrlich ascites carcinoma, L1210 and Lewis lung carcinoma.[2] During the course of the studies on MCR, a yellow macropeptide was crystallized and named auromomycin (AUR). The differences between MCR and AUR were described in a previous paper[3]. AUR contained a chromophore having a broad ultraviolet absorption maximum around 350~360 nm and converted to MCR by Amberlite XAD-7 (Rohm and Haas Co., U.S.A.) column chromatography which removed the chromophore.

SUZUKI *et al.* reported that the chromophore of AUR obtained by methanol extraction caused DNA strand scission and suggested that the activity of AUR might be due to its chromophore[4,5]. Recently, we found that a small amount of a chromophore was extracted from MCR with methanol. In this paper, the DNA strand scission activities of the native materials, the protein moieties and the methanol-extracted chromophores of MCR and AUR are described.

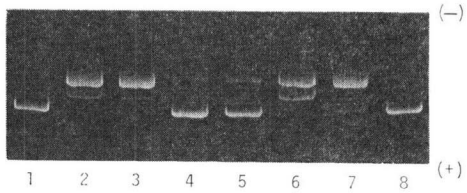
The chromophore was extracted as follows. MCR or AUR powder purified by the method described previously[3] was suspended in methanol at 1 mg/ml and stirred for an hour in a dark and cold place. By centrifugation at 10,000 rpm for 10 minutes, the supernatant (chromophore fraction) and the precipitate (protein fraction) were separated and both fractions were diluted with deionized water. Since the chromophores of MCR and AUR were extremely unstable, they were used immediately after preparation.

The assay of the DNA strand scission activity was carried out as follows. The reaction mixture consisted of 50 mM tris-HCl (pH 7.6), 25 μg/ml of PM 2 DNA (Boehringer Mannheim GmbH, Germany) and an indicated concentration of MCR, AUR, their chromophores or their protein fractions. Forty μl of the reaction mixture was incubated at 37°C for 30 minutes. Immediately after the addition of 5 μl of 0.0025% of bromophenol blue in 50% sucrose solution, 25 μl of the mixture was applied to an agarose slab gel. Electrophoresis was carried out according to the method described by SUZUKI *et al.*[6]. After electrophoresis, the gel was stained with 0.5 μg/ml of ethidium bromide and stained DNA bands were photographed.

As shown in Fig. 1, AUR and its chromophore exhibited DNA strand scission without any supplement, whereas MCR required a reducing agent such as sodium borohydride for DNA strand scission. These results were in accord with the findings described by SUZUKI *et al.*[6] However, as SUZUKI *et al.* described[6], the activity of MCR or AUR in causing DNA strand scission was lost when MCR or AUR was preincubated with sodium borohydride. A new finding shown in Fig. 1 was that the chromophore extracted from MCR exhibited DNA strand scission without the presence of reducing agent as well as native AUR or AUR chromophore, although native MCR did not. The protein moieties of both MCR and AUR after removal of their chromophores by methanol extraction were inactive even at 100 μg/ml. The results indicate that the activity of MCR in causing DNA strand scission *in vitro* is due to its chromophore.

Activities of MCR and AUR chromophores on DNA strand scission were compared in Fig. 2. MCR chromophore equivalent to 400~500 μg/ml of native MCR was as active as AUR chromophore equivalent to 50 μg/ml of native AUR.

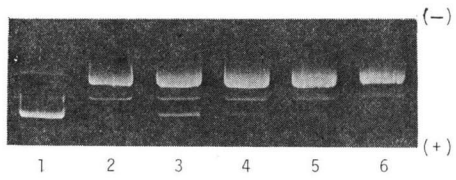
Fig. 1. DNA strand scission by MCR, AUR and their chromophores.



1; DNA (PM 2) alone, 2; AUR 50 μg/ml, 3; AUR chromophore 50 μg/ml, 4; AUR protein 100 μg/ml, 5; MCR 50 μg/ml, 6; MCR 50 μg/ml+$NaBH_4$ 1 mM, 7; MCR chromophore 100 μg/ml, 8; MCR protein 100 μg/ml.

Concentration of MCR or AUR chromophore in the reaction mixture is expressed by μg/ml of MCR or AUR before extraction, that is, 50 μg/ml of AUR chromophore was derived from 50 μg/ml of AUR.

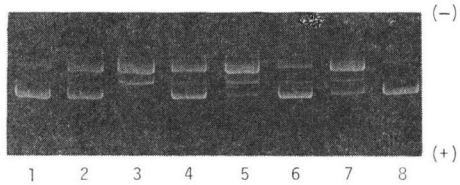
Fig. 2. Comparison of MCR and AUR chromophores on DNA strand scission.



1; DNA (PM 2) alone, 2; AUR chromophore 50 μg/ml, 3; MCR chromophore 200 μg/ml, 4; MCR chromophore 300 μg/ml, 5; MCR chromophore 400 μg/ml, 6; MCR chromophore 500 μg/ml.

Concentration of MCR or AUR chromophore in the reaction mixture is expressed by μg/ml of MCR or AUR before extraction.

Fig. 3. Stimulation effect of sodium borohydride on DNA strand scission by MCR chromophore, AUR chromophore and native AUR.



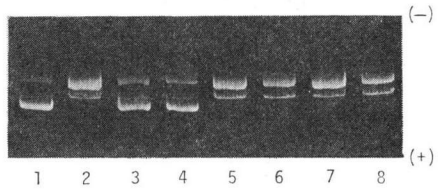
1; DNA (PM 2) alone, 2; AUR 2 μg/ml, 3; AUR 2 μg/ml+$NaBH_4$ 1 mM, 4; AUR chromophore 5 μg/ml, 5; AUR chromophore 5 μg/ml+$NaBH_4$ 1 mM, 6; MCR chromophore 10 μg/ml, 7; MCR chromophore 10 μg/ml+$NaBH_4$ 1 mM, 8; DNA+$NaBH_4$ 1 mM.

Concentration of MCR or AUR chromophore in the reaction mixture is expressed by μg/ml of MCR or AUR before extraction.

The stimulation of the activity of native AUR by reducing agents such as cysteine and dithiothreitol has been reported.[7] As shown in Fig. 3, a small amount of AUR causing only a little DNA strand scission was stimulated by the addition of sodium borohydride, showing the DNA-fragmentation activities of both MCR and AUR chromophores were stimulated by the addition of sodium borohydride as well as native MCR and AUR.

The influence of MCR protein and other proteins on DNA strand scission by MCR chromophore is shown in Fig. 4. DNA-fragmentation activity of MCR chromophore was remarkably inhibited by the addition of its protein moiety. But, bovine serum albumin, human fibrinogen and protein component of neocarzinostatin showed no inhibitory effect. The inhibitory effect of MCR protein moiety was thus shown to be specific to DNA-fragmentation by MCR chromophore.

Fig. 4. Inhibitory effects of various proteins on DNA strand scission by MCR chromophore.



1; DNA (PM 2) alone, 2; MCR chromophore 100 μg/ml, 3; MCR chromophore 100 μg/ml+MCR protein moiety 200 μg/ml, 4; MCR chromophore 100 μg/ml+MCR protein moiety 400 μg/ml, 5; MCR chromophore 100 μg/ml+bovine serum albumin 200 μg/ml, 6; MCR chromophore 100 μg/ml+human fibrinogen 200 μg/ml, 7; MCR chromophore 100 μg/ml+neocarzinostatin protein component 200 μg/ml, 8; MCR chromophore 100 μg/ml+MCR protein moiety 200 μg/ml+$NaBH_4$ 1 mM.

Concentration of MCR or AUR chromophore in the reaction mixture is expressed by μg/ml of MCR or AUR before extraction.

AUR protein also inhibited DNA-fragmentation by MCR and AUR chromophores (data are not shown).

The results described above suggest that MCR and AUR chromophores are similar but different, or MCR is the mixture of AUR and AUR (MCR) protein. The separation of MCR into AUR and AUR (MCR) protein has not yet been successful although various chromatographies were tried. Further efforts are under way to characterize the chromophores of MCR and AUR.

Noriyuki Naoi
Yoshiki Kumada
Toshiaki Yamashita

Biochemical Research Laboratories,
Kanegafuchi Chemical Industry Co., Ltd.,
Takasago, Hyogo, Japan

Tomio Takeuchi
Hamao Umezawa

Institute of Microbial Chemistry,
Kamiosaki, Shinagawa-ku, Tokyo, Japan

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