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BIOLOGICAL ACTIVITIES OF NON-PROTEIN CHROMOPHORES OF ANTITUMOR PROTEIN ANTIBIOTICS: AUROMOMYCIN AND NEOCARZINOSTATIN

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SUMMARY

A non-protein chromophore(s), λ_{max} 350 nm, was extracted by methanol from auromomycin. The methanol extracts of auromomycin and neocarzinostatin blocked growth of lymphoblastoma L5178Y cells. The degree of inhibition was similar to that by the original drugs. PM2 DNA was cleaved by auromomycin chromophore in the absence of reducing agents, whereas neocarzinostatin chromophore required 10 mM 2-mercaptoethanol for DNA strand scission. The DNA-cutting activities of the chromophores were slightly weaker than those of the native antibiotics. The protein fractions, left after methanol extraction, showed little activity on cell growth and isolated DNA. The results suggest that the chromophore moiety plays a predominant role in the inhibition of cell growth and cleavage of DNA.

The antitumor protein antibiotic auromomycin (AUR) has been isolated from Streptomyces macromomyceticus, a macromomycin (MCR)-producing organism. AUR is converted to MCR on a column of Amberlite XAD-7 by releasing a chromophore, which shows an absorption maximum at 350-360 nm. Both antibiotics are similar in molecular weight, isoelectric point and amino acid composition, but different in absorption spectra. AUR exhibits much stronger antibacterial and antitumor activities, as well as toxicity, than MCR (1,2). We have previously observed that AUR and MCR induce DNA strand scission in living cells (3-5), showing different modes of cleavage of isolated PM2 DNA (6). The results suggest that the chromophore of AUR molecule plays an important role in exhibiting the biological activity, although its chemical characteristics have not yet been elucidated because of its instability.

Neocarzinostatin (NCS), another antitumor protein antibiotic (7), also induces DNA break in vivo and in vitro (8). Recently, Napier et al. have extracted a non-protein chromophore from NCS with

Abbreviations: AUR, auromomycin; MCR, macromomycin; NCS, neocarzinostatin; MIa, the methanol-insoluble fraction of AUR; MIn, the methanol-insoluble fraction of NCS; MSa, the methanol-soluble fraction of AUR; and MSn, the methanol-soluble fraction of NCS.

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methanol, although they have not specified any biological activity of the chromophore (9).

We have found that the methanol-extractable non-protein fractions of AUR and NCS markedly inhibit growth of mouse lymphoblastoma L5178Y cells in culture and induce strand scission of isolated PM2 DNA, whereas the methanol-insoluble protein fractions hardly show any biological activity. The results are presented in this communication.

MATERIALS AND METHODS

AUR was kindly supplied by Dr. K. Watanabe, Kanegafuchi Chemical Industry Co., Takasago, Hyogo-ken, Japan. NCS solution (4,070 units/ml) was a product of Kayaku Antibiotics Research Co., Tokyo, Japan.

Mouse lymphoblastoma L5178Y cells were grown in Fisher's medium supplemented with 10 % horse serum, as described previously (4). DNA strand scission was detected in agarose gel electrophoresis, using PM2 phage DNA (Boehringer Mannheim, Germany), by the method described previously (6). Absorption spectra were recorded with a Hitachi double-beam spectrophotometer (model 200-10). Protein content was determined by the Lowry method, using bovine serum albumin as a standard, after methanol was evaporated in vacuo.

Methanol extraction of AUR and NCS: AUR was suspended in methanol usually at a concentration of 1 mg/ml and vortexed. The suspension was allowed to stand for an hour at room temperature, and then for two hours at -20°C. After centrifugation at 3,000 rpm for 30 minutes, the upper half of the supernatant was gently collected (the methanol-soluble fraction), and the sediment was repeatedly washed with methanol and dissolved in sterilized redistilled water (the methanol-insoluble fraction).

First we tried to lyophilize NCS for methanol fractionation, and found that NCS lost the biological activity by freeze-drying. Therefore, we were obliged to start with water solution of NCS, which was dialyzed against a large volume of redistilled water and mixed with methanol (usually NCS 500 units/ml in 88 % methanol). The NCS suspension in methanol was treated as in the case of AUR, except that it was kept overnight at -20°C and sedimented at 30,000 rpm for 60 minutes in a Beckman L2-65B ultracentrifuge, because NCS formed fine particles in methanol and were not readily sedimented.

During the procedure care was taken to exclude light. Since the chromophores of AUR and NCS were labile, they were used immediately after preparation. The concentrations of the methanol-soluble and -insoluble fractions are expressed by μg of AUR or NCS per ml of methanol before fractionation.

RESULTS

Absorption spectra of the methanol-soluble and -insoluble fractions of AUR and NCS:

As illustrated in Fig. 1A, native AUR showed a maximum at 273 nm and a lower broad peak around 350 nm. The methanol-insoluble material (MIa) gave a maximum at 280 nm with a shoulder around

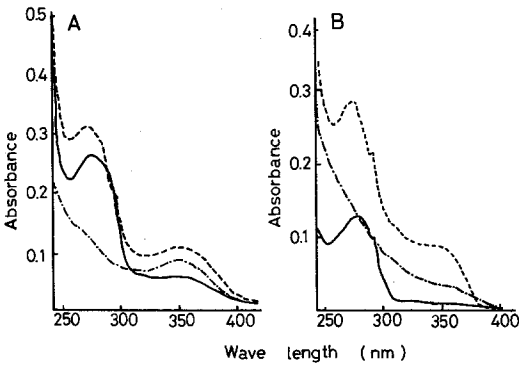

Fig. 1. Absorption spectra of auromomycin (AUR) and neocarzinostatin (NCS), and their methanol-soluble (MSa and MSn) and -insoluble (MIa and MIn) fractions.

A. AUR; ------native AUR (390 μg/ml), ——— MIa (330 μg/ml), and —·—·—MSa (330 μg/ml).

B. NCS; ------native NCS (225 μg/ml), ——— MIn (200 μg/ml), and —·—·—MSn (200 μg/ml).

350 nm; and the methanol extract (MSa) showed a maximum at 350 nm without any distinct peak at 280 nm. By the Lowry method, all the protein was recovered in MIa, but protein could not be detected in MSa. The results indicated that methanol extracted a non-protein chromophore(s) but not the protein of AUR.

Native NCS exhibited an absorption maximum at 276 nm and a broad shoulder between 320-380 nm. In the methanol precipitate (MIn), a peak at 280 nm was observed, but a broad shoulder (320-380 nm) disappeared. The methanol extract (MSn) gave a shoulder around 350 nm (Fig. 1B). The recovery of protein in MIn was ca. 93 % of the original dialyzed sample of NCS, and less than 4 % of protein was detected in MSn.

The effects on growth of lymphoblastoma L5178Y cells:

The methanol extracts of both antibiotics were diluted with sterilized redistilled water and added to the culture of L5178Y cells. The final concentration of methanol never exceeded 1 %, which did not significantly affect the cell growth (** in Table 1).

As summarized in Fig. 2A, the cell growth was markedly blocked by native AUR at drug concentration of 0.06 and 0.015 μg/ml, and slightly at 0.004 μg/ml. MSa exhibited a similar degree of growth inhibition to native AUR (Fig. 2B). The cell growth was hardly affected by MIa (Fig. 2C).

The cell growth of L5178Y was markedly prevented by the methanol extract of NCS (MSn) as well as native NCS, but not by the methanol

Table 1. Effects of neocarzinostatin (NCS), and its methanol-soluble (MSn) and -insoluble (MIn) fractions on growth of lymphoblastoma L5178Y cells.

Concentration of materials		Materials		
		NCS	MSn	MIn
5	μg/ml	21*(4.1)	22 (4.3)	517 (101)
1		32 (6.3)	32 (6.3)	505 (99)
0.2		35 (6.9)	64 (13.)	512 (100)
0.04		194 (38.)	366 (72.)	498 (98)
0		510 (100.)	507 (99.)**	

* The value represents cell number x 10^{-3}/ml on day 3, and that in the parentheses percent of control.
The cell number on day 0 was 16 x 10^{3}/ml.
** + 1 % methanol.

precipitate of NCS (MIn). The degree of inhibition by MSn was slightly less than that by the native drug. The results are presented in Table 1.

DNA-cleaving potentials of the methanol-soluble and -insoluble fractions of AUR and NCS:

The effect of each fraction on PM2 DNA was compared with the native antibiotic by agarose gel electrophoresis (Fig. 3). AUR at concentrations of 100 and 20 μg/ml distinctly caused strand break of covalently closed circular (ccc) PM2 DNA, forming open circular and linear DNA, without any supplement in the reaction mixture (Fig. 3A lanes 3,4). The methanol extract of AUR (MSa), at concentrations equivalent to 100 and 20 μg/ml of native AUR, cut PM2 DNA without any supplement (Fig. 3A lanes 5,6). The degree of DNA cleavage by MSa was slightly less than that by native AUR. The methanol-insoluble fraction of AUR (MIa)did not significantly

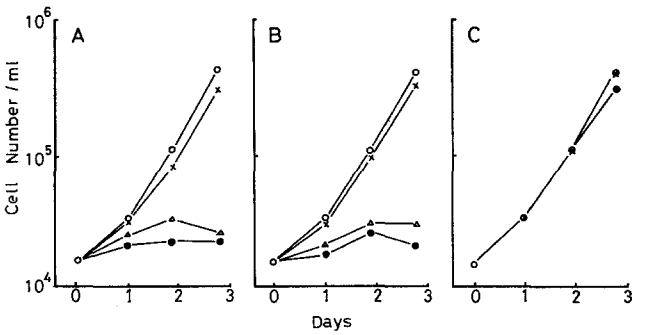

Fig. 2. Effects of AUR, and its methanol-soluble (MSa) and -insoluble (MIa) fractions on growth of lymphoblastoma L5178Y cells.

A. AUR, B. MSa, C. MIa; ○ 0, × 0.004, △ 0.015, ● 0.06 μg/ml.

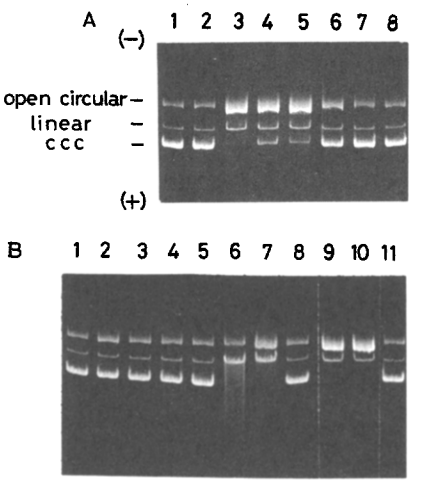

Fig. 3. Agarose gel electrophoresis of PM2 DNA, treated with AUR and NCS, and their methanol-soluble (MSa and MSn) and -insoluble (MIa and MIn) fractions.

A. AUR; 1. no addition, 2. 20 % methanol, 3. AUR 100 μg/ml 4. AUR 20 μg/ml, 5. MSa 100 μg/ml, 6. MSa 20 μg/ml, 7. MIa 100 μg/ml, 8. MIa 20 μg/ml.

B. NCS; 1. no addition, 2. NCS 100 μg/ml, 3. MSn 100 μg/ml, 4. MIn 100 μg/ml, 5. 2-mercaptoethanol (2-ME) 10 mM, 6. NCS 100 μg/ml + 2-ME 10 mM, 7. MSn 100 μg/ml + 2-ME 10 mM, 8. MIn 100 μg/ml + 2-ME 10 mM, 9. NCS 20 μg/ml + 2-ME 10 mM, 10. MSn 20 μg/ml+ 2-ME 10 mM, and 11. MIn 20 μg/ml + 2-ME 10 mM.

affect PM2 DNA even at a concentration of 100 μg/ml (Fig. 3A lanes 7,8).

In contrast to AUR, no significant strand scission of PM2 DNA was induced by native NCS and its fractions in the absence of reducing agents (Fig. 3B lanes 2-4). A dialyzed sample of NCS markedly caused DNA cleavage in the presence of 10 mM 2-mercapto-ethanol (2-ME) at drug concentrations of 100 and 20 μg/ml (Fig. 3B lanes 6,9). DNA strand break was induced by the methanol extract of NCS (MSn) but not by the methanol-insoluble fraction (MIn) in the presence of 2-ME (Fig. 3B lanes 7,8,10,11).

DISCUSSION

The current experiments show that a non-protein chromophore(s) is extracted from AUR by methanol, as reported with NCS (9), and the chromophore of AUR inhibits growth of L5178Y cells and causes a single strand scission of isolated DNA. The degree of these biological activities is similar to, but slightly less than, that of native AUR. The methanol-insoluble protein fraction of AUR

was inactive in preventing cell growth and cleaving PM2 DNA. The results suggest that the chromophore moiety may play a predominant role in the action of AUR. It is in accord with the previous report that AUR is converted to MCR by removing a chromophore by adsorption chromatography, and AUR shows a stronger biological activity than MCR (1).

It has been also demonstrated in the present study that the chromophore of NCS blocks cell growth and induces DNA cleavage, although the activity is slightly weaker than that of native NCS. The results suggest that the biological activity of NCS is attributed to the chromophore moiety.

The chromophores of AUR and NCS give a peak or shoulder at similar wavelength in the absorption spectra, indicating a certain resemblance between both molecules. 2-Mercaptoethanol is required for the NCS chromophore to cleave chromophore, but not for the AUR chromophore. A similar effect of 2-mercaptoethanol has previously been observed with native AUR and NCS (4,6,8,10). The results suggest that the active groups of the two antibiotics are similar to, but different from each other.

The biological potentials of AUR and NCS chromophores are less than those of the native antibiotics. This may be partly due to the recovery and lability of the chromophores. The protein portions of AUR and NCS seem to enhance or to stabilize the chromophores, because the chromophores are more labile than the native drugs. However, the biological activity of the protein moiety remains to be determined.

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