Beerman, T. A., & Goldberg, I. H. (1974) *Biochem. Biophys. Res. Commun. 59*, 1254.
Burger, R. M., Peisach, J., & Horwitz, S. B. (1978) *J. Biol. Chem. 253*, 4830.
Chien, M., Grollman, A. P., & Horwitz, S. B. (1977) *Biochemistry 16*, 3641.
D'Andrea, A. D., & Haseltine, W. A. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3608.
Hatayama, T., Goldberg, I. H., Takeshita, M., & Grollman, A. P. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3603.
Ishida, R., & Takahashi, T. (1976) *Biochem. Biophys. Res. Commun. 68*, 256.
Kappen, L. S., & Goldberg, I. H. (1978) *Nucleic Acids Res. 5*, 2959.
Kappen, L. S., & Goldberg, I. H. (1979) *Biochemistry 18*, 5647.
Kappen, L. S., & Goldberg, I. H. (1980) *Biochemistry* (third paper of three in this issue).
Kappen, L. S., Napier, M. A., & Goldberg, I. H. (1980) *Proc. Natl. Acad. Sci. U.S.A.* (in press).
Krugh, T. R. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1911.
Krugh, T. R., & Reinhardt, C. G. (1975) *J. Mol. Biol. 97*, 133.
Lown, J. W., Sim, S., Majumdar, K. C., & Chang, R. C. (1976) *Biochem. Biophys. Res. Commun. 76*, 705.
Maeda, H., Aikawa, S., & Yamashita, A. (1975) *Cancer Res. 35*, 554.
Marmur, J. (1961) *J. Mol. Biol. 3*, 208.
Mong, S., DuVernay, V. H., Strong, J. E., & Crooke, S. T. (1980) *Mol. Pharmacol. 17*, 100.
Müller, W. E. G., Yamazaki, Z., Breter, H.-J., & Zahn, R. K. (1972) *Eur. J. Biochem. 31*, 518.
Napier, M. A., Holmquist, B., Strydom, D. J., & Goldberg, I. H. (1979) *Biochem. Biophys. Res. Commun. 89*, 635.
Napier, M. A., Kappen, L. S., & Goldberg, I. H. (1980) *Biochemistry 19*, 1767.
Onishi, T., Iwata, H., & Takagi, Y. (1975) *J. Biochem. (Tokyo) 77*, 745.
Poon, R., Beerman, T. A., & Goldberg, I. H. (1977) *Biochemistry 16*, 486.
Povirk, L. F., Kohnlein, W., & Hutchinson, F. (1978) *Biochim. Biophys. Acta 521*, 126.
Povirk, L. F., Hogan, M., & Dattagupta, N. (1979) *Biochemistry 18*, 96.
Sausville, E. A., Peisach, J., & Horwitz, S. B. (1978) *Biochemistry 17*, 2740.
Someya, A., & Tanaka, N. (1979) *J. Antibiot. 32*, 839.
Takeshita, M., Grollman, A. P., Ohtsubo, E., & Ohtsubo, H. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5983.
Waring, M. (1970) *J. Mol. Biol. 54*, 247.

Requirement for Reducing Agents in Deoxyribonucleic Acid Strand Scission by the Purified Chromophore of Auromomycin†

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ABSTRACT: By methanol extraction and high-pressure liquid chromatography a nonprotein chromophore has been obtained from the antitumor protein antibiotic auromomycin (AUR) which possesses the cytotoxic and the in vivo and in vitro deoxyribonucleic acid (DNA) strand scission activities of the parent material. The rate of DNA strand breakage by the purified chromophore is markedly stimulated by reducing compounds (maximally at ~0.1 mM dithiothreitol), but DNA strand scission activity is lost upon pretreatment of the chromophore with these agents. Apoprotein specifically protects against such inactivation but blocks the activity of both the stimulated and unstimulated reactions, presumably by complexing the chromophore and making it less available to the target DNA. Dithiothreitol-dependent scission of DNA by chromophore is faster and more complete at 0 °C than at 37 °C. The reaction at 0 °C is almost entirely dependent on the presence of a reducing compound. Although 2-mercaptoethanol does not stimulate the reaction of either AUR or its chromophore at 37 °C, it has a significant stimulatory effect at 0 °C.

The antitumor antibiotic auromomycin (AUR)[1] is a polypeptide of molecular weight 12 500 produced by *Streptomyces macromomyceticus*, which also produces macromomycin (MCR) (Yamashita et al., 1979). AUR is identical with MCR in molecular weight, isoelectric point (p*I* of 5.4), and amino acid composition but differs from it by possessing UV–visible absorption above 300 nm (broad peak at ~355 nm). AUR can be converted into a material with the chemical,

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[1] Abbreviations used: AUR, auromomycin; NCS, neocarzinostatin; MCR, macromomycin; DTT, dithiothreitol; BME, 2-mercaptoethanol; EDTA, ethylenediaminetetraacetate; Tris, tris(hydroxymethyl)aminomethane.

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physical, and biological properties of MCR by chromatography on nonionic Amberlite XAD-7. The relationship between AUR and MCR appears to be similar to that between the nonprotein chromophore containing protein antibiotic neocarzinostatin (NCS) and its apoprotein (Napier et al., 1979, 1980; Kappen et al., 1979; Kappen & Goldberg, 1979). We have proposed that in both cases it is the nonprotein chromophore that is responsible for the cytotoxic and the in vivo and in vitro DNA strand scission properties of the parent antibiotic. This prediction has been shown to be valid for NCS, where the apoprotein is a specific carrier for the labile chromophore and acts to protect it and control its release (Kappen et al., 1980; Kappen & Goldberg, 1980) for interaction with DNA (Povirk & Goldberg, 1980). Similarly, the nonprotein chromophore has been extracted from AUR, and it has been found to possess the biological activities of the holoantibiotic (Kappen et al., 1980).

AUR, but not MCR, produces single-strand breaks in linear duplex and supercoiled DNA, and, unlike NCS, this reaction is not stimulated by 2-mercaptoethanol (Kappen et al., 1979; Suzuki et al., 1979); furthermore, MCR specifically blocks AUR-induced DNA breakage (Kappen & Goldberg, 1979; Napier et al., 1980). Suzuki et al. (1979) have found, however, that in the presence of other reducing agents, such as dithiothreitol (DTT) and $NaBH_4$, relatively high levels of MCR generate DNA strand breaks; on the other hand, the AUR reaction is not significantly affected by these compounds.

In an effort to clarify the relationship between the biological effects of AUR and MCR, we have isolated the nonprotein chromophore of AUR and have characterized its cytotoxic properties and its DNA strand scission activities with respect to reducing agents and its apoprotein.

Materials and Methods

MCR and AUR were obtained from Kanegafuchi Chemical Industries Co. through the courtesy of Dr. H. Umezawa. They were further purified to homogeneity, as determined by acrylamide gel electrophoresis and isoelectric focusing (p*I* 5.4), by chromatography on Sephadex G-100 and DEAE-cellulose (T. S. A. Samy, unpublished experiments). Purified MCR was chromatographed on nonionic Amberlite XAD-7 to remove any remaining chromophore. The conditions of the chromatography were identical with those described earlier (Napier et al., 1980) for NCS. The H_2O-eluted fraction was used. Preparation of pMB9 DNA was as described earlier (Kappen & Goldberg, 1979).

Preparation of Chromophore from AUR. AUR (2 mg) was extracted at 0 °C with dry, redistilled methanol (1 mL). The protein residue obtained after centrifugation was reextracted with methanol (1 mL). The combined supernatants which contain the nonprotein chromophore were stored at −70 °C. The protein residue was redissolved in distilled water and was stored frozen. The chromophore preparation contained a maximum of 5% protein contaminant as determined by the method of Lowry et al. (1951).

High-Pressure Liquid Chromatography (HPLC). A Model A2C/GPC-204 chromatograph from Waters Associates equipped with a Model 660 solvent flow programmer, a μBondapak C_{18} column (3.9 mm × 30 cm), a 254-nm absorbance detector, and a Schoeffel Model SF 970 fluorescence detector (360-nm excitation with 418-nm emission cutoff filter) was used.

The methanol-soluble chromophore was injected in a small volume and was eluted at 2 mL/min at ambient temperature into cold amber tubes in dim light with a concave gradient of 35–90% solvent B (methanol containing 0.1% acetic acid and 0.1% triethylamine) in solvent A (20% methanol, 0.1% acetic acid, and 0.1% triethylamine in water). One-minute fractions were collected. The fractions were stored at −20 °C in the elution buffer until used.

In Vitro DNA Scission. Activity was measured with supercoiled pMB9 DNA (form I) as the substrate. Standard incubations (100 μL) contained 50 mM Tris, pH 8.0, 0.25–0.5 μg of DNA (4×10^4 cpm/μg), and the drug at levels given in the legends. In experiments where the time course of the reaction was followed, the volume of the reaction was increased four- to fivefold. Portions, removed at various times, were made 0.5 mM in α-tocopherol by the addition of required volumes of a 5 mM stock solution. α-Tocopherol inhibits any further reaction (Kappen et al., 1979). After incubation (at 37 °C unless otherwise stated), the reaction mixture was analyzed on alkaline sucrose gradients, and the percent of nicked (form II) DNA was determined. Details of the procedure have been described earlier (Kappen & Goldberg, 1979). Control incubations lacking drug (with and without reducing compounds) showed no DNA strand breakage.

In Vivo Activity of Drugs. The effect of drugs on DNA synthesis in HeLa S_3 cells was determined as described earlier (Kappen et al., 1979). The cells (1 mL, 9×10^5mL) were preincubated with the drugs for 40 min at 37 °C before the addition of 0.5 μCi/mL [^{3}H]thymidine (50 Ci/mmol). The radioactivity incorporated into DNA for 40 min was measured. The samples were processed as described by Beerman & Goldberg (1977). DNA strand breakage in HeLa cells exposed to drugs for 30 min at 37 °C was measured by the procedure described earlier (Kappen et al., 1979). The effect of drugs on cell growth was determined under conditions previously reported (Kappen et al., 1980).

Results

Figure 1 shows the UV–visible absorption spectra of AUR and the methanol-extracted chromophore. AUR exhibits absorption maxima at 270 nm and a broad peak at 352 nm. The chromophore has a broad absorption maximum at 352 nm and a broad shoulder with a maximum at 270 nm. The spectrum of the residual protein after methanol extraction showed that ~30% of the 352 nm absorbing material is still bound to it (data not shown). AUR has fluorescence excitation maxima at 280 and 360 nm with respective emission maxima at 340 and 430 nm. The chromophore has a single excitation maximum at 360 nm with maximal emission at 430 nm (data not shown).

Both AUR and the chromophore induce strand scissions in supercoiled pMB9 DNA in the absence of reducing compounds; the chromophore is somewhat more active than AUR (Figure 2a). AUR and the chromophore are equally potent (50% inhibition was obtained at 5×10^{-5} A_{355}/mL, equivalent to 0.2 μg/mL AUR) in inhibiting DNA synthesis in HeLa cells (Figure 2b). The residual protein after methanol extraction is only 1% as active as AUR. HeLa cell growth is inhibited 50% by 6 ng/mL AUR and 2 ng/mL (equivalent of AUR) chromophore.

In agreement with earlier work on AUR, 2-mercaptoethanol (BME) has no significant effect on the activity of the chromophore, but other reducing agents, such as dithiothreitol (DTT), $NaBH_4$, and cysteine, stimulate the DNA strand scission reaction by the drug (Table I). Again, as with AUR, α-tocopherol is strongly inhibitory, while EDTA has no effect.

As shown in Figure 3, the active component in the methanol extract can be purified and isolated by high-pressure liquid chromatography. DNA strand scission activity is restricted to the major 254 nm absorbing component (fraction number

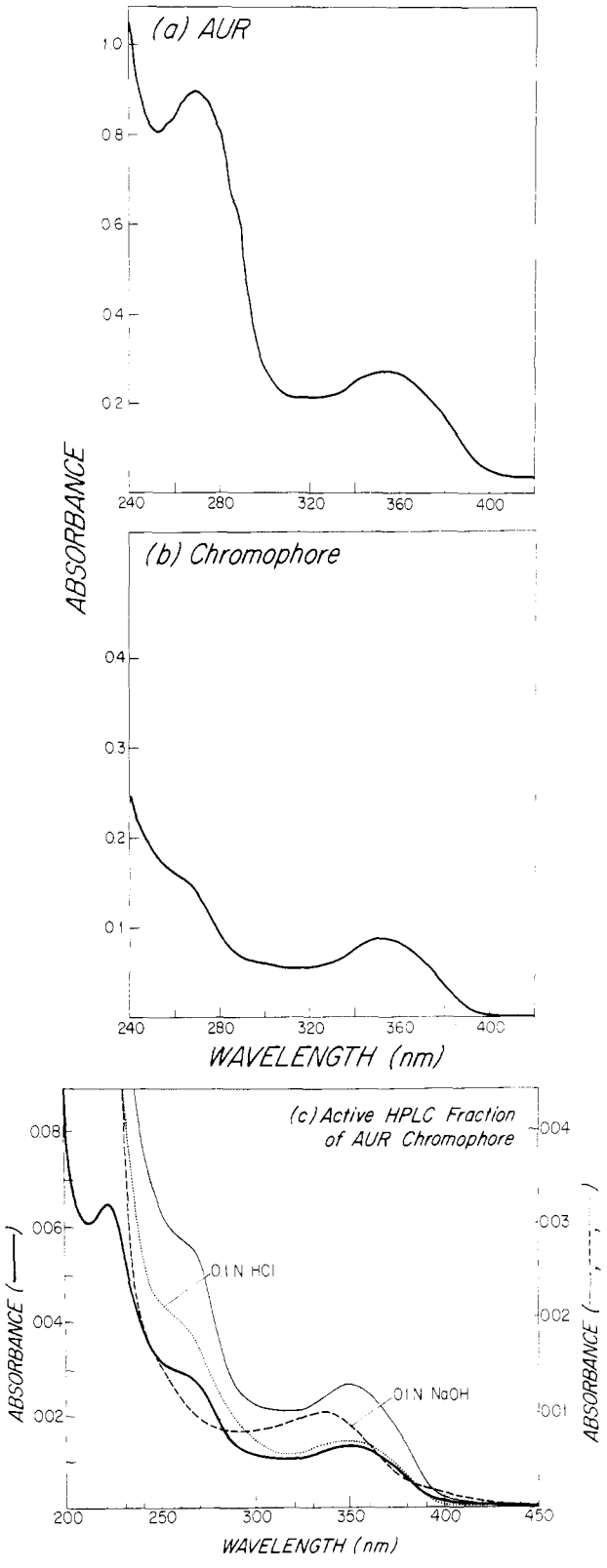

FIGURE 1: Ultraviolet–visible absorption spectra of AUR and its chromophore. (a) AUR (1 mg/mL) is dissolved in H_2O. (b) Methanol-extracted chromophore (equivalent to 1 mg/mL AUR) is dissolved in methanol. (c) HPLC-44 in 66% methanol in H_2O, 0.1% acetic acid, and 0.1% triethylamine. Spectra in acid and alkali are as indicated.

44; subsequently referred to as HPLC-44); this result was confirmed by measuring the absorbance in individual fractions and by repeat high-pressure liquid chromatography of the fractions around and including the peak UV–visible absorbance. DNA synthesis inhibitory action and DNA strand scission activity in HeLa cells were also confined to fraction number 44 (data not shown). The UV–visible absorption spectra of the active high-pressure liquid chromatography fraction (Figure 1c) resemble the spectrum of the crude methanol-extracted chromophore (Figure 1b). The fluorescence intensity (excitation at 360 nm) of the active peak is less than that of other inactive peaks (Figure 3) and no tryptophan fluorescence (excitation at 280 nm) is found (data not shown).

Table I: Effect of Different Agents on DNA Strand Scission Activity by AUR Chromophore[a]

	form II DNA formed (%)
no addition	59
+10 mM BME	56
+1 mM DTT	100
+1 mM $NaBH_4$	85
+1 mM cysteine	100
+2 mM EDTA	59
+0.25 mM α-tocopherol	14

[a] The activity of the chromophore was assayed with pMB9 DNA as the substrate. The reaction (30 min) was started by the addition of 0.001 A_{355} unit/mL (equivalent to 4 μg/mL AUR) of chromophore to the rest of the components. In the absence of AUR chromophore, the various additions do not result in any DNA strand breakage.

Table II: Activity of HPLC-44 after Preincubation with Varying Levels of DTT in the Absence and Presence of MCR[a]

DTT (mM)	form II DNA formed (%)			
	−preincubation		+preincubation	
	−MCR	+MCR	−MCR	+MCR
0	44	4.7	39	7
0.1	80	65	19	62
1.0	85	76	1.4	47

[a] HPLC-44 (7.5 μL) was preincubated for 2 min at 37 °C in 62.5 mM Tris buffer, pH 8, and DTT with and without 6 μg/mL MCR in a total volume of 80 μL. DNA (20 μL) was then added and the reaction mixture was incubated for 40 min at 37 °C. Where there is no preincubation, the reaction was started by the addition of the drug. MCR (6 μg/mL) in the presence or absence of 1 mM DTT did not produce any DNA strand breakage. Based on A_{355}, the HPLC-44 concentration is equivalent to 1.5 μg/mL AUR.

While DTT stimulates the DNA strand scission reaction when included in the incubation, it inactivates the isolated chromophore in a preincubation (Table II and Figure 4). Under similar pretreatment conditions AUR is not inactivated. All the reducing compounds that stimulate the reaction also inactivate the chromophore in a preincubation; 2-mercaptoethanol which failed to stimulate at 0.02–50 mM did, however, inactivate at 10 mM in 50 mM Tris, pH 8.0. Neither activation nor inactivation by DTT is affected by the addition of EDTA (3 mM), catalase (50 μg/mL) or superoxide dismutase (100 μg/mL). While MCR (freed of contaminating chromophore by XAD-7 chromatography) specifically inhibits the reaction in the absence of reducing agent (Figure 5), it protects the chromophore against DTT-induced inactivation (Tables II and III). Neither bovine serum albumin (10–50 μg/mL) nor the apoprotein of NCS (10 μg/mL) has such an effect on the chromophore from AUR.

The most striking effect of DTT is on the rate of DNA strand scission by HPLC-44 (Figure 6). The DTT-stimulated reaction is very fast and occurs more rapidly at 0 °C than at 37 °C. Futhermore, the reaction is more complete (two- to threefold) at the lower temperature (Figure 6 and Table IV). Maximal stimulation at both temperatures is found at ~0.1

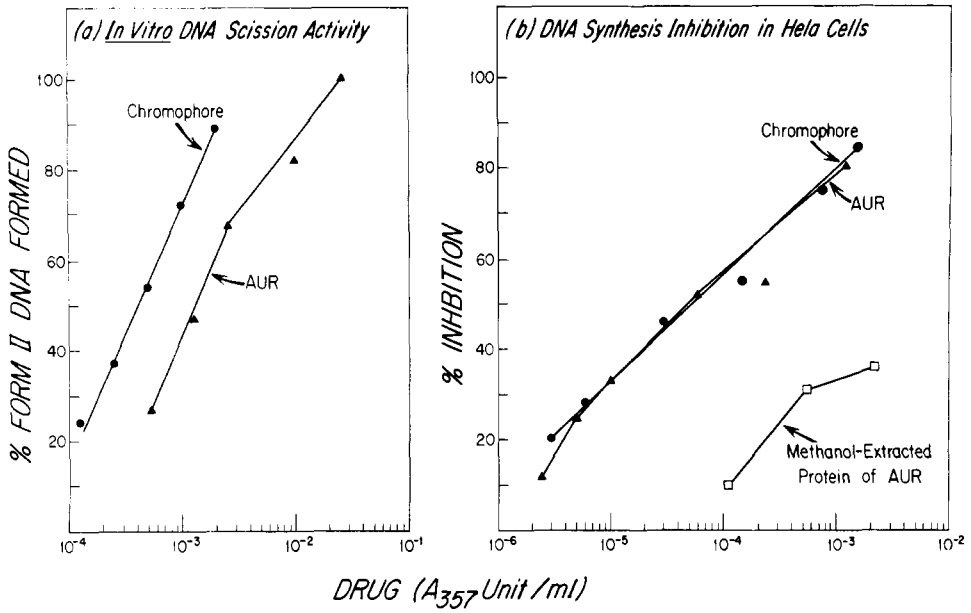

FIGURE 2: (a) In vitro DNA scission activity of AUR and chromophore. In the standard incubation (30 min) the levels of drug were varied. Chromophore addition in methanol gave a final level of 10% methanol in the incubation. Incubations with AUR also contained 10% methanol. (b) Inhibition of DNA synthesis in HeLa cells by AUR and chromophore. The conditions of the assay have been given under Materials and Methods. Chromophore addition in methanol gave a final level of 1% methanol in the assay. The assays with AUR did not contain methanol. In the absence of any drug, the cells incorporated 38 697 cpm into DNA and this was not affected by the addition of 1% methanol.

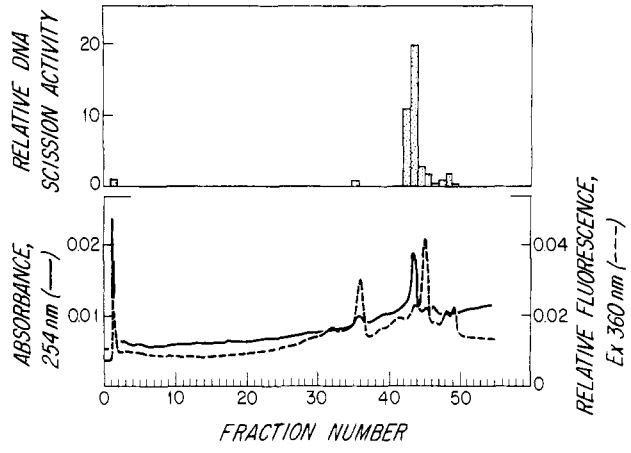

FIGURE 3: HPLC of AUR chromophore. AUR chromophore (150 μL) (1 mg equiv of AUR/mL in methanol) was injected and elution was with a gradient of 35–90% mobile phase solvent B in solvent A. One-minute fractions (2 mL) were collected. (—) Absorbance at 254 nm; (---) fluorescence emission (excitation at 360 nm, with 418-nm cutoff filter). The fractions (15 μL) were assayed for activity in the standard incubation (30 min) with pMB9 DNA as the substrate. Fraction 44 (15 μL) produced 47% of form II DNA. The total recovery of activity was 100%. The A_{355} of fraction 44 is 5×10^{-3}.

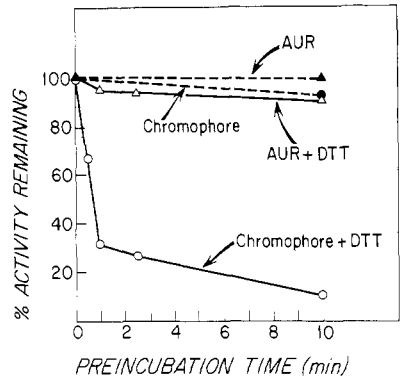

FIGURE 4: Effect of preincubation of AUR and HPLC-44 with DTT on their activities. The drug was preincubated in 62.5 mM Tris, pH 8.0, with or without 0.125 mM DTT in ice. The preincubation mixture with HPLC-44 and AUR contained 5% methanol. At the times indicated, 80 μL of the mixture was withdrawn and was assayed for its DNA scission activity in a 100-μL standard incubation for 40 min. In experiments where the drugs were preincubated in the absence of DTT, equivalent amounts of DTT were added in subsequent activity assays. The final level of AUR in the DNA-cutting reaction was 5 μg/mL which, with no preincubation, produced 68% of form II DNA (100% activity). Without preincubation HPLC-44 (8 μL/100-μL incubation) generated 56% of form II DNA (100% activity).

mM DTT (Figure 7 and Table II). The inclusion of excess (about 5:1 with respect to chromophore) apoprotein, MCR, in the reaction (Table II and Figure 6) slows the reaction considerably, especially at 0 °C, although the final extent of DNA strand scission with DTT at 37 °C is less affected. The MCR effect is specific, since equal concentrations of NCS apoprotein or bovine serum albumin have no effect. In the absence of DTT, AUR (at 3 times the equivalent concentration of HPLC-44) gives a time course pattern (data not shown) very similar to that shown for HPLC-44 in the absence of DTT or MCR (Figure 6). With DTT (0.1 mM) the AUR reaction at 37 °C is increased two- to fourfold. In confirmation of the results with AUR and its methanol-extracted chromophore, 10 mM 2-mercaptoethanol failed to stimulate DNA strand scission by HPLC-44 at 37 °C but caused about one-third the stimulation of 0.1 mM DTT at 0 °C.

Discussion

The extracted nonprotein chromophore of AUR possesses the cytotoxic and in vitro DNA damaging activities of the holoantibiotic. The biological activity is associated with a single UV–visible absorbing, but low fluorescing, peak on HPLC. Unlike that from NCS (Kappen et al., 1980), the AUR chromophore is relatively stable in aqueous solutions at neutral pH values (L. S. Kappen and I. H. Goldberg, unpublished data). Further, in contrast to NCS, extraction of the chromophore of AUR by methanol is incomplete. The AUR chromophore also differs from that of NCS in possessing considerably more DNA strand-breaking activity at 37 °C in the absence of reducing compounds, relative to their presence, although both free chromophores are rapidly inactivated by pretreatment with these compounds, including 2-mercapto-

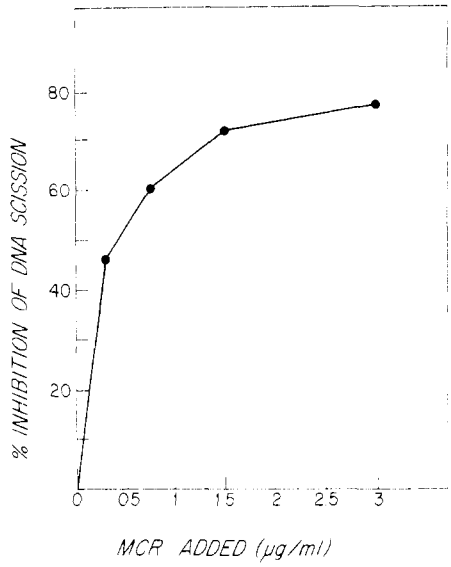

FIGURE 5: Inhibition of the DNA scission activity of HPLC-44 by MCR. Varying levels of MCR were added to standard incubations (40 min) containing 15 μL of HPLC-44. The reaction was started by the addition of DNA. HPLC-44 alone produced 50% of form II DNA. Based on A_{355}, the HPLC-44 concentration is equivalent to 3 μg/mL AUR.

Table III: Protection of HPLC-44 by Proteins against Inactivation by DTT[a]

protein added	% activity recovered
none	0.7
MCR, 0.5 μg/mL	15
MCR, 2 μg/mL	52
MCR, 10 μg/mL	78
apo-NCS, 10 μg/mL	0.9
BSA, 10 μg/mL	0

[a] HPLC-44 (10 μL) was preincubated in 62.5 mM Tris, pH 8.0, and 1.25 mM DTT with or without proteins for 1 min at 37 °C in a total volume of 80 μL. pMB9 DNA (20 μL) was then added and the complete reaction mixture was incubated for 40 min. Since apo-NCS may have (0.5–1%) residual activity of native NCS, all three proteins were heated at 65 °C for 2 h, a treatment which is known to destroy NCS activity. Identical protection was obtained with MCR not subjected to heat treatment. MCR, with or without heat treatment, does not have any DNA scission activity at the level used in this experiment. In the absence of preincubation, HPLC-44 generates 78% of form II DNA (taken as 100% activity). BSA, bovine serum albumin. Based on A_{355}, the HPLC-44 concentration is equivalent to 2 μg/mL AUR.

Table IV: Comparison of DNA Scission Activity of Varying Levels of HPLC-44 at 0 and 37 °C[a]

HPLC-44 (μL)	form II DNA formed (%)	
	at 0 °C	at 37 °C
2	37	15
4	59	24
6	68	37

[a] The reaction mixture (100 μL) contained varying amounts of HPLC-44, 0.1 mM DTT, and the other components. Incubation was for 20 min at 0 or 37 °C. Based on A_{355}, the HPLC-44 concentration in the stock solution is equivalent to 12.5 μg/mL AUR.

ethanol. At 0 °C, however, the AUR chromophore activity becomes almost entirely dependent on a reducing compound, and the scission of DNA is both faster and more extensive than at 37 °C. The latter results may reflect the longer life of the activated, possibly free-radical, form of the drug at the lower temperature. Nevertheless, NCS (Kappen et al., 1979) or its isolated chromophore (Kappen et al., 1980) in the presence of a mercaptan is much more potent than AUR or its isolated chromophore as a DNA strand breaking agent in vitro, although not in vivo, suggesting that optimal in vitro activation conditions have yet to be found. Apoproteins of both drugs afforded specific protection of their respective chromophores from inactivation by reducing compounds and regulate the release of the active component for interaction with DNA.

We find that low concentrations of DTT markedly stimulate the initial rate of DNA strand scission by AUR chromophore at 0 °C and somewhat less so at 37 °C, although the final levels of breakage with or without DTT after long incubation times at 37 °C are not so different (two- to threefold). Since removal of traces of chromophore from MCR by Amberlite XAD-7 chromatography results in virtually complete loss of DTT-stimulated DNA scission, it seems likely that this activity (Suzuki et al., 1979) was due to contaminating AUR. A similar result was obtained when the XAD-treated MCR was

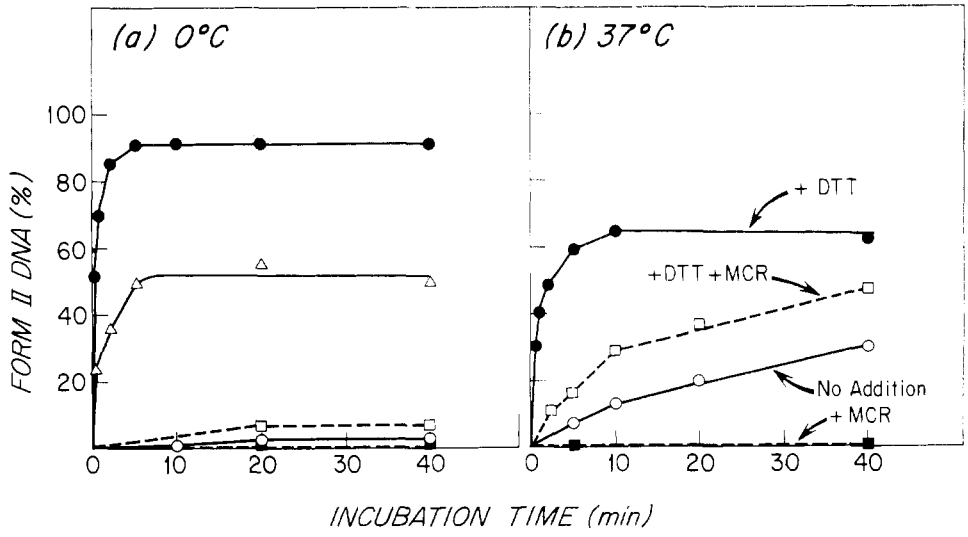

FIGURE 6: Comparison of the DNA scission activity of HPLC-44 at 0 and 37 °C in the presence and absence of DTT and MCR. Standard incubations (400 μL) were done at 0 and 37 °C with and without the indicated additions. The reaction was started by the addition of 24 μL of HPLC-44 to the rest of the components preequilibrated at the reaction temperature. At the indicated times 75 μL of the reaction was analyzed on alkaline sucrose gradients. When present, DTT was at a final level of 0.1 mM and MCR at 6 μg/mL. In (a) Δ–Δ has one-third the amount of HPLC-44 as in ●–●. Based on A_{355}, the HPLC-44 concentration at its higher level is equivalent to 1.2 μg/mL AUR. The same symbols are used in (a) and (b).

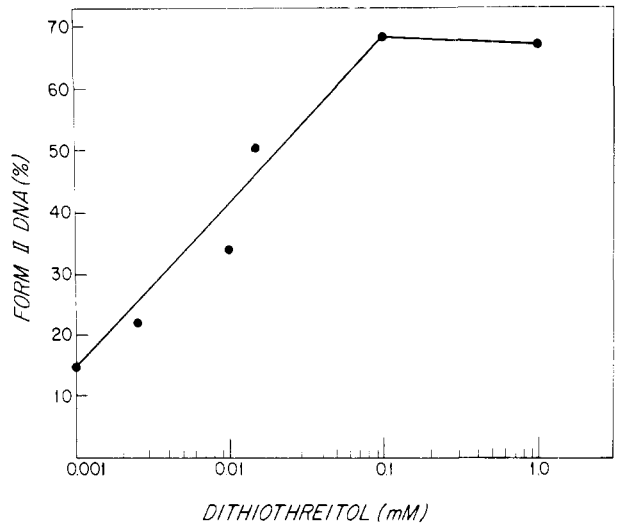

FIGURE 7: Effect of varying levels of DTT on DNA scission by HPLC-44. Standard incubations (100 μL) contained varying levels of DTT. The reaction was started by the addition of 3 μL of HPLC-44. Incubation was for 5 min in ice. In the absence of DTT, HPLC-44 produced less than 1% of form II DNA. Based on A_{355}, the HPLC-44 concentration is equivalent to 0.6 μg/mL AUR.

assayed for DNA synthesis inhibition in HeLa cells (Napier et al., 1980). By the addition of excess apoprotein to the purified AUR chromophore, we have generated a material resembling MCR containing a small amount of contaminating AUR (Table II and Figure 6). As expected, in the absence of DTT there is no DNA strand scission due to the tight binding of the chromophore to the protein, but on addition of the mercaptan significant DNA breakage is found at 37 °C. At 0 °C, however, chromophore remains bound to its apoprotein even in the presence of DTT and shows little strand-breaking activity. This is true for AUR, as well as for chromophore to which apoprotein has been added. In the case of AUR, however, where the ratio of apoprotein to chromophore is lower than that in Figure 6, DNA strand scission is found in the absence of DTT, and its stimulation by DTT is less dramatic (two- to fourfold) than when the apoprotein is in excess. These results suggest that the active form of the drug generated by DTT binds less tightly to the apoprotein and is more available for interaction with the DNA. The question arises as to whether the activity found in the absence of reducing agent is due to an already reduced form of the chromophore. The findings that both DTT-independent and DTT-dependent activities are specifically blocked by the apoprotein and that they elute as a single peak on HPLC indicate a close, if not identical, structural relationship, but further experiments are required to clarify this point.

The reason for the failure of 2-mercaptoethanol to stimulate the activity of AUR or of its isolated chromophore at 37 °C, while other reducing agents and mercaptans do, is not clear. Since 2-mercaptoethanol can inactivate, it is possible that the lack of observable stimulation at 37 °C is a coincidence of equal activation and inactivation. Since significant stimulation is found at 0 °C, this explanation seems reasonable. In further understanding this result it will be important to determine if the inactivated form is generated from a labile, activated form of the drug or is produced by an unrelated side reaction.

While distinct differences exist in various aspects of the actions and of the chemical and physical properties of AUR and NCS, sufficient similarities exist to permit grouping them into a novel family of protein antibiotics in which the apoprotein acts as a highly specific carrier and regulator of release of an active and labile prosthetic group. It is of particular interest that DNA strand breakage appears to account for the main actions of both agents. Whether this will also hold for other less well characterized members of this group, such as actinoxanthin (Khokhlov et al., 1976), sporamycin (Komiyama et al., 1977; Okamoto et al., 1979), and others [see Sekizawa et al. (1962)], remains to be determined.

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References

Beerman, T. A., & Goldberg, I. H. (1977) *Biochim. Biophys. Acta 475*, 281.

Kappen, L. S., & Goldberg, I. H. (1979) *Biochemistry 18*, 5647.

Kappen, L. S., & Goldberg, I. H. (1980) *Biochemistry* (following paper in this issue).

Kappen, L. S., Goldberg, I. H., & Samy, T. S. A. (1979) *Biochemistry 18*, 5123.

Kappen, L. S., Napier, M. A., & Goldberg, I. H. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1970.

Khokhlov, A. S., Reshetov, P. D., Chupova, L. A., Cherches, B. Z., Zhigis, L. S., & Stoyachenko, I. A. (1976) *J. Antibiot. 29*, 1026.

Komiyama, K., Sugimoto, K., Takeshima, H., & Umezawa, I. (1977) *J. Antibiot. 30*, 202.

Lowry, O. H., Rosebrough, N. J., Fari, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265.

Napier, M. A., Holmquist, B., Strydom, D. J., & Goldberg, I. H. (1979) *Biochem. Biophys. Res. Commun. 89*, 635.

Napier, M. A., Kappen, L. S., & Goldberg, I. H. (1980) *Biochemistry 19*, 1767.

Okamoto, M., Kamiyama, K., Takeshima, H., Yamamoto, H., & Umezawa, I. (1979) *J. Antibiot. 32*, 386.

Povirk, L. F., & Goldberg, I. H. (1980) *Biochemistry* (preceding paper in this issue).

Sekizawa, Y., Inouye, S., & Kagino, K. (1962) *J. Antibiot. 15*, 236.

Suzuki, H., Miura, K., & Tanaka, N. (1979) *Biochem. Biophys. Res. Commun. 89*, 1281.

Yamashita, T., Naoi, N., Hidaka, T., Watanabe, K., Kumada, Y., Takeuchi, T., & Umezawa, H. (1979) *J. Antibiot. 32*, 330.