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Effect of Some Aminoquinone Compounds on Nucleic Acid Synthesis catalyzed by *E. coli* DNA Polymerase I and RNA Polymerase

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The effect of DNA-interacting aminoquinones, 2-amino-1,4-naphthoquinone imine (ANQI), 2,5-diamino-1,4-naphthoquinone imine (DANQI) and 2-hydroxyamino-1,4-naphthoquinone (HANQ), which had been reported to inhibit cellular DNA synthesis in Ehrlich ascites carcinoma and *E. coli*, was studied on cell-free DNA and RNA syntheses catalyzed by *E. coli* DNA polymerase I and RNA polymerase, respectively.

These aminoquinones inhibited both the polymerase reactions to a similar degree (30—56% at 1×10^{-3} M). The inhibition of DNA synthesis was in nearly the same extent as that observed in *E. coli* whole cells, but lower than that found in intact cells of Ehrlich ascites carcinoma.

The inhibition of the DNA polymerase reaction by the aminoquinones was competitive with calf thymus DNA used as the template. Any of the aminoquinones received higher spectral change by the native DNA than by the heat-denatured, and also more inhibited the DNA synthesis directed by the double-stranded DNA than that by the single-stranded. These results suggest that the inhibition is due to the interaction between the DNA and the aminoquinones.

Keywords——drug action mechanism; aminoquinone compounds; DNA polymerase, inhibition of; RNA polymerase, inhibition of; interaction with DNA

It has been reported in the previous papers[2-6] that, among several aminoquinone compounds, 2-amino-1,4-naphthoquinone imine (ANQI), 2,5-diamino-1,4-naphthoquinone imine (DANQI) and 2-hydroxyamino-1,4-naphthoquinone (HANQ) interacted with DNA[7] *in vitro* at the base moieties and potentially inhibited DNA synthesis in Ehrlich ascites carcinoma cells. The inhibition, which was remarkably higher than that by mitomycin C, was speculated to be a result of that the aminoquinones penetrated into the cell nuclei and interacted with the DNA.[4-6]

To ascertain this speculation, the effect of these aminoquinones was studied on cell-free reactions of DNA-directed DNA and RNA[7] polymerases. The result that these amino quinones inhibited both the reactions possibly through the interaction with the template DNA is consistent with this speculation.

Experimental

Materials——ANQI was synthesized according to the procedure of Fieser[8] as described previously.[2] DANQI was prepared by the reduction of 2,4,5-trinitro-1-naphthol, obtained by the fuming HNO_3 nitration

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2) S. Okada, *Chem. Pharm. Bull.* (Tokyo), **17**, 105 (1969).
3) S. Okada, *Chem. Pharm. Bull.* (Tokyo), **17**, 113 (1969).
4) S. Okada, *Chem. Pharm. Bull.* (Tokyo), **17**, 1057 (1969).
5) N. Mochizuki, S. Okada, and O. Tamemasa, *Chem. Pharm. Bull.* (Tokyo), **23**, 1077 (1975).
6) N. Mochizuki, S. Okada, and O. Tamemasa, *Yakugaku Zasshi*, **97**, 968 (1977).
7) Abbreviations: DNA—deoxyribonucleic acid, RNA—ribonucleic acid, *E. coli*—*Escherichia coli*, TCA—trichloroacetic acid, EDTA—ethylenediamine tetraacetic acid, tris—tris-(hydroxymethyl)-aminomethane, dATP—deoxyadenosine-5′-triphosphate, dGTP—deoxyguanosine-5′-triphosphate, dCTP—deoxycytidine-5′-triphosphate, dTTP—deoxythymidine-5′-triphosphate, ATP—adenosine-5′-triphosphate, GTP—guanosine-5′-triphosphate, CTP—cytidine-5′-triphosphate, UTP—uridine-5′-triphosphate.
8) L.F. Fieser, "Experiments in Organic Chemistry," 3rd ed., D.C. Heath and Co., Boston, 1957, pp. 234—238.

of 2,4-dinitro-1-naphthol, as reported in the previous paper.[6] HANQ was obtained by the H_2O_2 oxidation of ANQI as described earlier.[9] *E. coli*[7] DNA polymerase I (EC 2.7.7.7) (Grade II) and *E. coli* RNA polymerase (EC 2.7.7.6) (MRE 600) were purchased from Boehringer Mannheim GmbH. Calf thymus DNA (Type I, "highly polymerized") was the product of Sigma Chemical Co. Heat-denatured calf thymus DNA was prepared by heating the native DNA at 100° for 15 min and then by cooling rapidly to 0°.

Assay for DNA Synthesis——The conversion of ^{3}H-dTTP[7] into cold TCA[7]-insoluble products by *E. coli* DNA polymerase I was measured according to the methods of Richardson *et al.*[10] and of Englund.[11] The reaction mixture (0.3 ml) contained 20 μmol of potassium phosphate buffer (pH 7.4) or glycine buffer (pH 9.2), 2 μmol of $MgCl_2$, 0.3 μmol of 2-mercaptoethanol, 10 μg of calf thymus DNA, 10 nmol each of dATP,[7] dGTP,[7] dCTP[7] (P-L Biochemicals) and dTTP-methyl-^{3}H (Daiichi Pure Chemicals, 20 μCi/μmol), 0.3 unit of *E. coli* DNA polymerase I and the indicated amount of the aminoquinone. The incubation was carried out at 37° for 30 min. The reaction was stopped in an ice bath by adding 0.2 M sodium pyrophosphate (0.2 ml) and 7% perchloric acid (0.5 ml), followed, 5 min later, by the addition of 0.1 M sodium pyrophosphate containing 1 N HCl (3 ml). The precipitate formed was collected on a Whatman GF/C glass fiber filter (diameter: 2.4 cm) and washed 3 times with 3 ml of 0.1 M sodium pyrophosphate containing 1 N HCl and once with 95% ethanol. The filter was dried and its radioactivity was determined in an Aloka LSC-602 liquid scintillation spectrometer.

Assay for RNA Synthesis——The assay using *E. coli* RNA polymerase was done by the method of Burgess and Travers.[12] The reaction mixture of a total volume of 0.25 ml contained 10 μmol of tris[7]–HCl buffer (pH 7.9), 2.5 μmol of $MgCl_2$, 0.025 μmol of EDTA,[7] 0.025 μmol of dithiothreitol, 37.5 μmol of KCl, 0.1 μmol of K_2HPO_4, 0.125 mg of bovine serum albumin, 10 μg of calf thymus DNA, 0.0375 μmol each of ATP,[7] GTP,[7] CTP[7] (Sigma) and UTP[7]-5,6-^{3}H (Daiichi Pure Chemicals, 20 μCi/μmol), 1 unit of *E. coli* RNA polymerase and the indicated amount of the aminoquinone or actinomycin D (Calbiochem). The incubation was performed at 37° for 10 min. The reaction was stopped in an ice bath by adding 3 ml of ice-cold 5% TCA containing 0.01 M sodium pyrophosphate. After leaving for 15 min, the precipitate formed was collected on a Whatman GF/C glass fiber filter and washed 4 times with 3 ml of 2% TCA containing 0.01 M sodium pyrophosphate and then once with 95% ethanol. The filter was dried and its radioactivity was counted in an Aloka LSC-602 liquid scintillation spectrometer.

Difference Spectrum——The measurement was done in the same manner as described earlier[2,3,5,6] in a Hitachi spectrophotometer, Model EPS-3T.

Table I. Effect of Aminoquinones on DNA Synthesis Catalyzed by *E. coli* DNA Polymerase I

Aminoquinone	Concentration (M)	^{3}H-dTMP residues incorporated (nmol/mg protein)	Inhibition (%)
ANQI[a]	0	299	—
	1×10^{-5}	297	0.7
	1×10^{-4}	249	16.6
	1×10^{-3}	130	56.5
DANQI[a]	0	243	—
	1×10^{-5}	242	0.4
	1×10^{-4}	234	3.8
	1×10^{-3}	115	52.8
HANQ[b]	0	136	—
	1×10^{-5}	132	2.9
	1×10^{-4}	129	4.8
	1×10^{-3}	79	41.8

Experimental conditions are in the text.
a) Potassium phosphate buffer (pH 7.4) was used.
b) Glycine buffer (pH 9.2) was used.

9) N. Mochizuki, S. Okada, and O. Tamemasa, *Yakugaku Zasshi*, **94**, 744 (1974).
10) *a*) C.C. Richardson, C.L. Schildkrant, H.V. Aposhian, and A. Kornberg, *J. Biol. Chem.*, **239**, 222 (1964); *b*) C.C. Richardson, "Procedures in Nucleic Acid Research," Vol. 1, ed. by G.L. Cantoni and D.R. Davis, Harper and Row, Inc., New York, 1966, pp. 263—276.
11) P.T. Englund, "Procedures in Nucleic Acid Research," Vol. 2, ed. by G.L. Cantoni and D.R. Davis, Harper and Row, Inc., New York, 1971, pp. 864—874.
12) *a*) R.R. Burgess, *J. Biol. Chem.*, **244**, 6160 (1969); *b*) R.R. Burgess and A.A. Travers, "Procedures in Nucleic Acid Research," Vol. 2, ed. by G.L. Cantoni and D.R. Davis, Harper and Row, Inc., New York, 1971, pp. 851—863.

Result

Inhibition of DNA and RNA Syntheses

As shown in Table I, ANQI, DANQI and HANQ inhibited DNA synthesis catalyzed by *E. coli* DNA polymerase I. The extents of the inhibition by 1×10^{-3} M aminoquinones were in a range of 41—56%, and no remarkable difference was observed among the inhibition percentages by the three aminoquinones.

These aminoquinones also inhibited *E. coli* RNA polymerase reaction as presented in Table II. The degree of the inhibition by any of the aminoquinones at 1×10^{-3} M was in a range of 30—50%. This percentage was similar to that of DNA synthesis (Table I) and much lower than that by actinomycin D.

Table II. Effect of Aminoquinones on RNA Synthesis Catalyzed by *E. coli* RNA Polymerase

Aminoquinone	Concentration (M)	^{3}H-UMP residues incorporated (nmol/mg protein)	Inhibition (%)
None	—	6.24	—
ANQI	1×10^{-5}	5.52	11.5
	1×10^{-4}	5.25	15.9
	1×10^{-3}	4.31	31.1
DANQI	1×10^{-5}	5.28	15.5
	1×10^{-4}	4.95	18.5
	1×10^{-3}	4.19	32.9
HANQ	1×10^{-5}	4.62	24.0
	1×10^{-4}	4.11	32.4
	1×10^{-3}	3.03	50.1
Actinomycin D	1×10^{-5}	0.79	86.9

Experimental conditions are in the text.

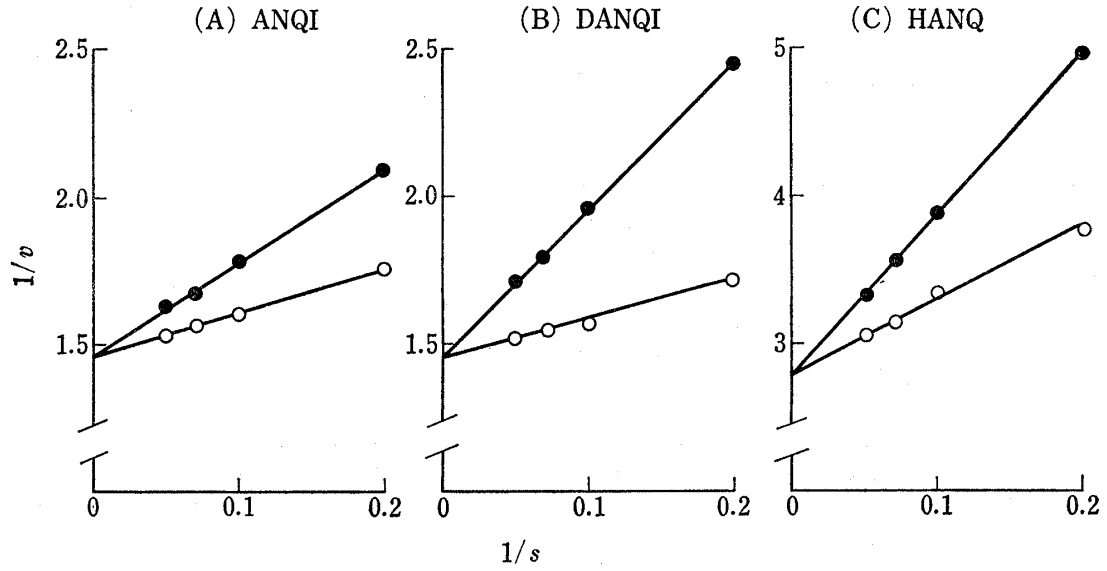

Fig. 1. Competitive Inhibition of DNA Synthesis by Aminoquinones

Experimental conditions, described in the text, were the same as in Table I except DNA concentrations.
v —— nmol of ^{3}H-dTMP incorporated per assay; s —— μg of calf thymus DNA per assay.
○——○ control, ●——● with aminoquinone (2×10^{-4} M).

Competition with the Template DNA in the Inhibition of DNA Synthesis

As the aminoquinones were known to interact with DNA *in vitro*,[2,3,5,6] it was expectable that the inhibition occurred by lowering the template activity of the preexisting DNA through the interaction. If it was the case, the inhibition extents might be changed by varing the amount of the template DNA. In fact, the inhibition of the DNA synthesis by the aminoquinones at a constant concentration, 2×10^{-4} M, was reduced with increase in the concentration of the template DNA. The result expressed by the Lineweaver–Burk's double–reciprocal plots is shown in Fig. 1. In any case of the three aminoquinones the plots gave a straight line, and each line intersected with the control line on the vertical axis. This result shows that the aminoquinones competed with the template DNA in the reaction. Therefore, it seems likely that the inhibitory effect of the aminoquinones was due to the interaction with the template DNA, although another possibility, such as the competitive attachment to the active site in the enzyme between the DNA and the aminoquinones, can not be ruled out.

Relationship between the Interaction with DNA and the Inhibition of DNA Synthesis

The aminoquinone–interacting activity of native and heat–denatured calf thymus DNA was compared with those template activity in the presence of the aminoquinones, because *E. coli* DNA polymerase I was active for both double– and single–stranded DNA as the template.

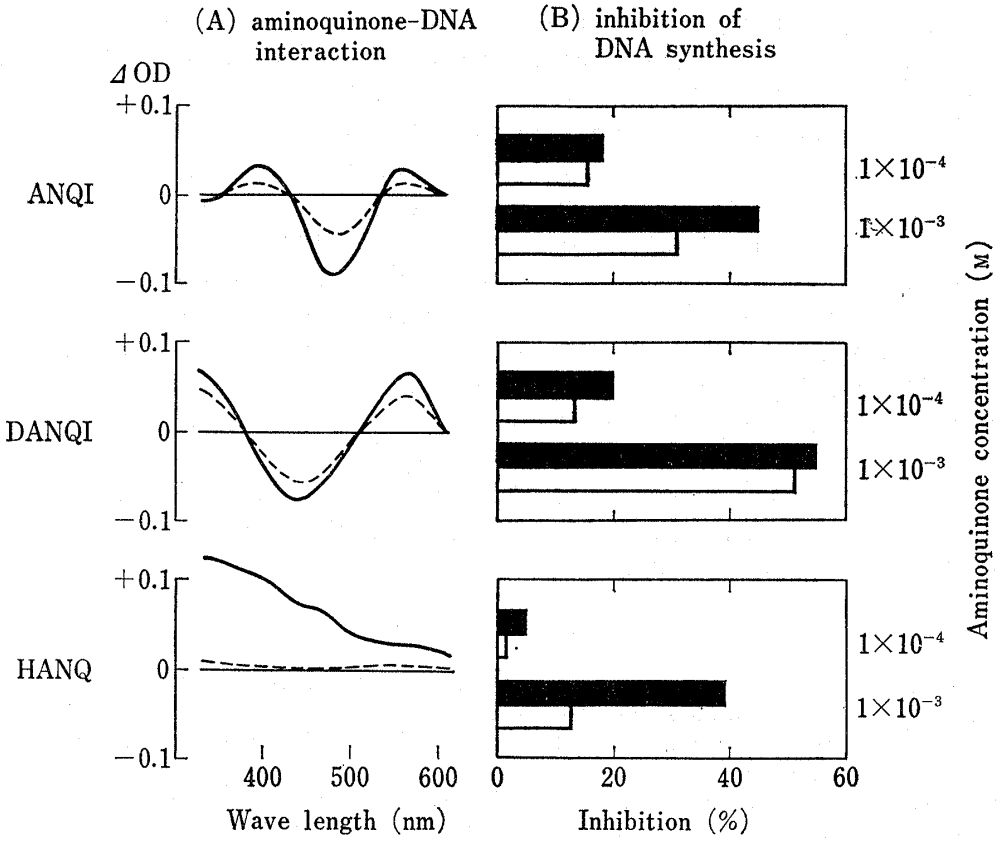

Fig. 2. Relationship between Aminoquinone-DNA Interaction and Inhibition of DNA Synthesis

(A) Difference spectra of aminoquinones and calf thymus DNA against aminoquinones. Concentrations of aminoquinones and DNA (as nucleotide) are 1×10^{-4} M and 1×10^{-3} M, respectively, in 0.01 M NaCl-0.01 M tris-HCl, pH 7.4.
——— native DNA, ------ heat-denatured DNA.
(B) Inhibition by aminoquinones of *E. coli* DNA polymerase I reaction directed by native or heat-denatured calf thymus DNA. The assay conditions, described in the text, were the same as in Table I except that two kinds of the DNA, native and heat-denatured, were used.
■ native DNA, □ heat-denatured DNA.

As shown in Fig. 2, all the aminoquinones received higher spectral change by the native DNA than by the heat-denatured, and also inhibited the DNA synthesis directed by the double-stranded DNA more than that by the single-stranded. This relationship was particularly notable in case of HANQ; the heat denaturation of calf thymus DNA led to almost complete disappearance of both the spectral change and the inhibition.

Discussion

A number of antineoplastic antibiotics have been known to inhibit DNA or RNA synthesis in tumor cells through the interaction with the cellular DNA. Of these antibiotics, mitomycin C,[13] porfiromycins,[13] streptonigrin,[14] pluramycin,[15,16] actinomycins,[17] daunomycin,[18] adriamycin[18] and kanchanomycin[16,19] have either an aminoquinone or a quinone moiety in the molecule as a functional group for the interaction, although the modes of the interaction are not necessarily in the same manner.[20] The three synthetic aminoquinones, *i. e.*, ANQI, DANQI and HANQ, have been reported to inhibit DNA synthesis in Ehrlich ascites carcinoma cells and to interact with DNA *in vitro*.[2-6] However, the *in vitro* interaction may not prove the *in vivo* intraction, because cells have a number of barriers, *e. g.* cell membranes, nuclear envelopes, nuclear proteins, *etc.*, for the aminoquinones to reach nuclear DNA. The present experiment using cell-free system which eliminated these barriers may be a way to know if the inhibition resulted from the interaction with nuclear DNA in living cells.

As a result, these aminoquinones inhibited both DNA and RNA syntheses catalyzed by *E. coli* DNA polymerase I and RNA polymerase, respectively, to a similar degree (Table I, II). The inhibition of the DNA synthesis by any of the aminoquinones was competitive with the template DNA (Fig. 1). There may be two possibilities, as mentioned above, in the competition between the aminoquinones and the DNA; one is by the interaction and the other is by the competitive attachment to the active site in the enzyme. The data that the inhibition extents were parallel to the degrees of the spectral changes (Fig. 2) support the former possibility for the following reason; the extent of the spectral change may reflect that of the interaction, since the mode of interaction of the aminoquinones was known to be nearly in the same manner between single- and double-stranded nucleic acids.[3,5,6]

The inhibition percentages of the cell-free DNA synthesis obtained in this study (41—56% at 1×10^{-3} M aminoquinone, Table I) were nearly equal to those observed on *E. coli* whole cells reported previously (50—60% at 1×10^{-3} M aminoquinone).[5,6] Therefore, the inhibition of DNA synthesis in living cells of *E. coli* might be mainly due to the aminoquinone-DNA interaction in the cells, although it should be noted that the intracellular concentration of the aminoquinones could be different from the extracellular and that there were many differences between the features of DNA synthesis in living cells and in the experimental cell-free system.

13) W. Szybalski and V.N. Iyer, "Antibiotics," Vol. I, ed. by D. Gottlieb and P.D. Shaw, Springer Verlag, Berlin-Heidelberg-New York, 1967, pp. 210—245.
14) *a*) N.S. Mizuno, *Biochim. Biophys. Acta*, **108**, 394 (1965); *b*) N.S. Mizuno and D.P. Gilboe, *Biochim. Biophys. Acta*, **224**, 319 (1970).
15) *a*) N. Tanaka, K. Nagai, H. Yamaguchi, and H. Umezawa, *Biochem. Biophys. Res. Commun.*, **21**, 328 (1965); *b*) K. Nagai, H. Yamaki, N. Tanaka, and H. Umezawa, *J. Biochem.* (Tokyo), **62**, 321 (1967); *c*) K. Nagai, N. Tanaka, and H. Umezawa, *J. Biochem.* (Tokyo), **67**, 655 (1970).
16) The complete structure remains unknown.
17) E. Reich, A. Cerami, and D.C. Ward, "Antibiotics," Vol. I, ed. by D. Gottlieb and P.D. Shaw, Springer Verlag, Berlin-Heidelberg-New York, 1967, pp. 714—725.
18) A. DiMarco, F. Arcamone, and F. Zunino, "Antibiotics," Vol. III, ed. by J.W. Corcoran and F.E. Hahn, Springer Verlag, New York-Heidelberg-Berlin, 1975, pp. 101—128.
19) I.H. Goldberg, "Antibiotics," Vol. III, ed. by J.W. Corcoran and F.E. Hahn, Springer Verlag, New York-Heidelberg-Berlin, 1975, pp. 166—173.
20) I.H. Goldberg and P.A. Friedman, *Ann. Rev. Biochem.*, **40**, 775 (1971).

On the other hand, when the inhibition percentages were compared with those found on whole cells of Ehrlich ascites carcinoma, a remarkable difference was noticed. The percent inhibition of DNA synthesis in the carcinoma cells was in a range of 70—80% at 1×10^{-5} M aminoquinone, which was the concentration of 1/100 as much as the case of *E. coli*, as reported earlier.[4-6] This suggests that the interaction with cellular DNA may not be the only mechanism for the aminoquinone inhibition of DNA synthesis in the intact carcinoma cells. In fact, another mechanism is being suggested by preliminary experiments, which will be reported later, on the strand scission of cellular DNA of Ehrlich ascites carcinoma.