Inhibitory Effects of Nalidixic Acid Group Compounds on Two DNA-DNAase Systems

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Inhibitory effects of nalidixic acid (NA) group compounds were spectrophotometrically measured on two DNA-DNAase systems: calf-thymus DNA *vs.* bovine pancreas DNAase and *E. coli* DNA *vs. Staph. aureus* nuclease. A close correlation between antibacterial activity and ability to inhibit both systems was demonstrated. These findings indicate that the inhibition of DNAase linked to DNA replication might be involved in the antibacterial action of NA drugs and this inhibition should be mainly due to chelation with Mg^{2+} (DNAase) and Ca^{2+} (nuclease).

INTRODUCTON

Nalidixic acid (NA) and related drugs are recently developed synthetic antibacterial agents which are used in clinics. They selectively inhibited DNA synthesis in bacteria (Goss et al., 1964; 1965; Shimizu et al., 1971) with only secondary inhibitory effects on the synthesis of RNA and proteins (Winshell & Rosenkranz, 1970; Javor, 1974). Unlike usual inhibitors of DNA synthesis, NA showed no inhibitory effect on DNA-polymerase I in vitro and was highly active against a mutant strain of *Escherichia coli* which lacked this enzyme (Boyle et al., 1969). On the other hand, NA blocked repair synthesis of DNA induced by u.v. irradiation (Eberle & Masker, 1971). As the important role of DNAase has been recognized in the repair synthesis of DNA (Lehman, 1967; Howard-Flanders, 1968), it appeared to be an interesting project to investigate effects of NA drugs on the activity of DNAase. The present paper describes the spectrophotometric determination of the effects of NA drugs on the animal and microbial DNA-DNAase systems and provides evidence of metal chelation mechanism for the DNAase inhibition which might be correlated with the antibacterial action.

MATERIALS AND METHODS

Enzyme systems. System I: calf thymus DNA (Sigma, Type I) *vs.* bovine pancreas DNAase I (Sigma) and system II: *E. coli* DNA (Sigma, Type VIII) *vs. Staph. aureus* nuclease (Sigma, Grade VI) were used.

Drugs. NA and oxolinic acid (OXA) were obtained from the commercial source. Piromidic acid (PA), pipemidic acid (PPA), AT-22 and AT-70 were obtained from Dainippon Pharmaceutical Research Laboratory.

Reagents. Adenine (Ad), 8-hydroxyquinoline (HQ), EDTA, tris-(hydroxymethyl) aminoethane hydrochloride (Tris-HCl) and other chemicals were of analytical reagent quality.

Enzyme assay. The rate of enzyme reaction was measured by the partly modified Kunitz's method (Kunitz, 1950); the increase of absorbance at 260 nm of the system was followed with a Shimadzu UV-180 double beam spectrophotometer. The reaction mixtures for system I contained 0.1 mg/ml DNA, 2 mM $MgSO_4$, 2 μg/ml DNAase and 25–80 μM inhibitor (NA drugs and other compounds) in 10 mM Tris-HCl buffer (pH 7.5). The reaction mixtures for system II contained 0.1 mg/ml DNA, 2 mM $CaCl_2$, 4 μg/ml nuclease and 80 μM inhibitor in the buffer. A reference cuvette also contained same DNA and inhibitor as a sample cuvette. Measurements were carried out at 23°C.

Differential absorption spectra. Sample solutions in a 10 mm-path cuvette contained 0.25 mM NA drug and 30 μg/ml DNAase and reference solutions in two 5 mm-path cuvettes contained 0.5 mM NA drug and 60 μg/ml DNAase separately. Sample solutions to measure the effect of enzyme concentration or $MgSO_4$ contained 7.5–3.75 μg/ml DNAase or 5 mM $MgSO_4$ in addition.

RESULTS

Fig. 1 shows the effects of six NA group compounds and two general chelators on system I. The effect was dependent on the concentration as seen for OXA and PPA and a comparison of the effects of these compounds at an equimolar level indicated their inhibitory order as: PPA>OXA>PA>NA>AT-70>EDTA>AT-22>HQ.

Fig. 2 shows the effects of six NA group compounds on system II. The same inhibitory order as above was estimated for these compounds.

Differential absorption spectra of the NA drug-DNAase systems are given in Fig. 3. NA drugs showed their perturbation peaks with DNAase at 280–330 nm, the heights of which were in the order: PPA>OXA>PA>Ad>NA. These peaks were affected by enzyme concentration and $MgSO_4$ addition.

DISCUSSION

Correlation between DNAase inhibition and antibacterial action. The order of inhibitory activity of NA group compounds on DNAase parallels that for their antibacterial activity which was reported in the previous study. This fact indicates that NA drugs might interfere with the repair synthesis of DNA through DNAase inhibition.

Comparison of inhibitory effects on systems I and II. NA drugs inhibited a microbial DNA-DNAase system mediated by Ca^{2+} as well as an animal DNA-DNAase system mediated by Mg^{2+}. This fact implies that under suitable conditions the inhibition of DNA synthesis by NA drugs might occur in the growing cells other than the specified bacterial cells hitherto studied. In this context the prevention of the mitochondrial DNA replication by NA in yeast (Whittaker, Hammond & Luha, 1972) is noticeable.

Style of DNAase inhibition. The inhibition was evident not only in the retardation of reaction rate but also in the reduction of hyperchromisity, $\triangle$Abs which is the increase (%) in absorbance at 260 nm. As the $\triangle$Abs was reciprocally parallel to the average

unit number of oligonucleotides (Bernardi et al., 1974), the n values for PPA, OXA, PA and NA were larger than that of the standard system in this order.

Metal chelation mechanism. NA drugs might inhibit the DNAase mainly by chelation with Mg^{2+} and Ca^{2+} which are essential for the binding of DNA with DNAase and nuclease, respectively. The facts supporting this mechanism have been previously obtained: NA drugs stimulated the electron transfer from Fe^{2+} to cytochrome *c* (Yamabe, 1976) and divalent metal ions other than Fe^{2+} inhibited this stimulation according to their stability constants (Yamabe, 1978).

Interaction of NA drugs with DNAase molecule. As the DNAase inhibition by general chelators such as EDTA and HQ was apparently weak, additional factor(s) *e. g.* structural similarity to DNA-purine and/or affinity for active centre of the enzyme should be considered for the high inhibitory activity of NA drugs. The spectral findings supporting this consideration are: (1) a similarity in the perturbation spectra of NA drugs to that of Ad which has been assigned to the interaction with RNAase (Hummel, Ver Ploeg & Nelson, 1961; Irie & Sawada, 1967) and (2) a close correlation between spectral intensity and ability to inhibit the enzyme.

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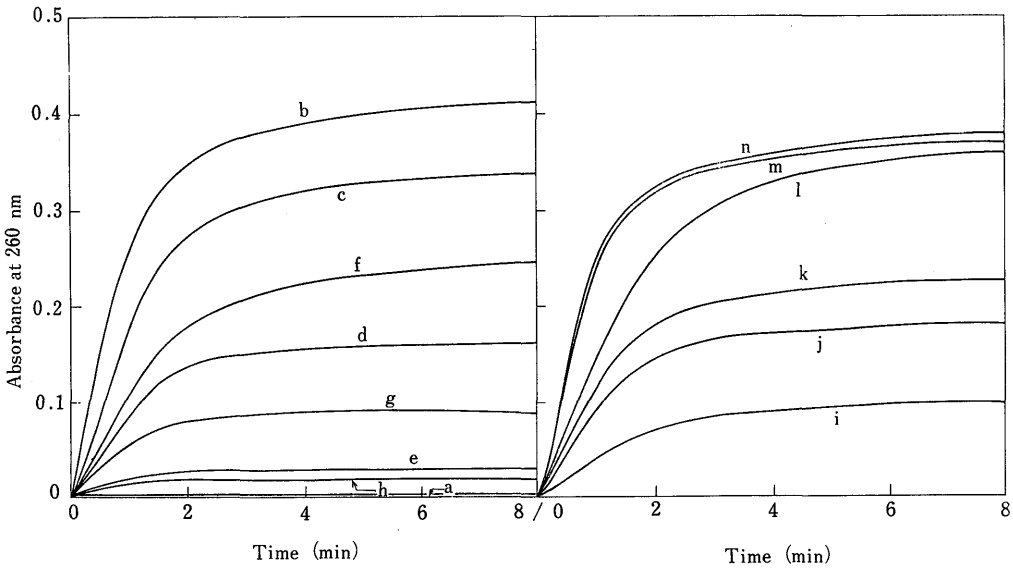

Fig. 1. Inhibitory effects of NA group compouds and chelators on system I. The assay procedure is described in Methods. a, System without $MgSO_4$ and inhibitor; b, standard system without inhibitor; c to e, systems containing OXA (25, 50, 80 μM); f to h, systems containing PPA (25, 50, 80 μM); i to n, systems containing PA, NA, AT-70, EDTA, AT-22 and HQ (all at 80 μM), respectively.

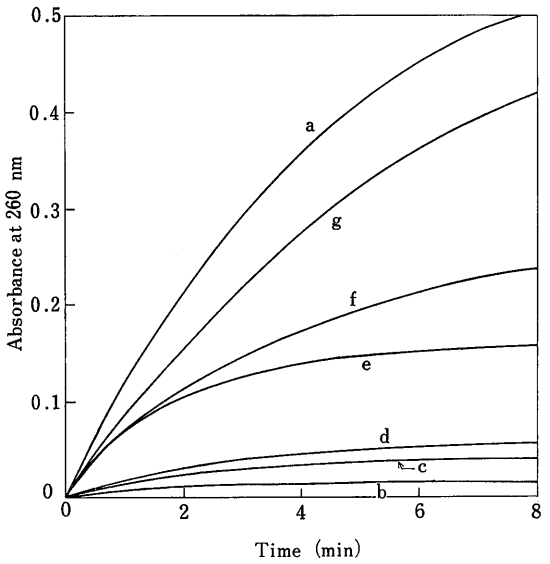

Fig. 2. Inhibitory effects of NA group compounds on system II. a, Standard system without inhibitor; b to g, systems containing PPA, OXA, PA, NA, AT-70 and AT-22 (all at 80 μM), respectively.

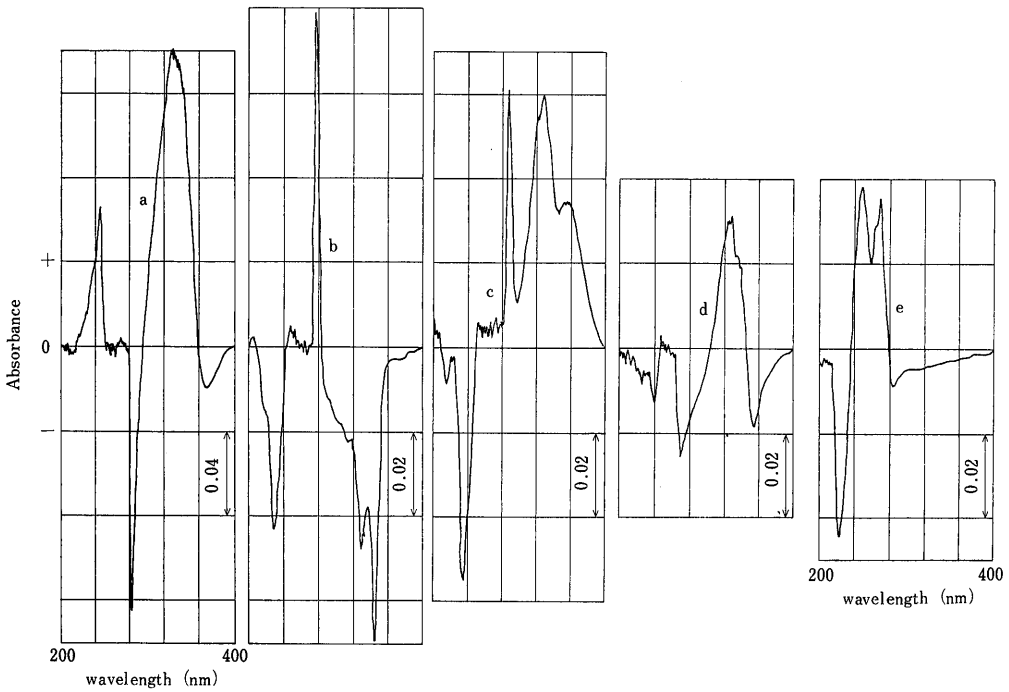

Fig. 3. Differential absorption spectra of DNAase (bovine pancreas)-NA drug systems. The assay procedure is described in Methods. a to e; Systems containing PPA, OXA, PA, NA and Ad, respectively. Arrow indicates the scale of absorbance.

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