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Phthalanilide interaction with nucleic acids*

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THE substituted phthalanilides are a series of basic compounds which have been shown to have exceptional therapeutic activity against certain experimental neoplasms, notably leukemias in rodents.[1, 2]

In preliminary experiments we have observed that a significant portion of the phthalanilide present in a mouse ascitic leukemia cell (P-388 line), after treatment *in vivo*, is localized in the nuclear fraction after centrifugal fractionation of the cell components. These findings, and reports of interactions of DNA with polybasic compounds such as spermidine[3] and with the actinomycins[4] which inhibit DNA-dependent RNA synthesis,[5, 6] have led us to investigate the phthalanilides for their capacity to interact with DNA and RNA.

MATERIALS AND METHODS

The phthalanilides used in this study were supplied by the Cancer Chemotherapy National Service Center and are as follows: NSC 35843: 4′,4″-di(2-imidazolin-2-yl)terephthalanilide dihydrochloride; NSC 53212: 4′,4″-di(2-imidazolin-2-yl)isophthalanilide dihydrochloride; NSC 60339: 2-chloro-4′,4″-di(2-imidazolin-2-yl)terephthalanilide.

Highly polymerized salmon-sperm DNA, yeast RNA, and polyadenylic acid were obtained from commercial sources. The DNA preparations from ascitic leukemia cells, which were either sensitive or resistant to NSC 60339, were prepared by the method described by Marmur.[7]

Spectral observations were made at room temperature with a Zeiss spectrophotometer, model PM QII.

RESULTS AND DISCUSSION

Mixtures of NSC 60339 (2 to 20 μg/ml) and salmon-sperm DNA (5 to 125 μg/ml) in dilute aqueous solutions result in the formation of a complex, as is evidenced by a 13-mμ bathochromic shift of the absorption maximum of the phthalanilide from 292 to 305 mμ (Fig. 1, a). The hyperchromic maximum of the difference spectrum at 325 mμ is taken to be an index of complex formation and is proportional to phthalanilide concentration at saturating levels of DNA (Fig. 1, b). It was determined that increasing the DNA concentration above 1·2 μg DNA/μg NSC 60339 did not increase the amount of complex formed (Fig. 2).

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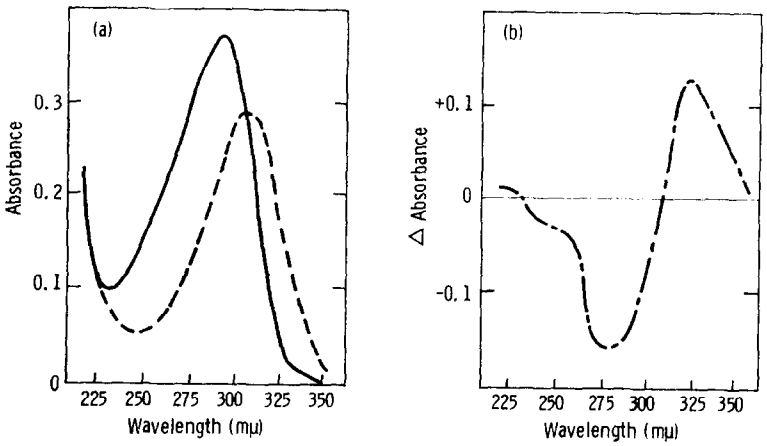

FIG. 1. Interaction of salmon-sperm DNA with NSC 60339.
(a) NSC 60339, 4 μg/ml (——); NSC 60339, 4 μg/ml, with DNA, 125 μg/ml vs. DNA, 125 μg/ml (– – – –). (b) Difference between spectra of Fig. 1, a.

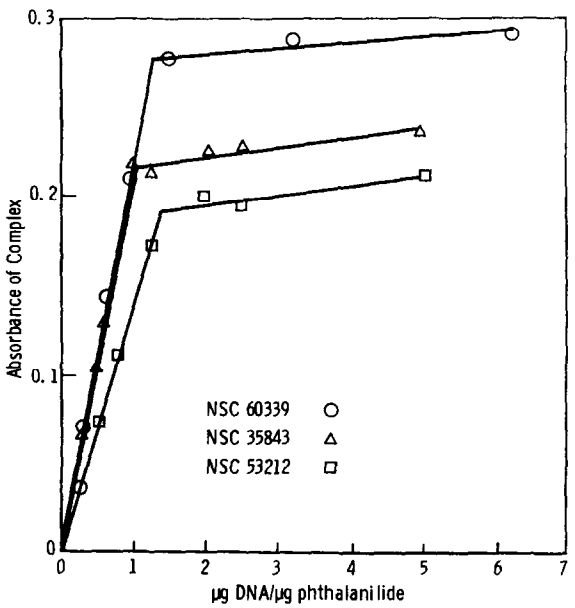

FIG. 2. Stoichiometry of DNA-phthalanilide interaction; concentration of phthalanilide, 10 μg/ml.

Similarly, with two other active phthalanilides (NSC 35843 and 53212) the amount of DNA per microgram of drug, required to obtain maximum levels of complex ranged from 1·1 to 1·4 μg (Fig. 2). Based on the average molecular weight (630) of a base pair of DNA, 1 molecule of phthalanilide can complex with a single base pair equivalent—i.e. two phosphate groups. Possibly, the imidazoline nitrogens of the phthalanilides are bound by the negatively charged phosphate groups of the nucleic acid polymer, resulting in interactions that give rise to the observed spectral shifts.

Uridine, thymidine, adenine, deoxyadenine, and their monophosphates were found to be without effect on the spectrum of NSC 60339, indicating that some structure which is at least more than a monomeric unit is required for the formation of a nucleic acid–phthalanilide complex and that the complex involves more than a simple nucleotide phosphate–imidazoline nitrogen attraction. Preliminary results obtained from binding experiments done in the presence of various cations or high

concentrations of urea suggest that both ionic and hydrogen bonding forces are operative. Hydrophobic attractions, which are important in polymer interactions, also may play a significant role in the formation of the phthalanilide–nucleic acid complex.[8]

Yeast RNA and polyadenylic acid also interact with NSC 60339 to produce a bathochromic shift in the absorption maximum of the phthalanilide in the presence of either of these ribose nucleic acid polymers. Unlike the complex observed with DNA, which appears to form a stable complex immediately after addition of phthalanilide, the RNA–phthalanilide complex results in a continuing aggregation of particles, giving rise to a visible Tyndall effect after several minutes. This aggregation increases slowly over a 24-hr period. The DNA–phthalanilide complex, on the other hand, is stable over a 24-hr interval at room temperature.

That the interaction of phthalanilides with both ribose- and deoxyribosenucleic acid polymers is relatively nonspecific is further supported by experiments showing that DNA preparations isolated from either NSC 60339-sensitive or -resistant mouse leukemia cells react with NSC 60339 in essentially the same way as does the salmon-sperm DNA.

Whether the interaction with nucleic acids bears any relationship to the antitumor activity of the basic phthalanilides cannot be determined with certainty at this time. However, it is intriguing to speculate on the possibility that the phthalanilides may mimic the histones in their ability to combine with DNA and thereby possibly repress or completely inhibit certain information transfer mechanisms—i.e. they may possess "anticodic" activity.[2] Although the DNA preparations from resistant and sensitive cells interact with phthalanilides to the same degree under conditions *in vitro*, interactions *in vivo* may differ. In fact, mouse leukemia cells resistant to NSC 60339 retain less than 20 per cent of the amount of phthalanilide bound by sensitive cells after exposure for 24 hr *in vivo*.[9]

Experiments are in progress to clarify the possible importance of the DNA-phthalanilide interaction in inhibiting the growth of mouse leukemia cells.

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A specific esterase in experimental inflammation

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DURING the investigation of the anti-inflammatory activity of the protease-inhibitor from potatoes (IPP),[1-3] an attempt was made to characterize the hypothetical enzyme involved in the development of inflammation and inhibited by IPP. For this purpose, a peptidic preparation of IPP was used possessing a high anti-inflammatory activity and, with the exception of chymotyrpsin, almost no inhibitory activity against some common proteolytic enzymes (trypsin, plasmin, papain). The hydrolysis of synthetic substrates (tosyl-L-arginin-methyl ester, L-lysin-ethyl ester and acetyl-L-tyrosin ethylester) was studied with a modified photometric indicator method[4] by extracts of inflamed tissue (kaolin-arthritis in rats, paws frozen to −40°, homogenized and extracted with saline). Only acetyl-L-tyrosin ethyl ester (ATEE) was hydrolyzed much more by the extracts from the inflamed tissue than by extracts from the normal tissue or tissue from inhibitor-treated rats (3 mg/kg of IPP i.p., 30 min before the kaolin injection Fig. 1). The hydrolytic activity of the inflamed tissue on ATEE increased in a parallel way to