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SEQUENCE SPECIFICITY OF THE DAUNOMYCIN-DNA INTERACTION

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The interaction of the important anticancer antibiotic daunomycin (daunorubicin) with DNA is thought to be important in the mechanism by which the drug inhibits transcription and DNA replication. While both equilibrium and kinetic aspects of the daunomycin-DNA interaction have been intensively studied for over two decades, the sequence specificity of daunomycin binding to DNA remains poorly defined, and contradictory results are found in the literature (1-3). A recent theoretical study, however, suggests that daunomycin selectively recognizes a triplet sequence comprised of adjacent GC base pairs, flanked by an A-T base pair (4). We report results from equilibrium binding experiments, deoxyribonuclease footprinting experiments, and experiments that examine the effect of daunomycin on the digestion of linearized pBR322 DNA by restriction endonucleases Pvu I and Eco RI. Our results provide the clearest experimental evidence to date that daunomycin does indeed recognize, and preferentially bind to, specific sites along the DNA lattice. In equilibrium binding studies using synthetic deoxypolynucleotides, daunomycin was found to bind more tightly to alternating purine-pyrimidine sequences (i.e., poly(dAdT)-poly(dAdT)) relative to the nonalternating sequence (i.e., polydA-polydT). Thermodynamic studies show that binding to alternating sequences is driven by a large, negative enthalpy, whereas binding to nonalternating sequences is essentially isoenthalpic, and therefore entropically driven. This pattern suggests that binding to the nonalternating sequence may be accompanied by a substantial conformational change in the polynucleotide. Synthetic deoxypolynucleotdes may, therefore, not be the most appropriate models for probing sequence specificity, since they may adopt unusual conformations which may dominate the binding interaction. We, therefore, have studied the interaction of daunomycin with natural DNA of varying base composition and sequence in a series of additional studies, since we judge these natural sequences to be a more appropriate model system for the

study of sequence specificity. The binding constant for the daunomycin-DNA interaction is a strong function of the GC content of the DNA. For example, the binding constant for the interaction of daunomycin with M. lysodeikticus DNA (72% GC) is four times that of the binding constant found for C. perfringens DNA (31% GC). Quantitative analysis of these results suggests that this arises from preferential interaction at sites containing adjacent GC base pairs, perhaps occurring as part of a triplet sequence containing an AT base pair at one end of the triplet. Deoxyribonuclease footprinting experiments using the tyr t restriction fragment show that daunomycin protects specific sequences from digestion (figure 1). The protected sequences are found to be GC rich, and have GC and CC as the most frequent dinucleotides. Within all protected regions, a triplet sequence consisting of adjacent GC base pairs flanked by an AT base pair may be found in one or more copies. We conclude that triplets of this type are the preferred daunomycin binding sites, in agreement with the predictions of Chen et al. (4). More refined footprinting studies are underway that should resolve the exact hierarchy of preferred sequences found within that general framework. Consistent with our notion of the preferred daunomycin binding site, we found that daunomycin inhibited the restriction endonuclease Pvu I (recognition site 5'-CGATCG-3') to a greater extent than restriction endonuclease Eco RI (recognition site 5'-GAATTC-3'). The former site contains the putative recognition triplet, while the latter does not. Our results show that daunomycin indeed recognizes specific binding sites along the DNA lattice.

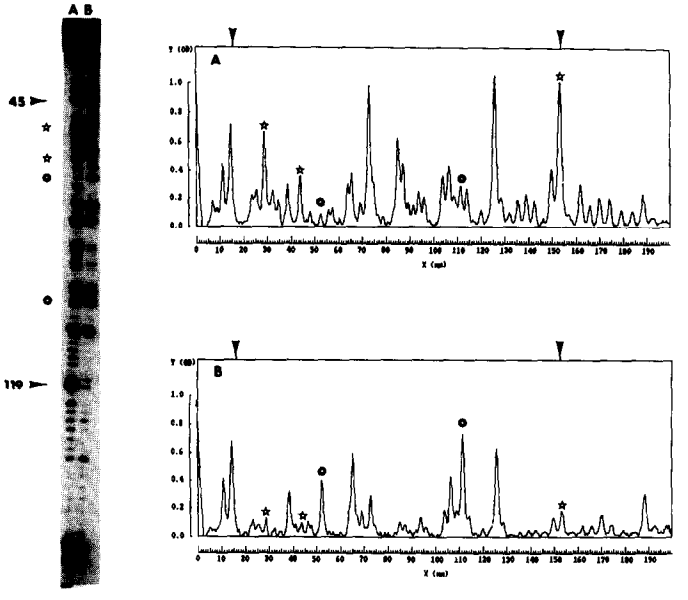

FIGURE 1. Deoxyribonuclease footprinting pattern of daunomycin bound to the tyr t DNA fragment. LEFT: Autoradiogram of a DNase I footprint of the tyr t fragment in the absence of daunomycin (A) or in the presence of 2 μM daunomycin (B). RIGHT: Densitometric scans of the data shown in the left panel. The stars show striking examples of bonds protected from DNase digestion by daunomycin, while the circled stars show bonds that are cleaved more readily in the presence of daunomycin. The footprinting experiment was conducted at 4°C.

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