Stability of DNase I in footprinting experiments

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The endonuclease DNase I is widely used as a footprinting agent for studying drug and protein binding to DNA (1-3). The commercially available enzyme contains the proteolytic contaminant chymotrypsin-B which slowly inactivates the enzyme in aqueous buffer solutions (4,5). Since the stability of the enzyme is important in all footprinting experiments, especially those attempting to derive thermodynamic information from the data (5-8), we investigated the ability of a commercial preparation of the enzyme (Sigma, D-4513) to cleave a 139 base pair Hind III/Nci I restriction fragment as a function of time. The cleavage reactions (37°C) were carried out in a total volume of 8 μ l containing 193 μ M (base pairs) sonicated calf thymus DNA, $\sim$ 1 μ M labeled fragment (32 P) and various concentrations of DNase I in buffer (50 mM Tris Cl, 8 mM MgCl₂, 2 mM CaCl₂, pH, 7.5). The concentration of enzyme chosen was such that at the end of the digest greater than 80% of the labeled fragment remained uncleaved. Enzyme concentration was reduced by 50% and reaction times respectively doubled for eight separate digests. In the absence of degradation, these conditions should result in identical amounts of total cleavage on the 139-mer. Analysis was by quantitative autoradiography/microdensitometry of the cleavage products (9). Although DNase I actively significantly decreases at long reaction times, eg. 128 min., it is constant in the time period 1-64 min., Figure. Thus, for relatively short digest times normally employed in footprinting experiments, enzymatic degradation will not present a problem.

Figure Caption Shown is the summed area (in for all observable products (93% of all possible products) produced by DNase I cleavage of the 139-mer as a function of digestion time. Gel loading errors can yield variations of $\pm$ 10%.

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