Effects of Hydrostatic Pressure on DNA Minor Groove Binding as Studied by Restriction Endonuclease Protection Assay (REPA)

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The effect of pressure on the interaction of minor groove binders with the pBR322 DNA was investigated by using the restriction endonuclease protection assay. The pressure employed alone did not provide measurable enzyme protection whereas it improved the DNA protection ability of drugs. Hydration change terms were proposed to be responsible for the observed pressure effect.

Distamycin A (Dst) binds to at least four successive A/T bps in the B-DNA minor groove without causing great distortion of the DNA structure.[1,2] Two binding modes have been clearly resolved.[2] Close van der Waal's contacts between the groove wall and the crescent and flexible drug molecule, and hydrogen bonds between Dst's amides and N-3 of A and O-2 of T in the minor groove primarily contribute to their binding specificity and affinity. A negative electric field in A.T-rich minor groove is a potential binding inducer for charged Dst.[3] The binding also disrupts the spine of hydration in the minor groove.[4] Structural and biochemical analysis indicate that Hoechst 33258 (Hst) and 4',6-diamidino-2-phenylindole (DAPI) share similar specificity and binding modes as Dst while they prefer three or four successive A.T bps regions.[5,6] DAPI can bind to G.C region by nonclassical intercalation.[7] There have been increasing number of studies designing DNA specific binders based on these and other molecules.[8,9]

Restriction endonuclease protection assay (REPA) has been used to study DNA minor groove binding since the 1970s. This methodology has contributed to the rational design of drug derivatives with great sequence specificities.[10,11] A principle governing REPA is that the simultaneous occlusion of cleavage site by ligands inhibits an attack by restriction endonuclease. REPA also has been used to study the binding to DNA by protein, peptide nucleic acid or oligonucleotide.[11-13]

Taking advantage of its moderate perturbation on a system to obtain molecular insights, many laboratories including ours have used hydrostatic pressure as an alternate variable to explore various biochemical processes.[14-16] Our past work indicated that the pressure sensitivity of some proteolytic enzymes and nucleases existed not only in activity but also in specificity.[16] We observed the reverse effect of pressure on the star activities of restriction endonucleases that were induced by organic solvents or high salt concentrations.[17] Here, we presented recent REPA observations of the pressure effect on DNA minor-groove binding, attempting to further our understanding of this binding nature. The study also showed REPA was very useful for the investigation of DNA-ligand interactions under high pressure since lack of sensitive detection methodology have restricted such studies.

The digestion product of supercoiled pBR322 DNA at GAATTC by the EcoRI enzyme is a full-length linear fragment.[18] Under 120 MPa, Dst showed a dose dependent protection of DNA from EcoRI activity as in controls while this ability was enhanced significantly (Figure 1). Pressure caused decreases of two r' limits at which Dst provided the least and complete protection by a magnitude of 2 and 1 at least within 2 h, respectively. The protection

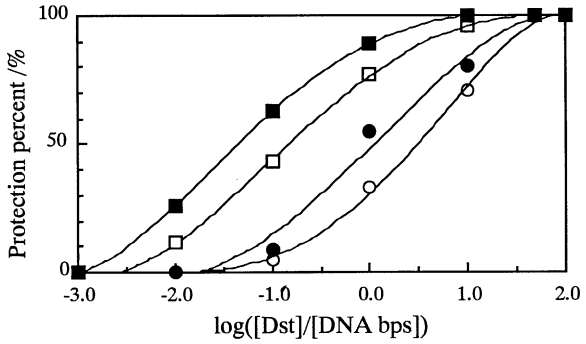

Figure 1. Dst dose-dependent protection curves for EcoRI cleavage of the pBR322 DNA at various pressures.
○, 0.1 MPa; ●, 50 MPa; □, 120 MPa; ■, 200 MPa.
[Dst] = 0 - 2 mM, [DNA bps] = 19 uM, [EcoRI] = 0.2 U/ul.
2 h reaction. Averaged values for seveal tests.

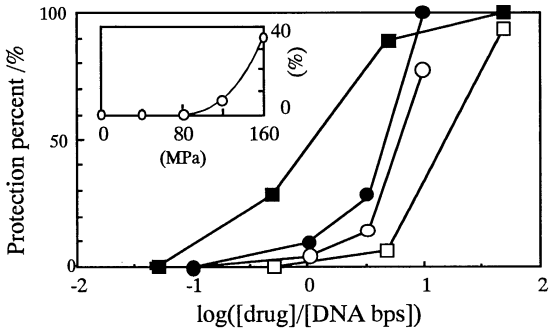

Figure 2. Dose-dependent REPA for DAPI-EcoRI (circles) and ActD-HindIII (squares) system at 0.1 MPa (open) and 120 MPa (filled), respecively.
Inset. Pressure-dependence of EcoRI protection by Hst (r'=1).
[DNA bps]= 19 uM, [enzyme] = 0.2 U/uM. 1 h reaction.

enhancement was pressure-dependent. The drug concentration needed to produce 50% protection (PC_{50}) was notably reduced from about 60 μM at 0.1 MPa to 0.8 μM at 200 MPa. In the absence of Dst, we did not find the measurable EcoRI enzyme protection by pressure alone.[19]

The generality of the pressure effect observed above also has been examined on systems with type II endonucleases of EcoRI, EcoRV, HindIII or BglI and structurally different groove binders of Hst, DAPI and one DNA intercalator ActD (Figure 2, and data not shown). Hst and DAPI provided less efficient nuclease protection for DNA than Dst both in 0.1 MPa and high pressures. No clear correlations were established between their protection ability and apparent DNA-binding affinity.[1, 2, 20,21] Pressure also moderately enhanced the HindIII enzyme protection ability of ActD possibly by favoring its stacking with GpC on the enzyme

cognate site (Figure 2).

Assuming a simplified association between the EcoRI enzyme inhibition and the competitive DNA-binding of Dst to the EcoRI cognate site, as well as the unchanged DNA-binding mechanism under pressure,[22] we have estimated the binding free energy ($\triangle G_{app}$ at 37 ℃) of -12.4 kcal/mol at atmospheric pressure as compared to literature data,[24,25] and an apparent volume change ($\triangle V_{app}$ at 37℃) of about -55 ml /mol upon the binding. The estimated volume change was compared to the data of netropsin and analogue binding to synthetic or natural DNA.[26, 27] The origins of the negative volume change in this ternary system (arbitrarily not including Mg^{2+}) should be responsible for observed pressure effects. The fact that no direct pressure-induced protection from endonuclease digestion occurred within the used pressure ranges allowed us to consider two possible pressure effects definitely on this system: the improvement of the drug-DNA complex stability and/or the pressure sensitivity of enzyme activity regained by drug binding to DNA. However, since the pressure-induced enhancement variations in the endonuclease protection were so greatly dependent on drug identities rather the enzyme, the second possibility appeared not to be a main pressure-sensitivity factor. Additionally, due to the increased stability of DNA in high pressure, volume changes from direct pressure effect on DNA structure transitions could be minimal.[14]

Regarding the probably higher pressure-stability of drug-DNA complexes at specific sequences than that of enzyme-DNA complexes, the factors involved in the minor groove binding should play a role. Among them, van der Waal's contacts and hydrogen bonds[28,29] usually lead near zero and small negative volume changes[16] thus are favored by pressure elevations only slightly. In contrast, the ionic interaction between drugs and DNA minor grooves is strongly unfavored by an increase in pressure.[16] Thus we believe that others, mainly hydration change terms following the drug-DNA binding, probably contributed much to the proposed pressure stability of drug-DNA complexes.

The role of hydrostatic pressure in enhancing molecular solvation is probably applicable to the DNA-drug complex formation. There have been examples, including of activities and specificities of several restriction enzymes, where the pressure sensitivity was related to changes in molecular solvation.[17,30,31] Usually, the hydration-dehydration process provides explanations for DNA-related events such as ligand bindings and conformation transitions.[25,32-34] A spine of hydration in the minor groove has well been characterized for DNA.[33-36] This spine of hydration is removed when the minor groove binding takes place.[4] Such binding-induced hydration changes (magnitude and direction) were strongly dependent upon DNA sequences.[25-27,37] After the binding-caused decompression of water spine, however, a possibly additional uptake of water into the minor groove binder-DNA complexes was inferred to be dependent on DNA.[24,25,38] Taking together them with present observations, we proposed that the minor groove binding increased the general exposure extent of DNA-drug complex towards solvent. The enhanced water exposure gave rise to a combined negative volume change which mainly originated from the electrostriction around charged groups and the decreased imperfect stacking of free water molecules. The present result that pressure elevations favored the minor groove binding to specific areas in the pBR322 DNA in hydration provided another example that new hydration matrix could be formed around the drug-bound DNA lattice with the removal of hydration spine in the minor groove.

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18 125 ng pBR322 DNA (Böhringer) was incubated with corresponding drugs (Sigma or Wako) at various molar ratios of drug to DNA bps (r') for 30 min at room temperature; then digested by enzymes (EcoRI, BglI, or HindIII (Toyobo)) in a reaction buffer (as recommended by the supplier) until enough EDTA was added to end the digestion after 1 or 2 hs. The digestion product was assayed on garose gel, stained with ethidium bromide, photographed, and then analyzed by using NIH Image 1.59 software. Except for the extraneous pressure, other conditions for the high pressure experiment were the same as for 0.1 MPa. High pressure vessel constructed by the Yamamoto Suiatsu Co. (Toyonaka) was used.[17] The time to increase the pressure to the final value was about 2 min, relatively short compared with the reaction time (1 or 2 hs).
19 This finding coincided with a previous study in which the pressure of 200 MPa led to no observable differences in the k_{cat}/K_m of the EcoRI cleavage of several DNA samples including pBR 322 DNA.[17]
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