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Physiological Concentration of Magnesium Ions Induces a Strong Macroscopic Curvature in GGGCCC-containing DNA

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The bending propensity of non-A/T DNA sequence elements is well known, but helical phasing/gel mobility experiments fail to reveal an intensive macroscopic curvature if A/T tracts are not present in the sequence. Recent X-ray data prove on the other hand that a GGCC element is intrinsically curved toward the major groove, which seemingly contradicts the fact that macroscopic curvature at GGGCCC elements is hardly detectable with a conventional gel mobility assay. Here we show that GGGCCC containing DNA, with no A/T tracts in the sequence context, has a detectable, strong gel mobility anomaly only in the presence of divalent ions (10 mM Mg^{2+} or Ca^{2+}, 1 mM Zn^{2+}). Metal ions increase the gel mobility anomaly in A/T tracts as well, but the effect is substantially stronger for GGGCCC than for the rigid A/T tracts. Our data suggest that metal ions change the sequence-dependent dynamic features of DNA; on the other hand, there is no evidence of twist-mediated change of the planarity of curvature in the presence of metal ions. The results show that near-physiological concentrations of divalent cations (10 mM $MgCl_2$) have a strong and differential effect on various sequence elements, so that the current picture of sequence-dependent DNA curvature is changed not only in a quantitative, but also in a qualitative sense.

Keywords: DNA curvature; diavalent metals; GGGCCC sequence elements; gel mobility anomaly; DNA structure

Curvature of different DNA sequence elements is usually determined by gel mobility analyses or by X-ray crystallography of DNA. Gel mobility data show that the macroscopic curvature is connected with the presence of helically phased A_nT_m tracts ($n+m \geq 3$) (Koo *et al.*, 1986) and that this curvature is increased by divalent metal ions (Diekmann, 1986; Laundon & Griffith, 1987, Shlyakhtenko *et al.*, 1990). Even though curvature of non A/T containing sequence elements has been reported (Bolshoy *et al.*, 1991; Brukner *et al.*, 1991, 1993; McNamara & Harrington, 1991), helical phasing/gel mobility experiments failed to reveal an intensive macroscopic curvature if A/T-tracts were not present in the sequence context. An example is GGGCCC-containing DNA, known to show no or very little curvature in gel mobility experiments (Koo *et al.*, 1986, Shlyakhtenko *et al.*, 1990; Brukner *et al.*, 1991). On the other hand, nuclease digestion experiments suggest that GGCC elements may be curved (Satchwell *et al.*, 1986; Calladine & Drew, 1986; Brukner *et al.*, 1993). X-ray crystallography first showed that A_nT_m tracts are not curved, suggesting that the site of bending must lie outside these tracts (Nelson *et al.*, 1987; DiGabriele *et al.*, 1989; DiGabriele & Steitz, 1993). In addition, recent X-ray data have directly shown that a GGCC element is curved toward the major groove (Goodsell *et al.*, 1993), which seemingly contradicts the fact that macroscopic curvature at the GGGCCC elements is hardly detectable with conventional gel mobility assay. Since DNA crystals are always prepared in the presence of divalent cations, and gel mobility experiments are usually carried out in the absence of divalent metals, we decided to determine the effect of divalent metal ions on the gel mobility of GGGCCC-containing repeating sequence DNA, and to compare this effect to that found with A_nT_m tracts.

Gel mobility assays were performed with concatamers of differentially phased GGGCCC

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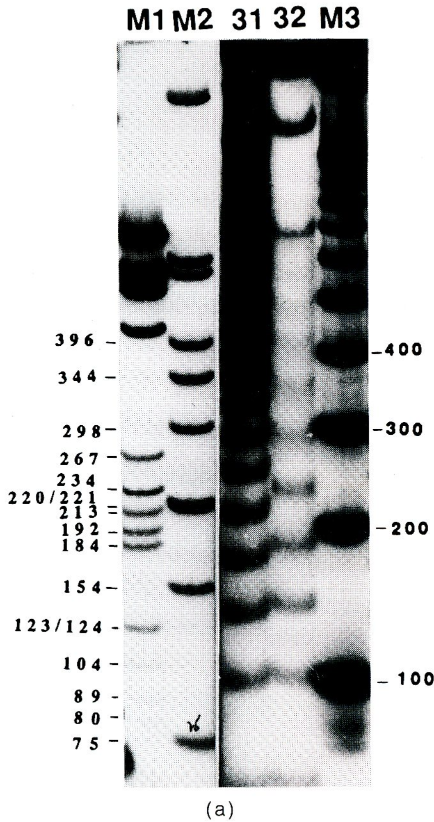


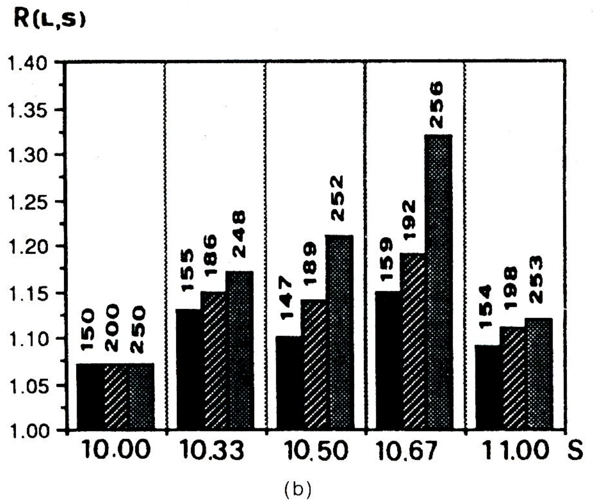


Figure 1. Determination of the average helical repeat of a curved DNA from ligation ladder experiments in the absence of divalent metal ions. (a) The gel mobility data of 31- and 32-mers on an 8% nondenaturing polyacrylamide gel. Lane M1, pBR 322 cut by *Hae*III; lane M2, pBR 322 cut by *Hin*fl; lane M3, 100 bp ladder (Pharmacia); the 31- and 32-mers are marked on the top of the gel line. We used gel mobility anomaly values for 10-, 11- and 21-mers, obtained under same conditions, which were previously published by us (Brukner *et al.*, 1991). (b) The retardation coefficient of the multimers, R, was plotted against sequence repeat (s = 10·00, 10·33, 10·50, 10·67 and 11·00 bp) for the length values closest to $L \approx 150$, 200 and 250 bp. The real lengths of the corresponding sequence repeats is written on the top of each bar. The experimental error for determination of R is $\pm 0{\cdot}03$. Note that the maximum of R (s) corresponds to s = 10·67 base-pairs.

elements, since this analysis provides information both on the magnitude and on the planarity of the macroscopic curvature. The oligos used to obtain the concatamer mixtures (ligation ladders) are shown in Table 1. Gel mobility analysis of the ligation ladders showed that the maximum macroscopic curvature is obtained if the spacing of the GGGCCC elements corresponds to 10·67 base-pairs (Fig. 1), so we decided to use this ligation ladder for monitoring the metal effect. The metals were added to the electrophoretic buffers in the following concentrations; 10 mM $MgCl_2$, 10 mM $CaCl_2$ and 1 mM $ZnCl_2$. In the case of $ZnCl_2$, 0·9% NaCl was also added in order to avoid precipitation. The gel mobility experiments were carried out as described by Diekmann (1986).

Mobility anomaly of GGGCCC-containing DNA is markedly increased by metal ions

Figure 2 shows that metal ions, in fact, markedly increase the gel mobility anomaly of GGGCCC-containing curved DNA. The relative increase in the coefficient of retardation, R (Koo *et al.*, 1986), is $R(\mathrm{Ca}) > R(\mathrm{Mg}) > R(\mathrm{Zn})$. There are few recent

Table 1

A. *Curved DNA without AA/TT*	Base pairs per GGGCCC repeat
10-mer AGGGCCCTAG	10·00
31-mer AGGGCCCTAGAGGGCCCTAGAGGGGCCCTAG	10·33
21-mer AGGGCCCCTAGAGGGCCCTAG	10·50
32-mer AGGGCCCTAGAGGGGCCCTAGAGGGGCCCTAG	10·67
11-mer AGGGCCCCTAG	11·00
B. *Curved DNA with AA/TT*	
42-mer AAAAACTCTCTAAAAACTCTCTAGAGGGGCCCTAGAGGGCCC	10·50

Sequences of the oligonucleotides used in ligation ladder experiments (5′-3′ direction). Complementary strands have the same length, but are shifted by 4 bp, so that the duplex could have a 5′ protruding end for the ligation reaction. Preparation of the oligonucleotides, radioactive labelling and the ligation reactions were done as described (Diekmann, 1986; Koo *et al.*, 1986; McNamara & Harrington, 1991).

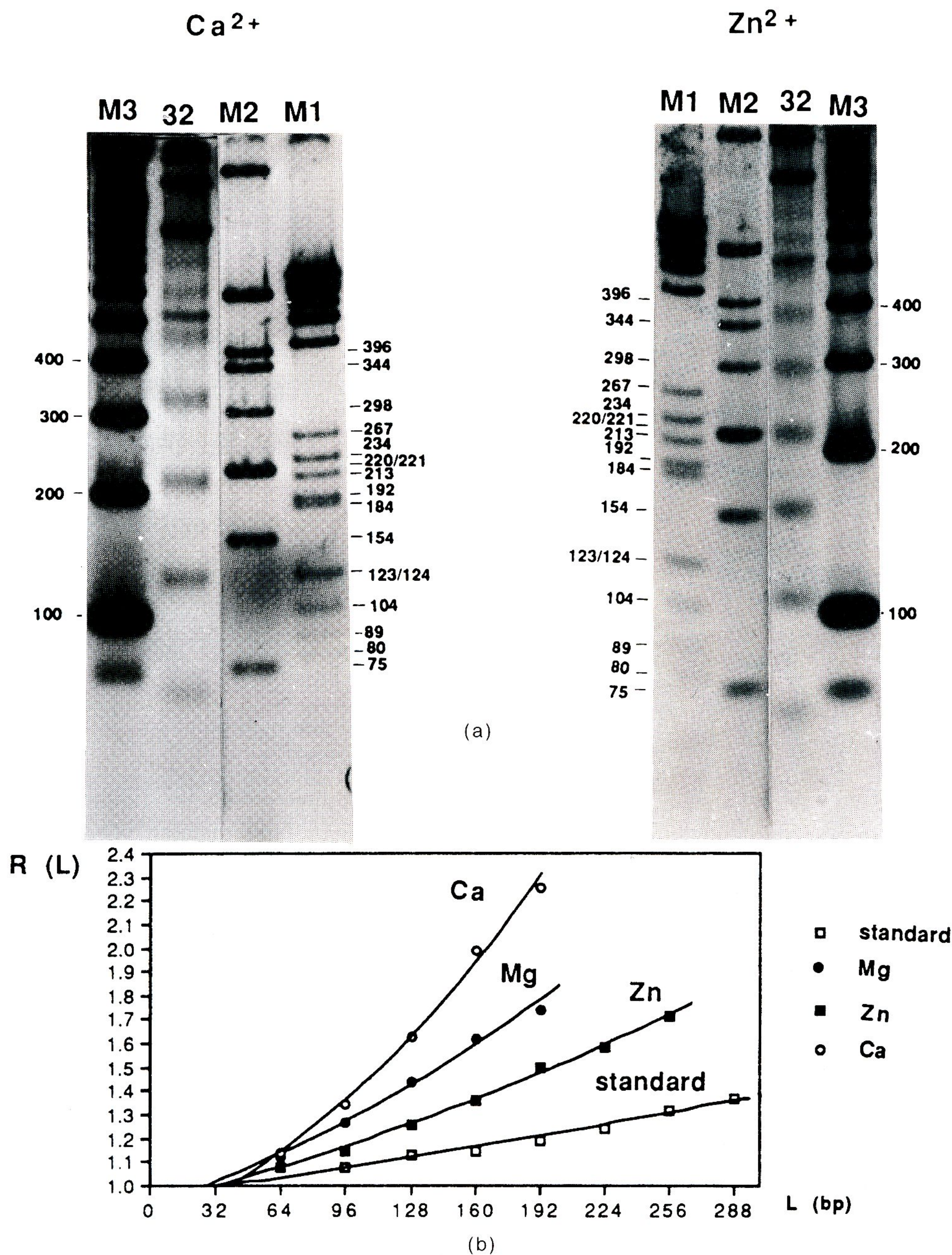
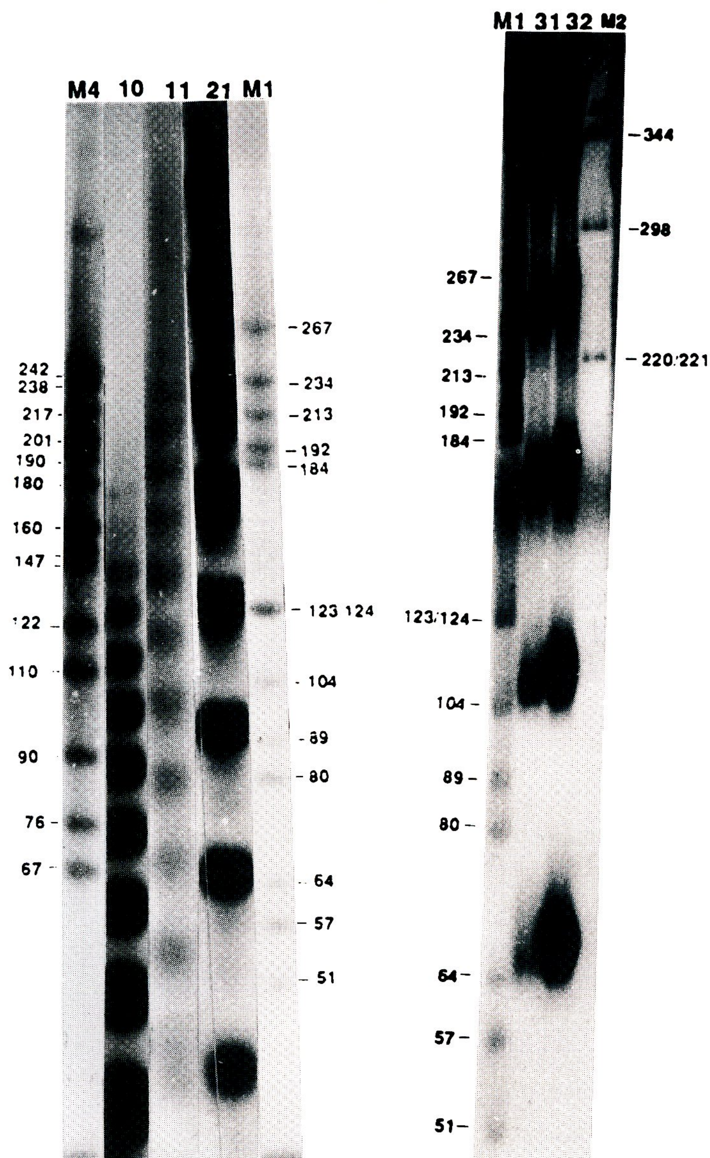


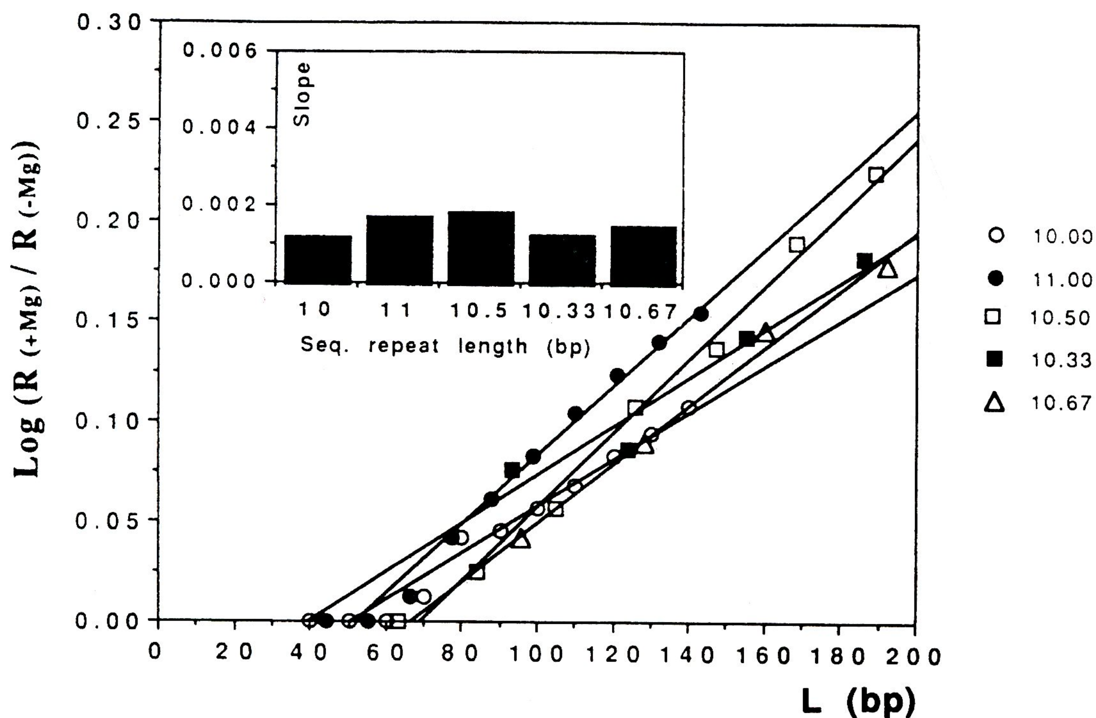
Figure 2. The effect of ions on gel mobility anomaly of curved GGGCCC, but not AA/TT containing 32-mer. (a) Ionic conditions are illustrated at the top of each photograph. The ligated 32-mer is depicted at the top of each gel lane, together with molecular weight markers are: M1, pBR 322 cut by *Hae*III; M2, pBR 322 cut by *Hin*fI; M3, 100 bp ladder (Pharmacia). The effect of Mg^{2+} ions is shown in Fig. 3(a). (b) The coefficient of retardation (R) is plotted as a function of length of the ligated multimers (L). The type of ions (Zn, Ca and Mg) is indicated by open or filled symbols. The data of usual electrophoretic conditions for gel mobility assay of curved DNA are noted as standard (Koo *et al.*, 1986, Brukner *et al.*, 1991, 1993; McNamara & Harrington, 1991).The experimental error for determination of R is $\pm 0{\cdot}03$.

reports which are compatible with our findings: (1) NMR results of Braunlin *et al.* (1992), who found that Ca^{2+} may preferentially bind to G+C rich regions in synthetic and natural DNA molecules; (2) a modest gel mobility anomaly of $(G_5C_5)_n$ DNA induced by $MgCl_2$ (Shlyakhtenko *et al.*, 1990); and (3) Zn-induced bending of 5 S RNA gene (Nickol & Rau, 1992) which is characterized by the absence of the typical A_nT_m motifs. We mention that the concentration of Mg^{2+} corresponds to the intracellular concentration of this ion (Saenger, 1988).

In order to determine if the planarity of curvature is changed by metals influencing the average twist angle, we measured the gel mobility anomaly



(a)



(b)

Fig. 3.

of all ligation ladders in the presence of 10 mM $MgCl_2$. The R_L values varied with the sequence repeat length (data not shown), but the ratio between mobility anomalies in the presence and the absence of Mg^{2+} was similar for all the differentially phased GGGCCC elements (Fig. 3(b)). Our data thus suggest that the effect of metal ions is not related to a change in twist angle that would modify the planarity of the curvature.

The metal effect is different on GGGCCC and A_nT_m elements

Figure 4 shows a comparison of the gel mobility anomaly data of a typical A/T tract-containing DNA, with those obtained on the GGGCCC-containing DNA. It can be seen that addition of $MgCl_2$ has a differential effect. Macroscopic curvature of GGGCCC-containing DNA is more strongly increased than that of the A tract-containing DNA and, as a result, the overall macroscopic curvature of both molecules in the presence of 10 mM $MgCl_2$ becomes quite similar.

Summarizing we can conclude that curvature data obtained in the presence of divalent metals are not only quantitatively, but also qualitatively different from those obtained in the absence of metals. Our data suggest that the discrepancy between X-ray crystallographic and nuclease digestion experiments on the one hand, and gel mobility data on the other, may originate from the fact that divalent metals are not usually used in conventional gel electrophoretic experiments. It seems that divalent ions change not only static, but also sequence-dependent dynamic features of DNA (Hagerman, 1988). The facts that the metal effect is not dependent on the planarity of the molecule (Fig. 3), and that the metal-induced increase in the case of the rigid A_nT_m tracts is relatively smaller (Fig. 4), support this view.

Since the gel mobility anomaly assay is the principal method for the quantification of DNA curvature (Koo & Crothers, 1988; Calladine *et al.*, 1988; De Santis *et al.*, 1990; Bolshoy *et al.*, 1991), the metal effect may also have consequences on how the constants of the predictive models of curvature are derived. Estimations of the wedge-tilt and wedge-roll values of sequence elements are usually determined from gel mobility experiments which are carried out without metal cations in the electrophoretic buffers. Our results show that near-physiological concentrations of Mg^{2+} have a profound and differential effect on various sequence elements, so the metal effect may have to be considered if predictive models are meant to reflect the *in vivo* behaviour of DNA.

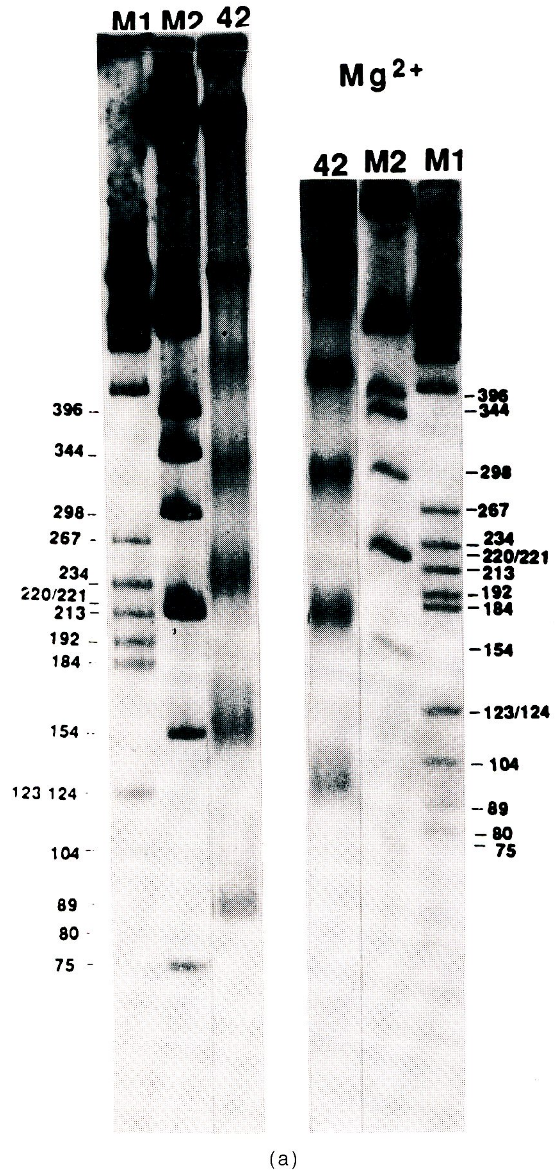


Figure 4. The differential effect of Mg^{2+} on the gel mobility anomaly of A/T and GGGCCC-containing curved DNA. (a) Gel mobility data of A/T-tract containing 42-mer. Ionic conditions are illustrated at the top of the photograph. The molecular weight markers are: M1, pBR 322 cut by *Hae*III; M2, pBR 322 cut by *Hinf*I. The mobility data of GGGCCC-containing 21-mer in the presence or in the absence of Mg^{2+} are presented in Fig. 3(a) and in our previous report (Brukner *et al.*, 1991).

Figure 3. The relative influence of 10mM $MgCl_2$ on curved DNA containing GGGCCC but not AA/TT, with different sequence repeat lengths (s = 10·00, 10·33, 10·50, 10·67 and 11.00 bp per turn). (a) The gel mobility data of 10-, 11-, 21-, 31- and 32-mers are labelled at the top of each gel line. The markers are: M1, pBR 322 cut by *Hae*III; M2, pBR 322 cut by *Hinf*I; M4, pBR cut by *Hpa*II. (b) The effect is presented as a logarithmic value of a ratio $R(+\text{Mg})/R(-\text{Mg})$ between retardation coefficients *versus* length L. $R(-\text{Mg})$ data from Fig. 1(a) and from Brukner *et al.* (1991) were used. The error in measuring R values is $\pm 0{\cdot}03$. Note that Mg^{2+} increases mobility anomaly with a similar slope for all sequence repeat lengths (insert).

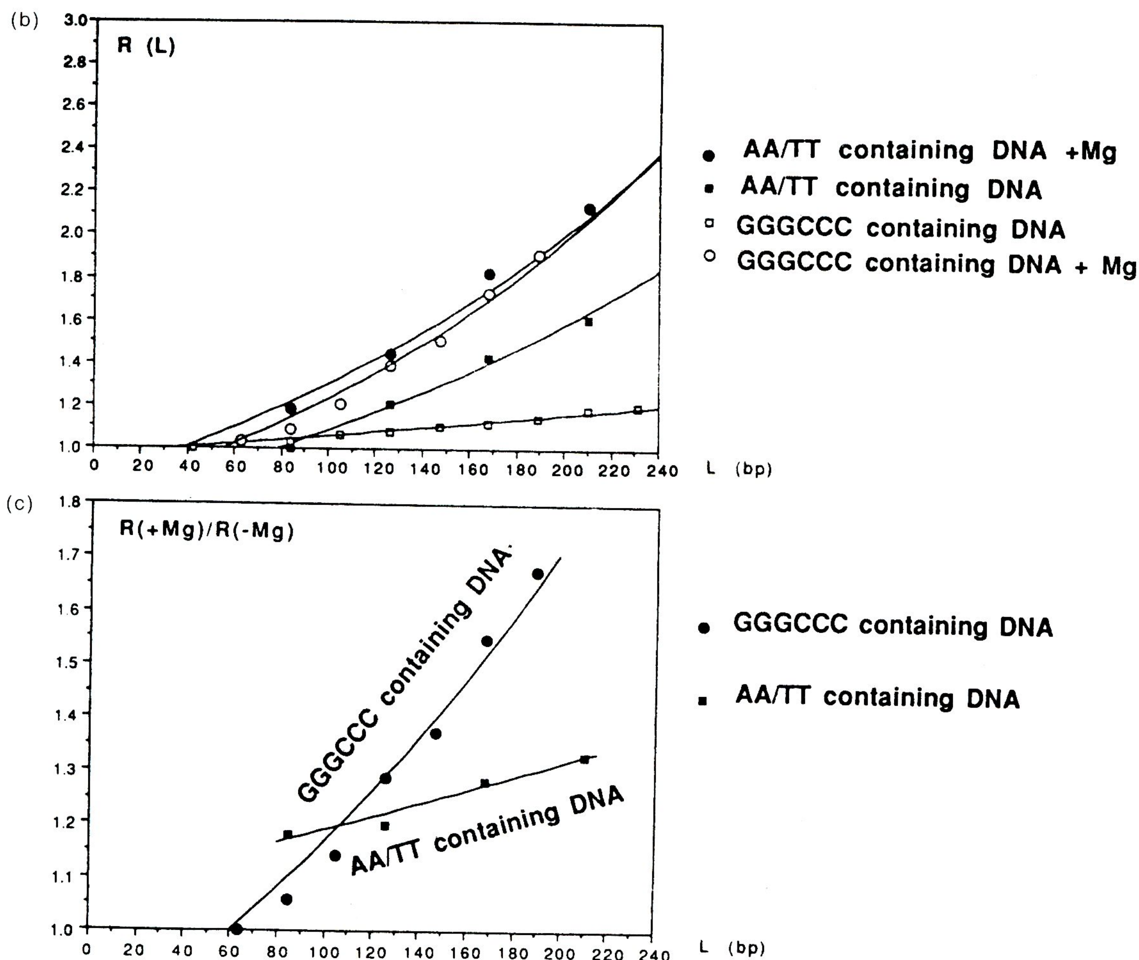


Figure 4. (b) The retardation coefficients (R) are plotted *versus* length of ligated 21- and 42-mers. The experimental error for determination of R is $\pm 0{\cdot}03$. (c) The ratio between retardation coefficients obtained in the presence and the absence of Mg^{2+} *versus* L. Note that the effect of 10mM $MgCl_2$ on GGGCCC-containing curved DNA is much stronger than on the curved DNA containing A_nT_m tracts.

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