Vol. 116, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 14, 1983 Pages 217-221

Mg^{2+}-, Ca^{2+}-DEPENDENT UNWINDING OF DNA BY POLY-L-GLUTAMIC ACID

Michiteru Yoshida

Laboratory of Biochemistry
Department of Agricultural Chemistry,
Tohoku University, Sendai 980, Japan

Received September 1, 1983

In order to examine a possibility that the high acidic amino acid region in the nonhistone protein HMG(1+2) is concerned with the Mg^{2+}-, or Ca^{2+}-dependent unwinding of DNA by the HMG(1+2) (1,2), poly-L-glutamic acid was employed as an acidic model peptide for thermal melting temperature analysis. The poly-L-glutamic acid bound to DNA either in the presence or absence of Mg^{2+}. The poly-L-glutamic acid unwound DNA double-helix to a similar extent to HMG(1+2) in the presence of Mg^{2+} or Ca^{2+}, but not in the absence of them. These results may suggest that the high acidic amino acid region in HMG(1+2) participates in Mg^{2+}-, Ca^{2+}- dependent unwinding of DNA double-helix.

High Mobility Group (HMG) proteins are a small group of nonhistone proteins (3) that are under intensive investigations in many laboratories. Large HMG proteins, HMG1 and HMG2, are a small set of the HMG protein of homologous nature. They exhibit a preferential affinity for single-stranded DNA (2,4,5). The binding of HMG1 and HMG2 to circular double-stranded DNA causes a change in the linking number of the double-helix (6). Recently, we have shown that HMG(1+2), a preparation containing HMG1 and HMG2, from pig thymus, unwinds partially DNA double-helix at low weight protein to DNA ratios (1,2). The unwinding reaction depended on the presence of Mg^{2+} or Ca^{2+}. The primary structures of both HMG1 and HMG2 have been determined (7). An interesting feature in the structures of HMG1 and HMG2 is the fact that both proteins contain a continuous sequence of 30-40 aspartic and glutamic acid residues in the C-terminus of the molecule. However, the function of the high acidic amino acid region is yet to be determined. In the present work, poly-L-glutamic acid (PGA) was employed as model peptide of the high acidic amino acid region in order to examine whether the acidic region participates in the

Abbreviations: HMG, high mobility group; PGA, poly-L-glutamic acid; T_m, melting temperature.

0006-291X/83 $1.50

unwinding reaction. The thermal melting temperature analysis showed that the PGA bound to DNA unwinds the DNA double-helix in the presence of Mg^{2+} or Ca^{2+}.

METHODS

DNA from pig thymus was prepared as described previously (1,2). The DNA preparation was treated in advance with EDTA. The DNA solution was dialyzed three times against 100 volumes of 10 mM Tris-HCl, pH 7.8, containing 1 mM EDTA·4H, and then three times against 100 volumes of 10 mM Tris-HCl, pH 7.8. The DNA concentration was determined spectrophotometrically by the absorbance at 260 nm. Poly-L-glutamic acid (m.w. 8,800, D.P.=68) was obtained from Miles Laboratories. All the solutions were prepared with doubly distilled water by a glass distiller.

The complex formation of DNA with PGA was monitored by gel filtration. A mixture (1 ml) containing DNA (300 µg) and PGA (30 µg) in 2 mM Tris-HCl, pH 7.8, or 2 mM Tris-HCl, pH 7.8, containing 0.005 mM $MgCl_2$ was applied to a Sephadex G-200 column (0.9 × 15 cm) equilibrated with the same solutions, respectively. An aliquot of the eluate was used for the determination of PGA by the method of Lowry et al. (8).

The thermal melting transition of DNA was monitored by a Beckman Acta CIII spectrophotometer. During the melting experiments, temperature was increased at a constant rate of 0.7 °C per min using a Beckman automatic TM programmer, and the actual temperature of the sample solution in a 4 ml cell measured directly by a glass-seathed R.T. probe was recorded on an XY recorder with the simultaneous changes of absorbance at 260 nm. The sample solution was prepared by mixing directly DNA and protein solutions at desired weight ratios at room temperature. PGA was added lastly in the mixing of all solutions.

RESULTS AND DISCUSSION

As previously shown (2), a large amount of HMG(1+2) binds to DNA either in the presence or absence of Mg^{2+}. In order to determine whether PGA can bind DNA, a mixture of DNA and PGA at a weight ratio of 0.1 was subjected to gel filtration on Sephadex G-200. As shown in Fig. 1, only a part of PGA applied was eluted with DNA at the void volume of the column in the presence of $MgCl_2$

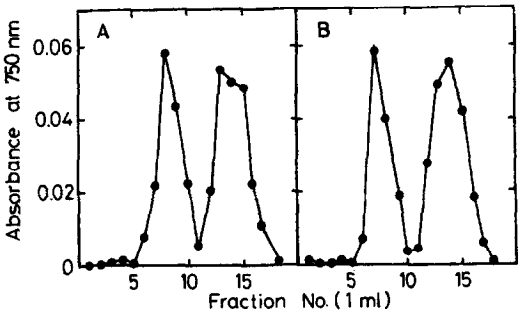
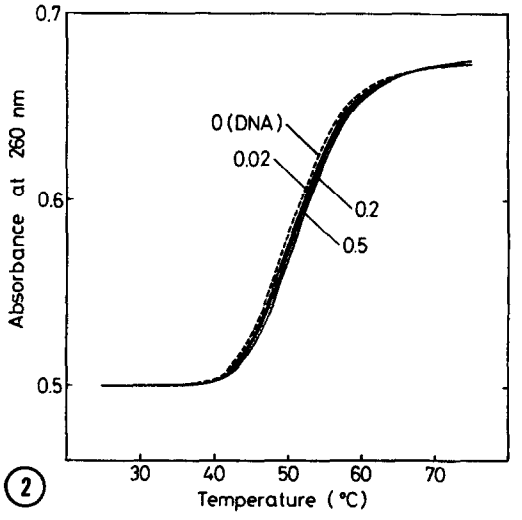


Fig. 1. The elution profiles of PGA from Sephadex G-200 gel column chromatography. PGA (30 µg) mixed with DNA (300 µg) in (A) 2 mM Tris-HCl, pH 7.8, or (B) 2 mM Tris-HCl, pH 7.8, containing 0.005 mM $MgCl_2$ was applied to the column equilibrated with the same solution, respectively.

as well as in the absence of it, indicating that a limited amount of PGA binds to DNA irrespective of the presence of Mg^{2+}.

Figure 2 shows the thermal denaturation profiles of DNA in the presence of PGA in 2 mM Tris-HCl, pH 7.8. There were no decreases of the thermal melting temperature (Tm) by PGA under the condition. There was a slight, if any, increase of Tm even at low PGA/DNA ratio. On the other hand, the thermal melting profiles of DNA changed markedly in the presence of 0.005 mM $MgCl_2$. As presented in Fig. 3. the Tm of control DNA was higher by 13 ^{o}C than that in the absence of Mg^{2+}. The Tm's of DNA in the presence of PGA decreased by increasing the weight ratio of it to DNA. At a ratio of 0.5 which gave almost maximum transition of Tm, the Tm decreased by 4 ^{o}C. A similar decrease of Tm was observed in the presence of 0.005 mM $CaCl_2$ as shown in Fig 4, but not in the presence of 0.005 mM $CuCl_2$ or 0.005 mM $MnCl_2$ (data not shown). The dependency of the decrease of Tm by PGA on the divalent cations such as Mg^{2+} or Ca^{2+} is consistent with the results by HMG(1+2) at low weight protein to DNA ratios (2). One probable explanation of the decrease of Tm by PGA is that



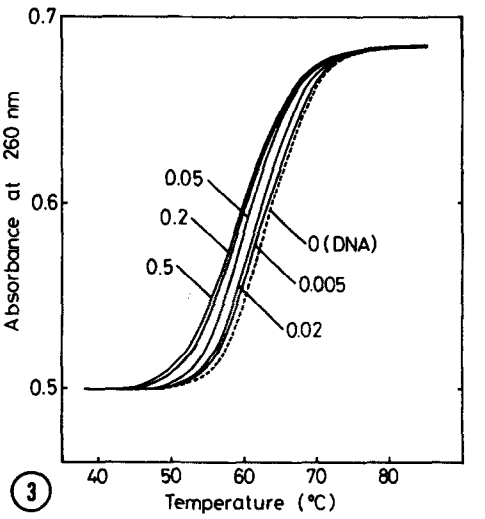


Fig. 2. Integral thermal denaturation profiles of DNA in the presence of PGA in 2 mM Tris-HCl, pH 7.8. The weight ratios of added PGA to DNA are given in the figure.

Fig. 3. Integral thermal denaturation profiles of DNA in the presence of PGA in 2 mM Tris-HCl, pH 7.8, containing 0.005 mM $MgCl_2$. The weight ratios of added PGA to DNA are given in the figure.

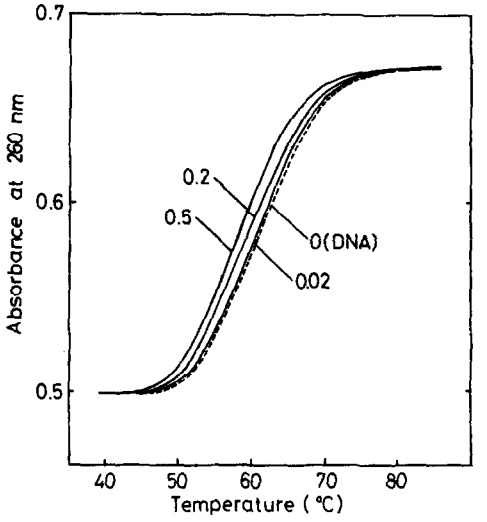


Fig. 4. Integral thermal denaturation profiles of DNA in the presence of PGA in 2 mM Tris-HCl, pH 7.8, containing 0.005 mM $CaCl_2$. The weight ratios of added PGA to DNA are given in the figure.

the PGA deprived Mg^{2+} or Ca^{2+} from DNA, thus decreasing the Tm. This possibility, however, may not be likely; if we assume the PGA deprived Mg^{2+} or Ca^{2+} from DNA, the Tm might decrease to that of control DNA without presence of Mg^{2+} or Ca^{2+} according to their concentrations under the first order kinetics. This was not the case. In addition to this, the decrease of Tm was not observed with poly[d(A-T)·d(A-T)] used instead of DNA under the same condition (data not shown). These results confirmed that the decrease of Tm by PGA in the presence of Mg^{2+} or Ca^{2+} is due to the unwinding of DNA double-helix by PGA.

If we assume that 1 % non-matching of DNA strands lowers the Tm by 1 °C (9), about 4 % region in the whole DNA strands might be unwound by PGA. This extent of unwinding is similar to that by HMG(1+2) (1,2). In addition to this, the result of a limited binding of PGA to DNA (Fig. 1) may support the previous induction that HMG(1+2) binds and unwinds some restricted regions in the whole DNA. The complete amino acid sequence and tertiary structure of the high acidic amino acid region in HMG(1+2) have not been determined, and it is ambiguous that the results obtained using a model peptide, PGA, reflect the real functional effect of the HMG protein. Still, it is suggestive that the

high acidic amino acid region participates in the unwinding reaction of DNA double-helix by the HMG protein.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. H. Nagayama, Tohoku University, for his critical reading of the manuscript. This research was supported in part by a grant from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. Yoshida, M. and Shimura, K. (1983) J. Biochem. Submitted
2. Yoshida, M., Makiguchi, K., Chida, Y. and Shimura, K. (1983) J. Biochem. Submitted
3. Goodwin, G.H., Sanders, C. and Johns, E.W. (1973) Eur. J. Biochem. 38, 14-19
4. Bidney, D.L. and Reeck, G.R. (1978) Biochem. Biophys. Res. Commun. 85, 1211-1218
5. Isackson, P.J., Fishback, J.L., Bidney, D.L. and Reeck, G.R. (1979) J. Biol. Chem. 254, 5569-5572
6. Javaherian, K., Liu, L.F. and Wang, J.C. (1978) Science 199, 1345-1346
7. Walker, J.M., Gooderham, K., Hastings, J.R.B., Mayes, E. and Johns, E.W. (1980) FEBS Lett. 122, 264-270
8. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 264-275
9. Britten, R.J., Graham, D.E. and Neufeld, B.R. (1974) in Methods in Enzymology (Grossman, L. and Moldave, K. eds.) Vol. 29, pp.363-418, Academic Press, New York.