ANTISERUM TO Z-DNA

Bernard MALFOY and Marc LENG
Centre de Biophysique Moléculaire, CNRS, 1A, avenue de la Recherche Scientifique, 45045 Orléans cedex, France

Received 28 May 1981

1. Introduction

Nucleic acids duplexes have been thought to be conformationally related to A-DNA or B-DNA. However, X-rays studies on crystals of alternating d(CpG)-DNA fragments and on fibers of poly(dG–dC) · poly(dG–dC) have demonstrated a left-handed double helix termed Z-DNA [1–4]. In solution, poly-(dG–dC) · poly(dG–dC) can adopt the B- or Z-form depending upon the ionic strength of the medium [5–9]. Moreover, it seems possible to join sequences in B- and Z-form, respectively [10,11].

Physicochemical techniques for looking at the Z-form need rather large quantities of material and are hardly useful to in situ studies. On the other hand, immunochemical techniques are very sensitive and there are already many reports on the use of specific antibodies to detect small amounts of nucleosides, modified nucleosides or hybrids in nucleic acids (see [12–18] and references herein).

In this paper, we report that one can get rabbit antisera which reacts with poly(dG–dC) · poly(dG–dC) in Z-form and does not react with poly(dG-dC) · poly(dG–dC) in B-form. The rabbits were immunized with a poly(dG–dC) · poly(dG–dC) modified by chlorodiethylenetriamino platinum(II) chloride (dien-Pt). This polynucleotide, in which 12% of the bases are complexed to dien-Pt (on the N(7) of guanine residues), adopts the Z-form in physiological conditions [19] while the Z-form of unmodified poly(dG–dC) · poly(dG–dC) is stable at much higher salt concentration [5].

2. Materials and methods

Poly(dG–dC) · poly(dG–dC) from P. L. Biochemicals was purified as in [20,21]. The modifications of poly(dG–dC) · poly(dG–dC) by dien-Pt and by *N*-acetoxy-*N*-acetyl-2-aminofluorene were as in [19–21]. We write poly(dG–dC)dien-Pt(r_b) and poly-(dG–dC)AAF(r_b) for poly(dG–dC) · poly(dG–dC) modified by dien-Pt and *N*-acetoxy-*N*-acetyl-2-amino-fluorene, respectively. r_b is the number of dien-Pt or AAF residues bound/nucleotide.

Poly(d[8-^{3}H]G–dC) · poly(d[8-^{3}H]G–dC) (we write poly*(dG–dC) · poly*(dG–dC)) was synthesized with *Escherichia coli* DNA polymerase large fragments (Boerhinger, Mannheim) in a manner similar to that used in [22]. d[8-^{3}H]GTP was from New England Nuclear, dGTP and dCTP from Boerhinger, Mannheim.

Immunization and radioimmunoassays were performed as in [20,23]. Poly(dG-dC)dien-Pt(0.12) was injected to rabbits in 0.1 M NaCl, 1 mM $MgCl_2$, 5 mM Tris (pH 7.3) as a complex with methylated serum albumin. The rabbits were bled 1 week after the intravenous booster (~2.5 months after the first injection).

To achieve conformational equilibrium, all the solutions were heated at 50°C for 10 min and then cooled at room temperature before use. Circular dichroism spectra were recorded with a Roussel Jouan III dichrograph.

3. Results

Two antisera were studied; they behaved similarly. The results relative to one antiserum are reported here.

To study the reactivity of the antiserum, we used poly(dG–dC) · poly(dG–dC), poly(dG–dC)AAF and poly(dG–dC)dien-Pt. As judged by circular dichroism (fig.1), poly(dG–dC) · poly(dG–dC) in 2.5 M $NaClO_4$, poly(dG–dC)AAF(0.13) and poly(dG–dC)-dien-Pt(0.12) in 0.1 M NaCl, 1 mM $MgCl_2$ belong to

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.50

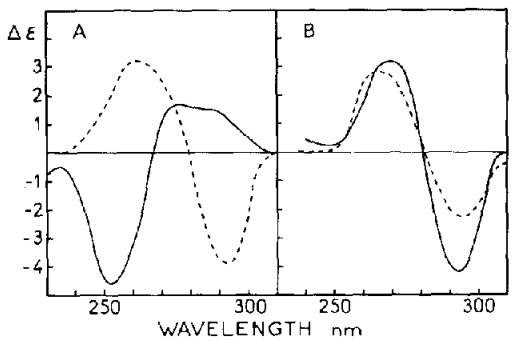

Fig.1. Circular dichroism spectra: (A) (——) Poly(dG–dC) · poly(dG dC) in 0.1 M NaCl, 1 mM $MgCl_2$, 5 mM Tris HCl, (pH 7.3) (– – –) in 2.5 M $NaClO_4$, 5 mM Tris–HCl (pH 7.3). (B) (——) Poly(dG- dC)dien-Pt(0.12); (– – –) poly(dG–dC)-AAF(0.13) in 0.1 M NaCl, 1 mM $MgCl_2$, 5 mM Tris–HCl (pH 7.3).

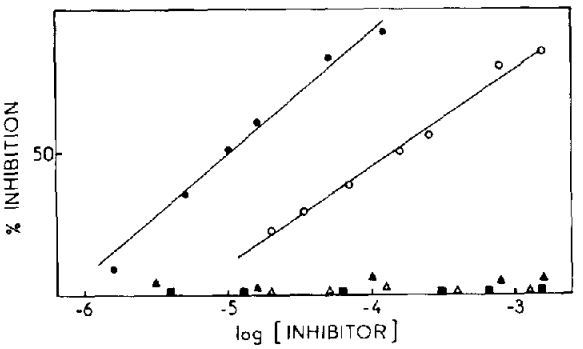

Fig.3. Radioimmunoassays: Percent of inhibition of tracer precipitation as a function of the logarithm of inhibitor concentration. Tracer poly*(dG–dC)dien-Pt(0.12), 5.7×10^{-6} M, antiserum dilution (1/100). Medium 0.1 M NaCl, 1 mM $MgCl_2$, 5 mM Tris–HCl (pH 7.3) inhibitors: (•) poly(dG–dC)dien-Pt(0.12); (○) poly(dG–dC)AAF(0.13); (■) poly(dG–dC) · poly(dG–dC); (△) native calf thymus DNA modified by dien-Pt $r_b = 0.05$; (▲) denatured calf thymus DNA modified by dien-Pt $r_b = 0.05$. The concentrations of inhibitors are expressed in mol/nucleotides.

the Z-family while poly(dG–dC) · poly(dG–dC) in 0.1 M NaCl, 1 mM $MgCl_2$ is in B-form. As noted in [19–21], dien-Pt and AAF residues stabilize the Z-form.

We first studied the precipitation of poly*-(dG–dC) · poly*(dG–dC) by the antiserum as a function of salt concentration. Poly*(dG–dC) · poly*-(dG–dC) and the antiserum in the right salt conditions were mixed and kept overnight at 4°C. After centrifugation, the amount of radioactivity in the supernatant was determined. In fig.2, we plotted the percentage of precipitation of poly*(dG–dC) · poly*(dG–dC) as a function of the salt concentration of the medium. Over 0.1–1.2 M NaCl, there is no precipitation of the polynucleotide. Above 1.2 M NaCl, the amount of precipitate increases suddenly as the salt concentration is slightly increased. The midpoint of the transition is at ~1.4 M. The variation of the circular dichroism at 290 nm ($\Delta\epsilon_{290}$) of poly(dG-DC) · poly(dG–dC) as a function of salt concentration is also presented for comparison (fig.2). The midpoint of the transition is at 1.8 M.

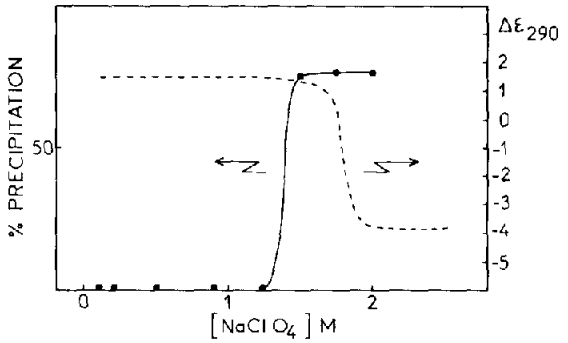

Fig.2. Conformation of poly(dG–dC) · poly(dG–dC) as a function of salt concentration. (–•–) Precipitation of poly*-(dG- dC) · poly*(dG–dC) by antiserum. Antiserum dilution (1/4); poly*(dG–dC) · poly*(dG–dC) 7.7×10^{-6} M. (– – –) Variation of circular dichroism at 290 nm ($\Delta\epsilon$) of poly-(dG -dC) · poly(dG–dC).

The specificity of the antiserum was then studied by radioimmunoassays (RIA) in 0.1 M NaCl, 1 mM $MgCl_2$, 5 mM Tris (pH 7.3). The tracer was poly*-(dG–dC)dien-Pt(0.12). The main results are shown in fig.3. Poly(dG–dC)AAF(0.13) competes with the tracer. It is less efficient than poly(dG–dC)dien-Pt-(0.12) but is inhibits up to 100%. Poly(dG–dC) · poly(dG–dC) does not inhibit, neither native or denatured DNA modified by dien-Pt. Deoxyguanosine, deoxycytidine and poly(G) · poly(C) are not competitors (not shown).

To gain some knowledge of the immunodeterminant group, we have determined the amount of poly*(dG–dC)dien-Pt(0.12) precipitated by a given amount of antiserum as a function of the salt concentration. The conditions were those used in the RIA. The amount of added antiserum was such that in 0.1 M NaCl, 1 mM $MgCl_2$, ~50% of the polynucleotide were precipitated. Over 0.1–1 M NaCl, the amount of precipitated polynucleotide was the same (not shown).

4. Discussion

In rabbit, B-DNA is not immunogenic. On the other hand, it has been well-demonstrated that specific antibodies can be elicited in rabbits immunized with double-stranded RNA, double-stranded polyribonucleotides and double-stranded hybrids (reviews [24–26]). Here we show that poly(dG–dC) · poly(dG–dC) in the Z-form is immunogenic.

We used for immunization poly(dG–dC) · poly(dG–dC) in which 12% of the bases were complexed to dien-Pt. In 0.1 M NaCl, 1 mM $MgCl_2$ poly(dG–dC)-dien-Pt(0.12) is in the Z-form (or at least belongs to the Z-family) as judged by circular dichroism (fig.1). Two rabbits were immunized with poly(dG–dC)-dien-Pt(0.12) electrostatically coupled to methylated bovine serum albumin. Similar results were obtained with the two antisera.

The antiserum does not react with poly(dG–dC) · poly(dG–dC) in B-form but does react with poly(dG–dC) · poly(dG–dC) in Z-form. This is clearly shown in fig.2. In 1.5 M $NaClO_4$, almost all the poly(dG–dC) · poly(dG–dC) is precipitated and is not at all precipitated below 1.2 M $NaClO_4$. It is known that poly(dG–dC) · poly(dG–dC) can undergo a salt-induced conformation transition from the B-form to the Z-form [5–10]. The midpoint of the transition is at 1.8 M. In 1.2 M $NaClO_4$, only a small fraction of poly(dG-dC) · poly(dG–dC) is in Z-form. However, since the antiserum binds to the Z-form, the equilibrium B-form ⇋ Z-form is displaced towards the right by addition of the antiserum. A similar result has been reported with poly(A) · poly(U), poly(A) · 2 poly(U) and the antibodies to poly(I) · poly(C). In presence of these antibodies, the equilibrium poly(A) · 2 poly(U) ⇋ poly(A) · poly(U) + poly(U) was displaced towards the right and the equilibrium poly(A) · poly(U) ⇋ poly(A) + poly(U) towards the left. Antibodies bind more strongly to poly(A) · poly(U) than to poly(A) · 2 poly(U), poly(A) or poly(U) [23,27].

The recognition of the Z-form by the antiserum has been confirmed by RIA in 0.1 M NaCl, 1 mM $MgCl_2$. Poly(dG–dC)AAF(0.13) inhibits the tracer–antiserum binding. It is less efficient than poly(dG–dC)-dien-Pt(0.12). This can be due to a steric hindrance of the bulky AAF residues and/or to slight differences in the conformation of the double helix. Moreover it inhibits up to 100%. Therefore we are essentially dealing with an antiserum which recognizes poly(dG–dC) · poly(dG–dC) in the Z-form. dien-Pt residues are not the main antigenic determinant. This is also confirmed by the finding that native or denatured DNA modified by dien-Pt (r_b = 0.05) do not inhibit.

The antigenic determinant is not yet known. One can say that it is not only guanine or cytosine residues since there are no inhibition by deoxyguanosine, deoxycytidine or denatured DNA. The phosphate groups do not seem to be involved. There were no variation of the amount of poly(dG–dC)dien-Pt precipitate as a function of the salt concentration (over 0.1–1 M NaCl). This is quite different from the case of the complexes between poly(I) · poly(C) and antibodies to poly(I) · poly(C) in which electrostatic interactions play an important role [23,28].

In conclusion, the antiserum reacts with poly(dG–dC) · poly(dG-dC) in the Z-form but not in the B-form. Work is in progress to detect Z-form in natural DNAs with this antiserum.

Acknowledgements

We thank Professor C. Hélène for his interest in this work. Thanks are due to Dr M. Granger for helpful discussions and to Mrs N. Rousseau for her excellent technical assistance. This work was supported in part by the Délégation Générale à la Recherche Scientifique et Technique (contract no. 79-7-0664).

References

[1] Wang, A. H. J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., Van Boom, J. H., Van Der Marel, G. and Rich, A. (1979) Nature 282, 680–686.
[2] Crawford, J. L., Kolpak, F. J., Wang, A. H. J., Quigley, G. J., Van Boom, J. H., Van Der Marel, G. and Rich, A. (1980) Proc. Natl. Acad. Sci. USA 77, 4016–4020.
[3] Drew, H., Takano, T., Takano, S., Itakura, K. and Dickerson, R. E. (1980) Nature 286, 567–573.
[4] Arnott, S., Chandrasekaran, R., Birdsall, D. L., Leslie, A. G. W. and Ratcliff, R. L. (1980) Nature 283, 743–745.
[5] Pohl, F. M. and Jovin, R. M. (1972) J. Mol. Biol. 67, 375–396.
[6] Pohl, F. M., Ranade, A. and Stockburger, M. (1974) Biochim. Biophys. Acta 335, 85–92.
[7] Patel, D. J., Canuel, L. L. and Pohl, F. M. (1979) Proc. Natl. Acad. Sci. USA 76, 2508–2511.
[8] Ramstein, J. and Leng, M. (1980) Nature 288, 413–414.
[9] Mitra, C. K., Sarma, M. H. and Sarma, R. H. (1981) Biochemistry 20, 2036–2041.
[10] Wang, A. J. H., Quigley, G. J., Kolpak, F. J., Van Der Marel, G., Van Boom, J. H. and Rich, A. (1981) Science 211, 171–176.

[11] Klysik, J., Stirdivant, S. M., Larson, J. E., Hart, P. A. and Wells, R. D. (1981) Nature 290, 672–677.
[12] Liebeskind, D., Hsu, K. C., Erlanger, B. and Bases, R. (1974) Exp. Cell Res. 83, 399–405.
[13] Cornelis, J. J., Rommelaere, J., Urbain, J. and Errera, M. (1977) Photochem. Photobiol. 26, 241–246.
[14] Lubit, B. W., Pham, T. D., Miller, O. J. and Erlanger, B. F. (1976) Cell 9, 503–509.
[15] De Murcia, G., Lang, M. C., Freund, A. M., Fuchs, R. P. P., Daune, M. P., Sage, E. and Leng, M. (1979) Proc. Natl. Acad. Sci. USA 76, 6076–6080.
[16] McKusic-Olson, H. and Glitz, D. G. (1979) Proc. Natl. Acad. Sci. USA 76, 3769–3773.
[17] Nahon-Merlin, E., Delain, E., Couland, D. and Lacour, F. (1980) Nucleic Acids Res. 8, 1805–1822.
[18] Rudkin, G. T. and Stollar, B. D. (1977) Nature 265, 472–473.
[19] Malfoy, B. and Leng, M. (1981) submitted.
[20] Sage, E. and Leng, M. (1980) Proc. Natl. Acad. Sci. USA 76, 6076–6080.
[21] Sage, E. and Leng, M. (1981) Nucleic Acids Res. 9, 1241–1250.
[22] Wells, R. D., Larson, J. E., Grant, R. C., Smertle, B. E. and Cantor, C. R. (1970) J. Mol. Biol. 54, 465–497.
[23] Guigues, M. and Leng, M. (1976) Eur. J. Biochem. 69, 615–624.
[24] Lacour, F., Nahon-Merlin, E. and Michelson, A. M. (1973) Curr. Top. Microbiol. Immunol. 62, 1–39.
[25] Stollar, B. D. (1975) Crit. Rev. Biochem. 3, 45–69.
[26] Stollar, B. D. (1973) in: The Antigens (Sela, M. ed) vol. 1, pp. 1–85, Academic Press, New York.
[27] Guigues, M. and Leng, M. (1976) Nucleic Acid Res. 3, 3337–3347.
[28] Leng, M., Guigues, M. and Genest, D. (1978) Biochemistry 17, 3215–3220.