ELECTROPHORETIC ISOLATION OF NUCLEOSOMES FROM *DICTYOSTELIUM* NUCLEI AND NUCLEOLI

Proteins associated with monomers and dimers

Roger W. PARISH, Sylvia SCHMIDLIN, Stelia FUHRER and Rosmarie WIDMER
Plant Biology Institute, University of Zürich, Zollikerstrasse 107, CH-8008 Zürich, Switzerland

Received 13 December 1979

1. Introduction

Cell differentiation is largely the result of regulated gene activity which may involve the interaction of DNA with regulatory proteins [1,2]. The nucleosome is the prime structural unit of chromatin [3]. The protein complement of nucleosomes and their secondary modifications may control transcriptional processes.

Dictyostelium discoideum is used to study differentiation since it possesses a simple genome and morphological changes occurring during the life cycle are accompanied by modulation of gene activity [4]. The DNA is packaged into nucleosomes [5,6] and we have isolated histone-like proteins from the nuclei [7,8].

Nucleosome monomers and oligomers from *Dictyostelium discoideum* were separated using gel electrophoresis. Satisfactory fractionation was not achieved using sucrose gradients. The mononucleosomes consisted of two subcomponents which were only partially separated. DNA and proteins associated with the nucleosomes were examined using 2 D gel electrophoresis or by extraction from the first dimension gel. The major basic nucleoproteins were associated with all nucleosome sizes, indicating that they do function as histones. More non-histone proteins were found in dinucleosomes than in mononucleosomes. Histones, but not non-histone proteins, were more readily lost from nucleolar nucleosomes during separation. This suggested histone–DNA interactions were on average weaker in nucleoli and many non-histone proteins interact directly with the DNA rather than via histones. Differences in the non-histone proteins associated with nucleolar and nuclear mono- and dinucleosomes were detected.

2. Materials and methods

Dictyostelium discoideum cells (strain Ax-3) were grown in HL-5 medium [9]. Cells were harvested at the end of the exponential growth phase ($5–6 \times 10^7$ cells/ml). Nuclei [8] and nucleoli [10] were isolated as described.

Nuclei or nucleoli were resuspended in 20 mM Tris–HCl (pH 7.8) and incubated for 5–10 min with 150 units (nuclei) or 75 units (nucleoli) micrococcal nuclease (Worthington) per ml at 37°C (15–20% of the DNA was then acid-soluble). Nuclei and nucleoli were $\sim 3 \times 10^9$/ml and 10^{10}/ml respectively. The reaction was stopped with 1/10th vol. 0.2 M EDTA (pH 7.5) and cooled at 0°C. The suspension was dialyzed overnight at 4°C against 10 mM Tris–HCl (pH 7.5), 1 mM EDTA and 1 mM *N*-α-*p*-tosyl-L-lysine–chloromethylketone–HCl. The dialysate was centrifuged at 12 000 × *g* for 5 min and the supernatant contained DNP particles.

Nuclei (3×10^9/ml) were digested with micrococcal nuclease (150 units/ml, 5 min) in 20 mM Tris–HCl (pH 7.8), containing 60 mM KCl, 15 mM NaCl, 1 mM $CaCl_2$, and dialysed against Tris–HCl/EDTA buffer as described for DNP-particles. After removal of the nuclear debris, the supernatant was made 3 mM with $MgCl_2$. The suspension was left 3 h at 0°C (or overnight at −15°C). DNA–histone complexes (crude nucleosomes) were pelleted by centrifuging 10 min at 10 000 × *g*.

Glycerine (10%) was added to the DNP-particle suspension before loading on a 2.5% acrylamide/0.5% agarose vertical slab gel. The gel and running buffer was 40 mM Tris–HCl, 3.2 mM sodium acetate, 0.32 mM EDTA (pH 8.0). Electrophoresis was carried out at 100 V for 3 h at 15°C. The gels were stained with ethidium bromide and photographed. Strips were cut from the first dimensional gel and equilibrated for 10 min in 1% SDS, 36 mM Tris–HCl, 30 mM Na_2HPO_4, 10 mM EDTA (pH 7.8). To further separate and characterise proteins and DNA fragments present in DNP-particles, strips were laid horizontally on 8% (for proteins) or 4% (for DNA) acrylamide–0.5% agarose vertical slab gels and polymerized in place with a 1% agarose solution. The gel and running buffer was 36 mM Tris–HCl containing 0.1% SDS, 30 mM Na_2HPO_4 and 10 mM EDTA (pH 7.8). Electrophoresis was overnight at 30 V. Gels were subsequently incubated for 15 min in isopropanol (25%)–acetic acid (10%) which permits gels to retain their shape during staining and drying. The SDS was removed using 50% methanol (3 washings, each 30 min) before staining with ethidium bromide.

Monomer and dimer nucleosomes were cut from the first dimension gel and extracted with 1% SDS, 10 mM Tris–HCl, 2 mM EDTA (pH 7.6). After removing the acrylamide by centrifugation the supernatants were extracted with phenol. DNA was recovered from the aqueous phase by ethanol precipitation, protein from the phenol phase by adding an equal vol. 1 M HCl and 12 vol. acetone.

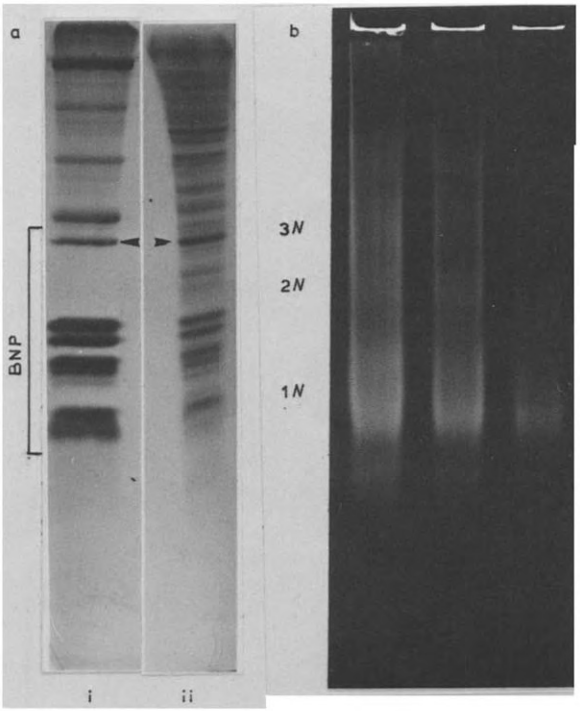

Fig.1. (a) Dodecylsulfate–polyacrylamide (15%) gels of proteins [11] in (i) crude nucleosomes and (ii) whole nuclei. BNP, basic nucleoproteins; arrow indicates H1-equivalent. (b) First dimension acrylamide–agarose gel of deoxyribonucleoprotein (DNP) particles stained with ethidium bromide. Three different concentrations are shown. 1 *N*, 2 *N* and 3 *N* represent mono-, di- and trinucleosomes.

3. Results

Many of the higher molecular weight proteins (>30 kilodaltons) found in nuclei were absent from the crude nucleosome fraction (fig.1a). All the major basic nucleoproteins [7,8] were present and dominant components of the crude nucleosomes. A considerable amount of the lysine-rich basic nucleoprotein (H1-equivalent) [8] was lost from the nucleosome fraction.

A number of bands were detected in gels stained with ethidium bromide following electrophoresis of nuclear DNP-particles on first dimension gels (fig.1b). Electrophoresis in the second dimension showed these bands corresponded to nucleosome monomers and oligomers (not shown). The two major mononucleosomes, containing DNA fragments of 168–185 and 146–152 base pairs [10], were only partially separated.

The protein content of nucleosomes from nuclei was examined with 2D gels (fig.2). The fast-migrating basic nucleoproteins were the major components and were present in all nucleosomes. Only relatively low concentrations of the H1-equivalent were present, indicating substantial losses occur during nucleosome preparation and electrophoresis. The H1-equivalent appeared to be preferentially associated with the larger of the two major mononucleosomes.

DNP isolated from untreated nuclei or nuclei treated with nuclease until over 50% of the DNA was acid-soluble were used as controls. A few high molecular weight proteins (mainly glycoproteins) were present or 2D gels, but no proteins in the monomer and dimer regions (not shown).

DNP-particles from nucleoli were also isolated and separated on 2D gels. The protein pattern differed

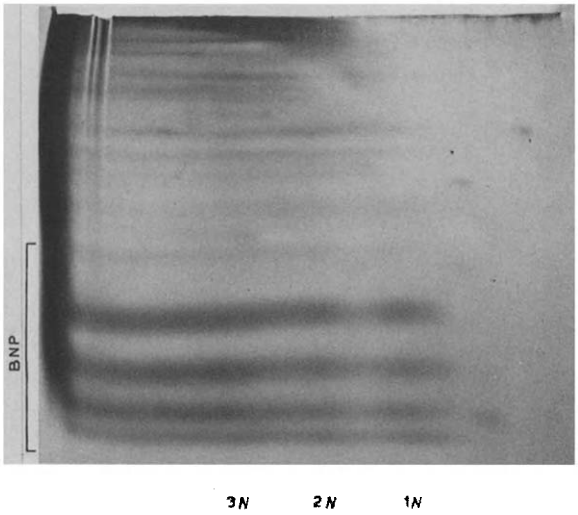

Fig.2. Two-dimensional gel stained for proteins; first dimension from right to left, second dimension from top to bottom. DNP isolated from micrococcal nuclease treated nuclei. Arrow indicates H1-equivalent.

markedly from that of whole nuclei (fig.3). In particular more higher molecular weight proteins (non-histone proteins) were associated with nucleosomes and the concentration of basic proteins was much lower and varied between preparations. Nevertheless, nucleoli initially contained a normal complement of basic proteins. These were apparently lost during DNP-particle isolation and also failed to migrate with DNA during electrophoresis in the first dimension.

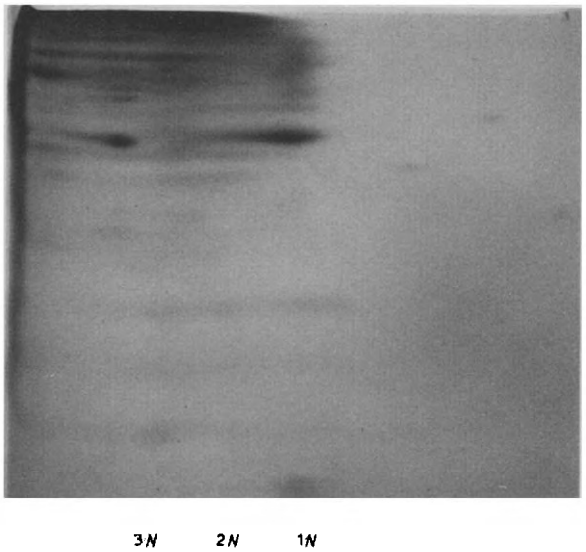

Fig.3. As in fig.2, except DNP were isolated from nucleoli.

The resolution of proteins on the 2D gels was limited and only a relatively small amount of material could be loaded. Hence, nucleosome monomers and dimers were extracted from first dimensional gels. Examination of DNA fragments with gel electrophoresis confirmed the purity of these nucleosomes (fig.4a).

The proteins of nuclear monomers and dimers are shown in fig.4b. The major proteins were basic nucleoproteins and presumably correspond to the core histones of higher eukaryotes. The concentration of H1-equivalent was lower in monomers than in dimers. The dimers contained more non-histone proteins than the monomers.

The proteins associated with nucleosome monomers and dimers isolated from nucleoli are shown in fig.5. The relatively low concentration of histones was again typical. Nucleosome dimers from nuclei and nucleoli are compared in fig.6. Differences between the non-histone proteins can be observed.

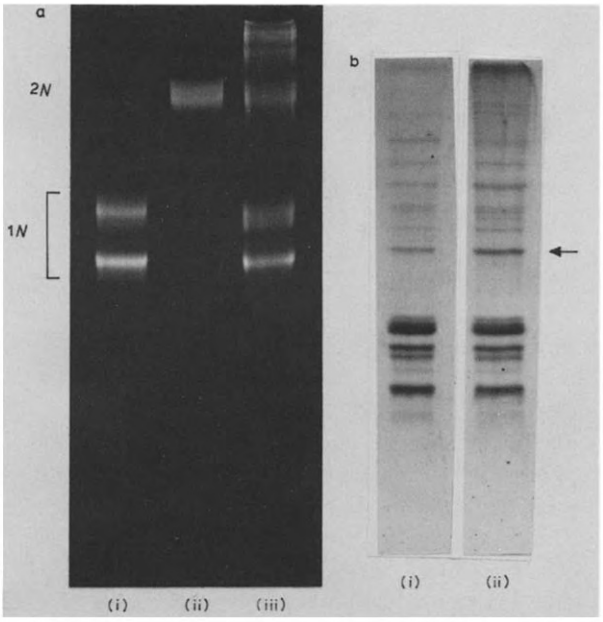

Fig.4. (a) Acrylamide–agarose (5.5% and 0.5%) gel [12] of DNA associated with DNP bands obtained by first-dimension gel electrophoresis as shown in fig.2. DNA was extracted from: (i) the mono-nucleosome band; (ii) the dinucleosome band; (iii) the total DNP fraction. (b) Dodecylsulfate–polyacrylamide (15%) gels of proteins extracted from mono-nucleosomes (i) and dinucleosomes (ii) isolated from nuclei. The arrow indicates H1-equivalent.

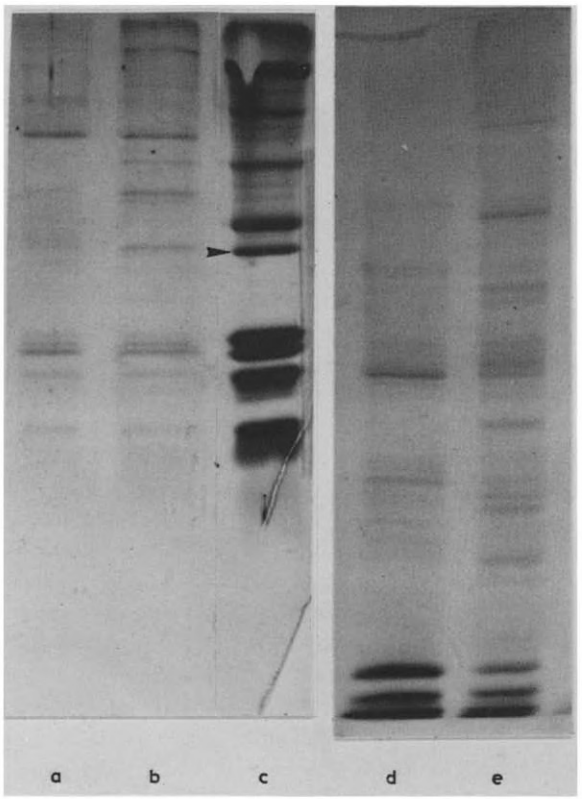

Fig.5. Dodecylsulfate–polyacrylamide gels of proteins extracted from mononucleosomes (a,d) and dinucleosomes (b,e) isolated from nucleoli. (c) Crude nucleosomes from nuclei. (a–c) 15% acrylamide gels; (d,e) 10% acrylamide gels.

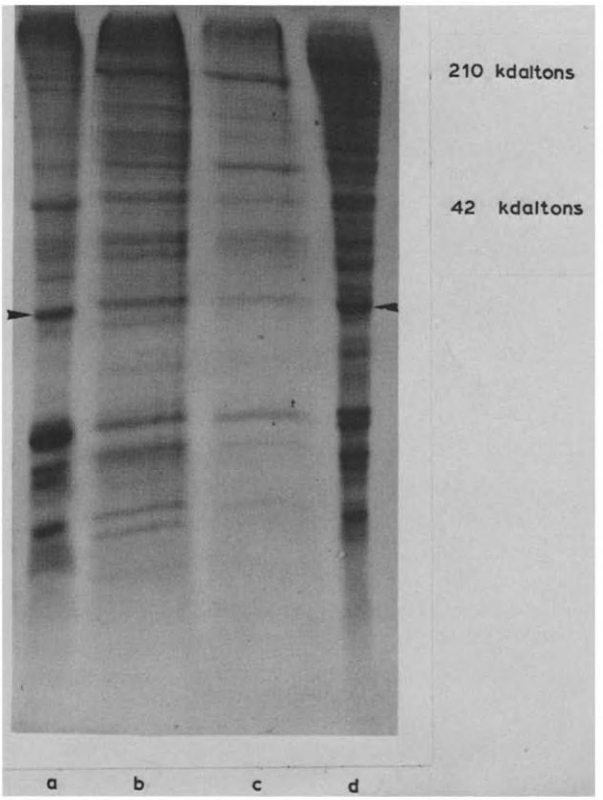

Fig.6. Dodecylsulfate–polyacrylamide (15%) gels comparing proteins associated with dinucleosomes isolated from nuclei and nucleoli. (a) Nuclear dinucleosomes; (b,c) nucleolar dinucleosomes, two different expt; (d) total proteins associated with nuclei.

4. Discussion

The nucleosomes of *D. discoideum* contained the major basic nucleoproteins [7,8], indicating that the latter do indeed function as histones. H1-equivalent was partially lost from nucleosomes during DNP-particle isolation and electrophoresis. This raises the possibility that other proteins, such as those bound to linker regions, were also lost.

In spite of initially conflicting data [14–19], probably related to isolation techniques, non-histone and high mobility group (HMG) proteins [20] are known to be present in mononucleosomes. They may be bound to DNA linker regions or to the core itself. We found at least 5 non-histone proteins were associated with *D. discoideum* mononucleosomes. The dimers carried additional proteins which may, for example, interact with the linker regions.

Histones, but not non-histone proteins, were more readily lost from nucleoli than nuclei during DNP-particle preparation and electrophoresis. This suggests that histone–DNA interactions are on average weaker in nucleoli. Non-histone proteins as well as secondary modifications to histones [21] may regulate these interactions and the non-histone complement of dinucleosomes from nucleoli and nuclei did somewhat differ. The results also imply that many non-histone proteins interact directly with DNA rather than via histones. Nuclease digestion indicates nucleoli chromatin from *D. discoideum* possesses a distinctive structure [10].

We are now examining the synthesis and modification of nucleosome proteins during differentiation.

Acknowledgements

This work was supported by the Schweizerische Nationalfonds zur Förderung der wissenschaftlichen Forschung (grant no. 3.637.-0.75).

References

[1] Ciba Foundation Symposium (1975) The Structure and Function of Chromatin, Elsevier/Excerpta Medica, Amsterdam, New York.
[2] Dawid, I. B. and Wahrli, W. (1979) Dev. Biol. 69, 305–328.
[3] Kornberg, R. D. (1977) Ann. Rev. Biochem. 46, 931–954.
[4] Firtel, R. A. (1972) J. Mol. Biol. 66, 363–377.
[5] Parish, R. W., Stalder, J. and Schmidlin, S. (1977) FEBS Lett. 84, 63–66.
[6] Bakke, A. C., Wu, J. R. and Bonner, J. (1978) Proc. Natl. Acad. Sci. USA 75, 705–709.
[7] Charlesworth, M. C. and Parish, R. W. (1975) Eur. J. Biochem. 54, 307–316.
[8] Charlesworth, M. C. and Parish, R. W. (1977) Eur. J. Biochem. 75, 241–250.
[9] Cocucci, S. M. and Sussman, M. (1970) J. Cell Biol. 45, 399–407.
[10] Widmer, R., Fuhrer, S. and Parish, R. W. (1979) FEBS Lett. 106, 363–369.
[11] Parish, R. W., Schmidlin, S. and Müller, U. (1977) Exp. Cell Res. 110, 267–276.
[12] Compton, J. L., Bellard, M. and Chambon, P. (1976) Proc. Natl. Acad. Sci. USA 73, 4382–4386.
[13] Bakayev, V. V., Bakayeva, T. G., Schmatchenko, V. V. and Georgiev, G. P. (1978) Eur. J. Biochem. 91, 291–301.
[14] Liew, C. C. and Chan, P. K. (1976) Proc. Natl. Acad. Sci. USA 73, 3458–3462.
[15] Bakayev, V. V., Bakayeva, T. G. and Varshavsky, A. J. (1977) Cell 11, 619–629.
[16] Honda, B. M., Baillie, D. L. and Candido, E. P. M. (1975) J. Biol. Chem. 250, 4643–4647.
[17] Augenlicht, L. H. and Lipkin, M. (1976) Biochem. Biophys. Res. Commun. 70, 540–544.
[18] Goodwin, G. H., Woodhead, L. and Johns, E. W. (1977) FEBS Lett. 73, 85–88.
[19] Musich, P. R., Brown, F. L. and Maio, J. J. (1977) Proc. Natl. Acad. Sci. USA 74, 3297–3301.
[20] Mathew, C. G. P., Goodwin, G. H. and Johns, E. W. (1979) Nucleic Acid. Res. 6, 167–179.
[21] Sealy, L. and Chalkley, R. (1978) Cell 14, 115–121.