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STRUCTURAL PROPERTIES OF BARLEY NUCLEOSOMES

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The structural properties of barley oligonucleosomes are investigated and compared to those of rat liver oligomers. Extraction of barley chromatin was performed using mild nuclease digestion of isolated nuclei leading to a low ionic strength soluble fraction. Oligonucleosomes were fractionated on sucrose gradients and characterized for DNA and histone content. Physico-chemical studies (sedimentation, circular dichroism and electric birefringence) showed that barley oligonucleosomes exhibit properties very close to those of the H1-depleted rat liver counterparts. Moreover, in situ, barley linker DNA was more sensitive to micrococcal nuclease digestion than that of rat liver. These results suggest that barley oligonucleosomes show a less compact structure than their rat liver counterparts and appear to be in contradiction with the very condensed organization of barley chromatin previously suggested.

1. Introduction

Nuclear DNA is organized into a repetitive nucleohistone subunit, called the nucleosome, in all eukaryotic organisms studied thus far (for a review, see refs. 1–3). The presence of this nucleosomal structure in very divergent groups such as vertebrates, micro-organisms, viruses or plants indicates the stability and conservation of the histone-DNA assembly during evolution. It thus appears that the nucleosomes have apparently the same basic architecture despite variations in DNA content and although the molecular weight and the chemical composition of several histones differ.

At very low ionic strength, chromatin appears as a beaded fibre where the beads represent the nucleosomes. The folding of the nucleosomal chain into the 20–30 nm thick fibre, variously described as a solenoidal [4] or a superbead structure [5], is still unclear. Particularly, the respective roles of histone H1, non-histone proteins and histone-DNA interactions between adjacent nucleosomes are not well understood. Fibres of similar appearance are found in nuclei or in isolated chromosomes [6–8] and transitions between the loose polynucleosomal chain and the compact higher-order structure must play a fundamental role in the template activity of the chromatin.

Concerning the structural properties of the nucleosome, it has been shown that the compaction of the nucleosome might be altered under certain conditions. In particular, nucleosomes associated with transcriptionally active genes and replicating chromatin exhibit a less compact structure than bulk chromatin [9–14]. This association structure-function of nucleosomes attracts current interest in the conformational state and transitions of these particles. Evidence has recently been presented which suggests that yeast nucleosomes are less constrained than those of higher eukaryotes [15]. It was inferred that changes in the H3 sequence and in the H3-H4 interactions might be responsible for this structural variation. In plants,

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very little is known concerning the compaction of nucleosomes. We were interested in the structural organization of chromatin in barley nuclei. This higher plant (*Hordeum vulgaris*) is of particular interest, since the chromatin shows a very highly condensed structure and exhibits a high degree of heterogeneity in histones H1 and H2 as do all Gramineaes [16–18]. We have previously reported the preparation and the physico-chemical study of a low ionic strength soluble chromatin fraction extracted from barley nuclei [19]. We observed the same transition in the electro-optical properties of oligonucleosomes as has been described in rat liver chromatin [20,21], suggesting changes in the orientation of nucleosomes.

In order to obtain more information about the structural properties of barley chromatin, we report here the study of low-order oligonucleosomes: monomers, dimers and trimers. We present the first detailed structural analysis of plant oligonucleosomes and we compare the results with those obtained with their rat liver counterparts, with or without H1. The chemical characterization, particularly the determination of the associated DNA length and of the histone content, was carried out in parallel with circular dichroism and electric birefringence studies.

2. Materials and methods

2.1. Preparation of barley chromatin oligomers

7-day-old barley leaves, grown at 25°C in darkness, were harvested. Nuclei were isolated according to the method of Muller et al. [17] slightly modified [19]. Extraction of barley oligonucleosomes was performed as described by Noll et al. [22]. Nuclei were suspended at a concentration of 10^8 per ml in a digestion buffer containing 5 mM Mes (pH 7.0), 1 mM $CaCl_2$, 250 mM sucrose and 1 mM sodium bisulfite as protease inhibitor. The suspension was incubated at 37°C and 10^{-2} U of micrococcal nuclease (Sigma) was added per 10^6 nuclei. 2 min 30 s later, the reaction was stopped by addition of 100 mM EDTA to a final concentration of 5 mM and quickly chilled in ice. After 5 min centrifugation at $1500 \times g$, pelleted nuclei were lysed in 1 mM sodium bisulfite and 1 mM EDTA (pH 7.5), with agitation at 4°C for 10 min. After 5 min centrifugation at $1500 \times g$, the supernatant containing the low ionic strength soluble chromatin was recovered. Oligonucleosomes were fractionated according to the method of Finch et al. [23]. Chromatin solution was layered on isokinetic sucrose gradients (5–28.2%) containing 1 mM sodium phosphate buffer (pH 7.4) and 1 mM EDTA. Centrifugation was for 19 h at 26000 rpm in a Beckman SW 27 rotor. The fractions were collected with an ISCO density gradient fractionator and dialysed overnight against 1 mM sodium phosphate buffer (pH 7.4) and 1 mM EDTA.

2.2. Preparation of rat liver oligonucleosomes

Rat liver nuclei were isolated as described by Hewish and Burgoyne [24]. Chromatin oligomers were prepared according to the method of Noll et al. [22]. Digestion of nuclei by micrococcal nuclease and fractionation of chromatin were performed under the same conditions as those for barley nuclei. H1-depleted oligomers were prepared either by fractionation on the isokinetic gradients described above but containing 450 mM NaCl [25] or from purified oligonucleosomes using the method of Bolund and Johns [26]. In this case, H1 was adsorbed on AG 50W × 2 cation-exchange resin in the presence of 1 mM phosphate buffer (pH 7.4) and 450 mM NaCl (0.2 ml equilibrated resin per ml chromatin solution). The suspension was gently stirred for 5 h at 4°C and centrifuged at $1000 \times g$ for 2 min. Supernatants were finally dialysed against 1 mM phosphate buffer (pH 7.4) and 1 mM EDTA.

2.3. DNA purification and analysis

The solutions containing soluble chromatin or digested nuclei were adjusted to a final concentration of 3 mM EDTA, 1% SDS and 1 M NaCl. They were incubated overnight at 37°C in the presence of 100 μg/ml of proteinase K. This treatment was further followed by four to five extractions with chloroform/isoamyl alcohol (24 : 1). The purified DNA was precipitated by addition of 2

vols. ethanol at −20°C and stored for 48 h at −20°C. Precipitated DNA was collected by centrifugation at 10000 × g for 10 min, washed twice with ethanol and dried under vacuum. The analysis on 0.5% agarose/2% polyacrylamide slab gels was performed as described by Philipps and Gigot [16].

2.4. Histone purification and analysis

The solutions containing nucleoproteins were adjusted to $A_{260\ nm} = 5$ with distilled water and dialysed overnight against 0.4 N H_2SO_4. After centrifugation at 20000 × g for 20 min, the supernatant was removed and dialysed for 48 h against ethanol. Histones were collected by centrifugation at 20000 × g for 20 min, washed twice with ethanol and dried under vacuum.

The analysis on polyacrylamide slab gels was performed as described by Laemmli [27]. The estimation of H1 percentage was performed by scanning gels and integrating peaks using a Vernon PH1 3 photometric densitometer.

2.5. Circular dichroism analysis

Circular dichroism measurements were performed with a Jobin & Yvon mark IV dichrograph using cuvettes of 1 cm path length and solutions showing an $A_{260\ nm} = 1$. The results are presented in terms of molar ellipticity $[\theta]$ in deg. cm^2 dmol^{-1}.

2.6. Electric birefringence measurements

Procedures for electric birefringence measurements on chromatin solutions have been previously described [20,21]. Single rectangular pulses were applied and birefringence signals were displayed on a transient recorder, then transferred to an XY plotter.

3. Results

3.1. Fractionation and sedimentation of nucleosome oligomers

Barley and rat liver nuclei were digested under identical conditions of micrococcal nuclease and $CaCl_2$ concentrations, as described in section 2. The lysis of digested nuclei led to low ionic strength soluble chromatin fractions. The sucrose gradient fractionation of rat liver chromatin allowed us to separate oligomeric nucleosomal components containing from one up to eight nucleosomes (fig. 1A), while only three nucleosomal oligomers could be separated from each other in the case of barley chromatin: monomer, dimer and trimer (fig. 1B). In the case of rat liver chromatin fractionated on sucrose gradients containing 450 mM NaCl (in order to remove histone H1), four nucleosomal oligomers could be separated from each other, the last peak to be detected being that of tetranucleosome (fig. 1A).

The Fritsch [28] equation for isokinetic gradients permits calculation of sedimentation coefficients of barley and rat liver oligonucleosomes.

The dependencies of s on the number of nucleosomes are presented in fig. 2. For rat liver

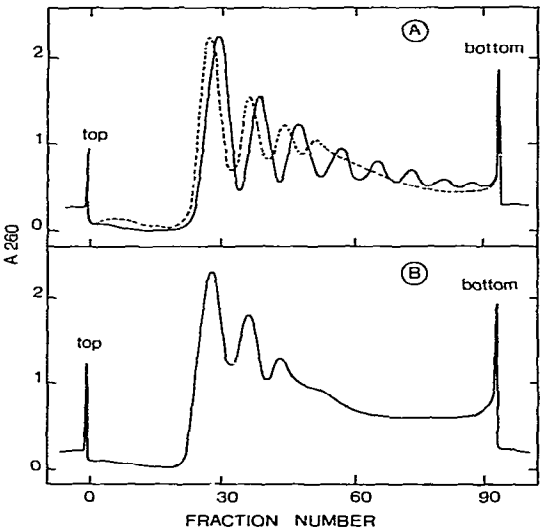

Fig. 1. Fractionation of micrococcal nuclease-digested chromatin by sucrose gradient centrifugation. (A) Rat liver chromatin. The isokinetic gradients contained 1 mM sodium phosphate buffer (pH 7.4), 0.2 mM EDTA and (———) no NaCl and (— — —) 450 mM NaCl. (B) Barley chromatin in low ionic strength.

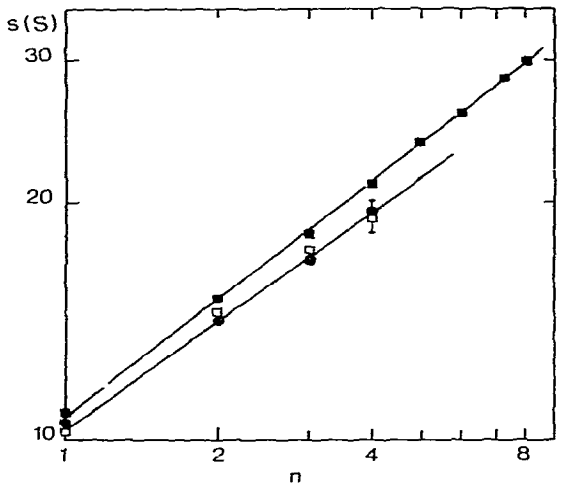

Fig. 2. Dependence of sedimentation coefficients on the number of nucleosomes in the oligomeric chain, plotted as a double-logarithmic scale. Native rat liver chromatin (■———■). H1-depleted rat liver chromatin (□———□), barley chromatin (●———●).

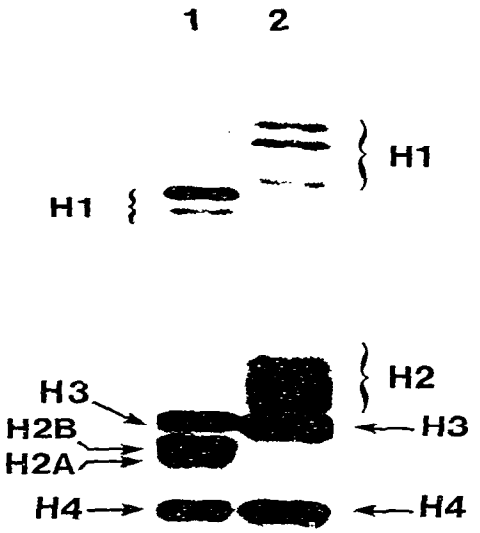

Fig. 3. Electrophoretic patterns of histones extracted from rat liver (lane 1) and barley oligonucleosomes (lane 2). Stacking gel (1 cm long) was 5% polyacrylamide, 125 mM Tris-HCl (pH 6.8), 0.1% SDS. Resolving gel (18 cm long) was 16% polyacrylamide, 375 mM Tris-HCl (pH 8.8), 0.1% SDS. Histones were dissolved in 62.5 mM Tris-HCl (pH 6.8), 2% SDS, 5% 2-mercaptoethanol, 10% glycerol and heated for 3 min at 100 °C before depositing. Migration was for 15 h at 40 mA in a buffer containing 25 mM Tris-HCl (pH 8.3), 192 mM glycine and 0.1% SDS. Gels were stained with Coomassie brilliant blue and destained by diffusion.

oligonucleosomes with H1, the experimental data, plotted on a double-logarithmic scale, are described by the relation:

$$s = 10.8 \times n^{0.50} \quad (1)$$

For the H1-depleted oligonucleosomes, the experimental data, plotted logarithmically, give a straight line having a lower slope and are described by the relation:

$$s = 10.3 \times n^{0.43} \quad (2)$$

For barley oligomers, our sedimentation results are described by the equation:

$$s = 10.6 \times n^{0.42} \quad (3)$$

3.2. DNA and histone analysis

Histones were extracted from every oligonucleosomal fraction. All rat liver oligomers exhibited the same histone pattern (fig. 3, lane 1), without any sign of proteolytic degradation. The approximate percentage of histone H1, determined by densitometric measurements, was approx. 19 ± 2% compared to total histones, for all rat liver oligonucleosomes. The patterns of histones extracted from barley were identical for mono-, di- and trinucleosomes and showed several characterized features (fig. 3, lane 2). There were three components having molecular weight greater than 21 500 and which corresponded to the H1 histones. H3 and H4 comigrated with their counterparts in rat liver histones and histones H2 appeared as at least four components with molecular weights between 15 800 and 17 000 [19]. The mean value of

the percentage of histone H1 was 17 ± 3% of total histones for all the oligomers.

On the other hand, DNA was extracted from soluble chromatin fractions of both species and analysed on 0.5% agarose/2% polyacrylamide slab gels in order to determine the length of DNA associated to the oligonucleosomes. The comparison with *Hae*III-digested ΦX174 DNA allowed us to determine mean repetitive lengths of 193 ± 5 and 198 ± 5 base-pairs for barley and rat liver, respectively. It is interesting to note that the DNA length associated to barley monomers is significantly shorter than the repetitive nucleosomal unit, in contrast to rat liver (fig. 4). Loss of H1 on the ends during digestion could explain the shorter DNA from barley monosomes but within the limits of experimental accuracy the percentage of H1 has always been found to be higher than 12–13% of total histones.

In the case of barley chromatin, a significant part of digested chromatin (at least 50%) remained insoluble at low ionic strength. The DNA contents of both fractions were analysed at different steps of the digestion. No difference was found in the electrophoretic pattern, DNA fragments of the same size being present in the soluble and insoluble fractions (fig. 4). This indicates that the insolubility is not due to a lack of digestion of part of the chromatin but results from an aggregation phenomenon. Moreover, the histone composition was exactly the same in soluble and insoluble chromatin.

3.3. Circular dichroism

Circular dichroism measurements were performed in 1 mM sodium phosphate buffer (pH 7.4) containing 1 mM EDTA. Unfractionated barley and rat liver chromatins showed approximately the same spectrum, typical of native chromatin [29,30] (fig. 5). It is characterized by a positive peak at 282 nm with a shoulder at 272 nm, a negative peak at 295 nm and a molar ellipticity $[\theta]^{20\,°C}_{282\ nm}$ ranging from 1800 up to 2000 deg. cm^2 dmol^{-1}. Rat liver oligonucleosomes fractionated on sucrose gradients exhibited the same spectrum

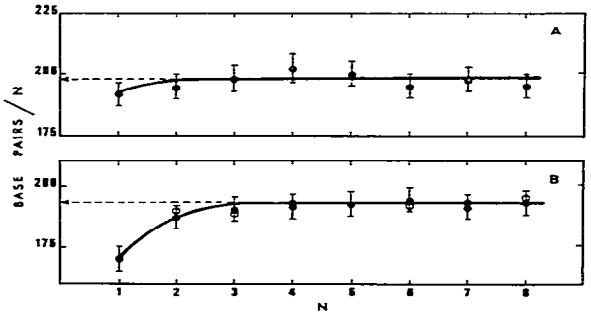

Fig. 4. Dependence of DNA content on the number of nucleosomes in the oligomeric chain. (A) Rat liver chromatin, (B) barley chromatin. Low ionic strength soluble oligonucleosomes (●——●) and low ionic strength insoluble oligonucleosomes (○——○). The gel (12 cm long) was 2% polyacrylamide/0.5% agarose, 0.1% SDS, 40 mM Tris-HCl (pH 7.8), 20 mM sodium acetate, 1 mM EDTA. DNA was dissolved in 40 mM Tris-HCl 10 mM (pH 8.0), 1 mM EDTA and 20% glycerol. Migration was for 4 h at 80 V in a buffer containing 40 mM Tris-HCl (pH 7.8), 20 mM sodium acetate and 1 mM EDTA. Gels were stained with ethidium bromide and photographed under ultraviolet illumination using an orange filter.

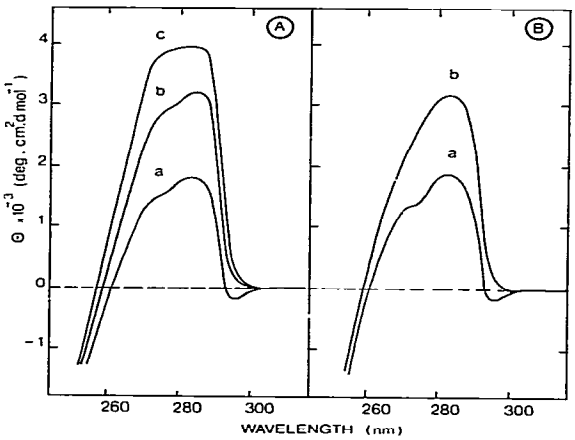

Fig. 5. Circular dichroism spectra. (A) Rat liver oligonucleosomes fractionated at low ionic strength (a), in the presence of 450 mM NaCl (b) and H1-depleted oligonucleosomes prepared by the method of Bolund and Johns [26] (c). (B) Barley chromatin: (a) soluble chromatin and (b) sucrose gradient fractionated oligonucleosomes.

Surprisingly, despite the fact that they have the same histone content as that of total soluble barley chromatin, isolated barley oligomers showed a different spectrum. The shoulder at 272 nm and the negative peak at 295 nm disappeared, whilst the molar ellipticity $[\theta]^{20°C}_{282\ nm}$ increased up to 3200–3300 deg. cm^2 dmol^{-1} (fig. 5B). A similar spectrum was obtained in the case of H1-depleted rat liver oligonucleosomes when H1 was removed by sedimentation on sucrose gradients containing 450 mM NaCl. In contrast, rat liver oligomers, depleted of H1 by using anion-exchanging resin, showed a more significant increase of $[\theta]^{20°C}_{282\ nm}$, which reached 4000 deg. cm^2 dmol^{-1} (fig. 5A).

3.4. Electric birefringence

Electric birefringence experiments were carried out in 1 mM phosphate buffer (pH 7.4) and 1 mM EDTA. As previously reported by us for barley [19] and rat liver [20], birefringence (Δn) is negative for small oligomers containing less than six nucleosomes and positive for higher-order oligomers. Rat liver oligomers corresponding to hexanucleosomes do not exhibit any birefringence [20,21]. Under similar experimental conditions, the values of specific electric birefringence ($\Delta n/A_{260}$) are lower for barley than for H1-containing rat liver oligomers and thus much lower than for H1-depleted rat liver oligomers [31]. For the mononucleosome, this may be explained by a highly compact spherical or disc-shaped structure, as shown by the very short relaxation times found, and consequently by a low degree of orientation. For barley dimer and trimer, the differences are no doubt due to the smaller DNA length than in rat liver oligomers.

Analysis of transient electric birefringence decay curves permits calculation of the oligonucleosome's relaxation times (τ) by using the well known relation of Benoit [32].

$$\Delta n(t) = \Delta n_{eq} e^{-t/\tau} \qquad (4)$$

where Δn_{eq} is the steady-state birefringence.

Fig. 6 shows the results obtained for barley oligomers in comparison with those obtained for their rat liver counterparts, with or without H1.

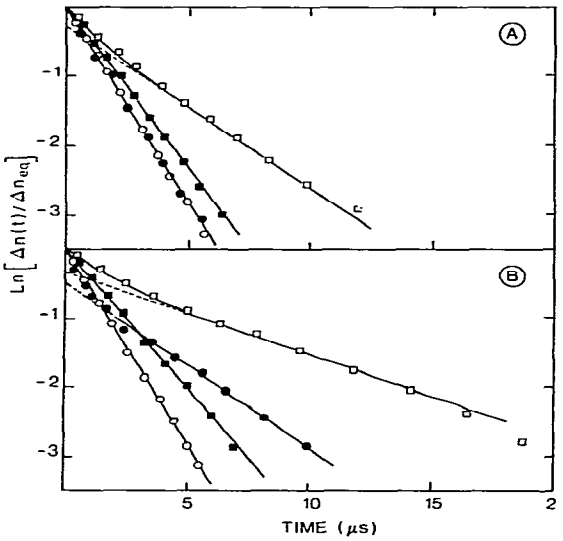

Fig. 6. Determination of relaxation times of dinucleosomes (A) and trinucleosomes (B). Rat liver oligomers containing H1 (○———○). H1-depleted rat liver oligomers obtained by centrifugation in high ionic strength (■———■) and by treatment with the cation-exchange resin (□———□). barley oligomers (●———●).

While the relaxation time exhibited by rat liver mononucleosome repeatedly found to be 0.6 ± 0.1 μs [20], the τ value ($\simeq 0.2$ μs) obtained for barley monomer is too close to the sensitivity limits of the apparatus to allow definitive calculations. However, one should bear in mind that both diffusion constants (rotational constant calculated from the relaxation time of 0.6 μs and translational constant determined from quasi-elastic light scattering measurements [33] have shown that rat liver mononucleosome must be hydrodynamically considered as a slightly elongated ellipsoid or as a lollipop with a spherical head of 110 Å diameter [32,33].

In contrast, a value of rotational relaxation time of 0.2 μs leads to hydrodynamical dimensions in good agreement with electron microscopy, since τ is the time exhibited by a spherical particle with a radius of 58 Å.

Rat liver dimer and trimer exhibit similar relaxation times which show that the trimer structure is highly compact, almost spherical [33,34]. The removal of H1 induces the appearance of an extended structure, this effect being rather limited when H1 is removed by centrifugation in high ionic strength. When H1 is removed in the presence of the cation-exchange resin, the decays of birefringence become multi-exponential showing slower relaxation times of 4.5 μs for the dinucleosome and of 7.5 μs for the trimer.

It is very interesting to note that barley oligomers show relaxation times which increase with the number of nucleosomes in the chain. τ is found to be 1.8 μs for the dimer with a monoexponential decay and 4 μs for the trimer for which shorter relaxation times are also found (fig. 6), no doubt due to slight contamination by dimer particles ($< 15\%$).

4. Discussion

The results reported here on soluble chromatin indicate that the barley oligonucleosomal chain exhibits a less compact structure than its animal counterpart, with rat liver as the model.

That barley monomers contain a length of DNA shorter than the repetitive value shows that the linker DNA of barley chromatin is more sensitive to micrococcal nuclease digestion (especially to the exonuclease activity) than that of rat liver chromatin. This strongly suggests a greater accessibility for the enzyme. Therefore, the difference in DNA length in mononucleosomes following digestion could be due either to a less condensed structure of barley chromatin or to different protection of linker DNA by non-histone proteins.

Circular dichroism experiments agree with both possibilities. Indeed, barley oligonucleosomes exhibit a higher molar ellipticity at 282 nm than their rat liver analogues. It is quite evident that the molar ellipticity at 282 nm depends on the proportion of free DNA in the nucleosome [35]. Concerning the organization of DNA in the rat liver nucleosome, it is now recognized that 140–145 base-pairs are associated to the histone octamer and 160 base-pairs to the chromatosome, whilst 35–40 base-pairs constitute the linker DNA [1–3]. It has been clearly shown that identical properties characterize the barley nucleosomal DNA organization [36]. Barley oligonucleosomes contain a shorter length of DNA than their rat liver counterparts (fig. 4) and they fully retain histone H1, as do those of rat liver. It then seems reasonable to believe that, if barley oligomers exhibit a shorter length of free DNA located at the nucleosome entry and exit, compared to those of rat liver, the higher values of $[\theta]^{20\,°C}_{282\ nm}$ result from the fact that the internal organization of their nucleosomal DNA is much less constrained. Indeed, the complete removal of H1 from rat liver counterparts is required to obtain similar values of $[\theta]^{20\,°C}_{282\ nm}$.

Sedimentation data agree well with these results. It is known that the value of the exponent a in the dependence on s of n may characterize the degree of compaction of the nucleosomal chain [37]. The exponent $a = 0.50$ in eq. 1 indicates relatively high compaction of the chain which decreases after dissociation of histone H1: $a = 0.43$ in eq. 2. Our results are in good agreement with similar data obtained for animal oligonucleosomes under low ionic strength conditions. a is equal to about 0.52 for oligonucleosomes with H1 [38–42] and about 0.40 for oligomers without H1 [39,40]. For barley oligomers containing H1, our sedimentation results give a value of 0.42 for a. This indicates that the barley nucleosomal chain seems to present the same unfolded structure as that of rat liver after removal of histone H1. Moreover, that it is not possible to separate barley oligomers containing more than three nucleosomes agrees with the work of Strätling.[43] who showed that the gradient separation of oligonucleosomes containing more than four nucleosomes depends on maximal compaction of the oligonucleosomes. It is noteworthy that attempts to separate H1-depleted oligonucleosomes of rat liver always lead to sedimentation profiles comparable to those of barley chromatin, the last peak to be detected being that of tetramer.

Confirmation of the extended structure of barley oligomers is given by the analysis of the birefringence decays which shows an increase of relaxation time with number of nucleosomes. One should bear in mind that rat liver oligomers ex-

hibit similar values for dimer, trimer, tetramer and pentamer (1.2–1.8 μs) [20].

These results may contrast with the fact that a significant amount of the digested chromatin remains insoluble at low ionic strength. An electron microscopy study led Muller et al. [17] to conclude that this poor solubility is due to a highly condensed structure of barley chromatin, on the basis that individual nucleosomes cannot be visualized at low ionic strength. Indeed, they only observed 20–30 nm thick fragments, even after micrococcal nuclease digestion. Our results disagree with these data, since we were able to separate oligonucleosomes containing a limited number of nucleosomes. It should be borne in mind that the low solubility of barley chromatin at low ionic strength is one of the two specific properties which may possibly be related to functional aspects. The other property is the multiplicity of histones H2 [16–18]. We have shown that the DNA of both the soluble and insoluble fraction was identically digested by DNases and that the histone composition was the same. Therefore, we are confident that the technique used for the preparation of soluble barley chromatin is valuable not only for mass isolation of plant histones but also for biochemical and biophysical studies giving useful information about native highly condensed chromatin. We believe that the insolubility could only arise from an aggregation phenomenon due to either differences in non-histone content or histone-histone interactions. Both effects result in the maintenance of oligonucleosomes in a condensed superstructure. However, we think that the low ionic strength insolubility is not incompatible with 'the less constrained structure of the barley oligonucleosomes' in solution.

In conclusion, it seems clearly established that barley oligonucleosomes exhibit little constrained structure, compared to their animal counterparts, even if strong interactions seem to be involved in higher-order structures of barley chromatin.

References

1 J.D. McGhee and G. Felsenfeld Annu. Rev. Biochem. 49 (1980) 1115.
2 I.L. Cartwright, S.M. Abmayr, G. Fleischman, K. Lowenhaupt, S.C.R. Elgin, M.A. Keene and G.C. Howard CRC Crit. Rev. Biochem. 13 (1982) 1.
3 T. Igo-Kemenes, W. Hörz and H.G. Zachau, Annu. Rev. Biochem. 51 (1982) 89.
4 J.T. Finch and A. Klug Proc. Natl. Acad. Sci. U.S.A. 73 (1976) 1897.
5 M. Renz, P. Nehls and J. Hozier, Proc. Natl. Acad. Sci. U.S.A. 74 (1977) 1879.
6 H.G. Davies, J. Cell. Sci. 3 (1968) 129.
7 K. Brasch, Exp. Cell Res. 101 (1976) 396.
8 H. Ris and J. Korenberg, in: Cell biology. A comprehensive treatise, eds. L. Goldstein and D.M. Prescott (Academic Press, New York, 1979) vol. 2.
9 H. Weintraub and M. Groudine, Science 193 (1976) 848.
10 H. Weintraub, A. Worcel and B. Alberts Cell 9 (1976) 409.
11 A. Garel M. Zolan and R. Axel, Proc. Natl. Acad. Sci. U.S.A. 74 (1977) 4867.
12 M. Bellard, F. Gannon and P. Chambon, Cold Spring Harbor Symp. Quant. Biol. 42 (1977) 779.
13 K.S. Bloom and J.N. Anderson, Cell 15 (1978) 141.
14 W.I. Wood and G. Felsenfeld, J. Biol. Chem. 257 (1982) 7730.
15 K.P. Lee, H.J. Baxter, J.G. Guillemette, H.G. Lawford and P.N. Lewis, Can. J. Biochem. 60 (1982) 379.
16 G. Philipps and C. Gigot, Nucleic Acids Res., 4 (1977) 3617.
17 A. Muller, G. Philipps and C. Gigot, Planta 149 (1980) 69.
18 J. Liberati-Langenbuch, A. Muller, G. Philipps, M.J. Robert, C. Schmitt and C. Gigot Physiol. Vég. 18 (1980) 357.
19 G. Mithieux and B. Roux, Eur. J. Biochem. 135 (1983) 443.
20 C. Marion and B. Roux, Nucleic Acids Res. 5 (1978) 4431.
21 B. Roux, C. Marion and J.C. Bernengo, in: Electro-optics and Dielectrics of macromolecules and colloïds, ed. B.R. Jennings (Plenum Press, New York, 1979) p. 163.
22 M. Noll, J.O. Thomas and R.D. Kornberg, Science 187 (1975) 1203.
23 J.T. Finch, M. Noll and R.D. Kornberg, Proc. Natl. Acad. Sci. U.S.A. 72 (1975) 3320.
24 D.R. Hewish and L.A. Burgoyne, Biochem. Biophys. Res. Commun. 52 (1973) 504.
25 H.H. Ohlenbusch, B.M. Oliviera, D. Tuan and N. Davidson, J. Mol. Biol. 25 (1967) 299.
26 L.A. Bolund and E.W. Johns, Eur. J. Biochem. 35 (1973) 546.
27 U.K. Laemmli, Nature 227 (1970) 680.
28 A. Fritsch, Anal. Biochem. 55 (1973) 57.
29 G. De Murcia, G.C. Das, M. Erard and M. Daune, Nucleic Acids Res. 5 (1978) 523.
30 R.L. Rill, in: Molecular Genetics III, ed. J.H. Taylor (Academic Press, New York, 1979) p. 247.
31 C. Marion and B. Roux, Biochem. Biophys. Res. Commun. 94 (1980) 535.
32 W. Benoit, Ann. Phys. 6 (1951) 561.
33 C. Marion, P. Bezot, C. Hesse-Bezot, B. Roux and J.C. Bernengo, Eur. J. Biochem., 120 (1981) 169.
34 J.C. Bernengo, P. Bezot, C. Hesse-Bezot, B. Roux and C. Marion, Ber. Bunsenges. Phys. Chem. 85 (1981) 657.

35 K. Watanabe and K. Iso, J. Mol. Biol. 151 (1981) 143.
36 A. Muller, Thesis, University Louis Pasteur, Strasbourg (1979).
37 C.R. Cantor and P.R. Schimmel, Biophysical chemistry (W.H. Freeman, San Francisco, 1980).
38 B. Ramsay-Shaw and K.S. Schmitz, Biochem. Biophys. Res. Commun. 73 (1976) 224.
39 T.N. Osipova, V.A. Pospelov, S.B. Svetlikova and V.I. Vorob'ev, Eur. J. Biochem.. 113 (1980) 183.
40 M. Noll and R.D. Kornberg, J. Mol. Biol. 109 (1977) 393.
41 E.C. Pearson, P.J. Butler and J.O. Thomas, EMBO J. 2 (1983) 1367.
42 J.M. Gottesfeld and P.J.G. Butler, Nucleic Acids Res. 4 (1977) 3155.
43 W.H. Strätling, Biochemistry 18 (1979) 596.