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The interaction of the histone H1-related protein ϕ_0 with chromatin

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Protein ϕ_0 is a unique protein which is present in the sperm of the sea cucumber, *Holothuria tubulosa*. It associates with histones, but its physiological role is unknown. From its amino acid composition and sequence, protein ϕ_0 can be considered as an H1-related protein. In this paper, we have studied its interaction with chicken erythrocyte chromatin particles of different complexity, from core particles to polynucleosomes. Addition of protein ϕ_0 results in marked chromatin insolubilization. The higher the molecular weight of the chromatin fragment, the lower is the ϕ_0/nucleosome molar ratio at which precipitation occurs, so that complete insolubilization of polynucleosomes is achieved at a ϕ_0/nucleosome molar ratio which is identical to that found in mature *H. tubulosa* spermatozoa. We have also found that the interaction of protein ϕ_0 with chromatin is cooperative. These findings contribute to clarification of the peculiar physico-chemical properties shown by *H. tubulosa* sperm chromatin and the role played by the ϕ_0 protein.

1. Introduction

The chromatin of the spermatozoa from the sea cucumber, *Holothuria tubulosa*, has a peculiar composition, in which the normal complement of histones is accompanied by a small amount (about 4%) of a strongly basic low molecular weight protein, which we have called ϕ_0 [1]. Previous experiments from our laboratory [2–5] have shown that *H. tubulosa* sperm chromatin is rather remarkable. On the one hand, purified nuclei swell only slightly at low ionic strength, and when they are subjected to micrococcal nuclease digestion only about 50% of the total DNA can be solubilized. All the ϕ_0 protein remains associated with the insoluble fraction. Furthermore, during micrococcal nuclease digestion protein ϕ_0 appears to displace histones from core particles, giving rise to anomalous nucleosomal particles which contain exclusively ϕ_0 and histone H1.

In order to establish whether protein ϕ_0 is responsible for this unusual physico-chemical behaviour, we have studied in detail the in vitro interaction of protein ϕ_0 with different chromatin particles of increasing complexity. Protein ϕ_0 is 78 amino acids long and contains 17.0% lysine, 25.9% arginine and 29.5% alanine [6]. In spite of its high arginine content, this protein is related to histone H1, since its sequence is very similar to the C-terminal region of histone H1 [6], as long as arginine is considered equivalent to lysine. Moreover, antisera induced against protein ϕ_0 cross-react with histone H1 [7]. Therefore, protein ϕ_0 appears to be equivalent to an H1 histone which lacks the central globular region. In this paper, we have found that addition of protein ϕ_0 to chromatin particles of increasing size, ranging from core particles to polynucleosomes, results in their insolubilization. We have observed that the higher the molecular weight of the particle, the lower is

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the molar ratio necessary for its insolubilization. Insolubilization of polynucleosomes occurs at a ϕ_0/nucleosome molar ratio which is very close to that found in mature *H. tubulosa* spermatozoa, suggesting that this protein is actually responsible for the high insolubility shown by *H. tubulosa* sperm chromatin. We have also found that the interaction of protein ϕ_0 with chromatin is cooperative.

2. Materials and methods

2.1. Preparation of nuclei and chromatin

Chicken erythrocyte nuclei were prepared according to ref. 8. Chromatin fragments of various lengths were obtained by micrococcal nuclease digestion, followed by fractionation through sucrose gradients as described previously [9,10]. To obtain short oligomers (up to octanucleosomes), purified nuclei were digested at an enzyme/DNA ratio of 50 Boehringer U/mg DNA for 15 min. Polynucleosomes were obtained after digestion with 15 enzyme U/mg DNA for 1.5 min. The DNA and protein contents of the samples used in these experiments were analyzed by gel electrophoresis (not shown).

2.2. Protein ϕ_0 purification

Protein ϕ_0 was obtained from *H. tubulosa* spermatozoa by differential acid extraction and purified to homogeneity by chromatography as described elsewhere [6].

2.3. Protein ϕ_0-chromatin interaction

Purified protein ϕ_0 was dissolved in 10 mM Tris-HCl, 1 mM EDTA (pH 7.5) (buffer A) to a concentration of 2 mg/ml. The actual concentration was determined by turbidometry [11].

Chromatin in buffer A was diluted to 1 ml ($A_{260} = 0.3$) with the same buffer and the salt concentration adjusted by the addition of 4 M NaCl. Protein ϕ_0 was then added at different ϕ_0/nucleosome molar ratios. Following a 10 min incubation at room temperature, complexes were centrifuged in an Eppendorf microfuge for 10 min. The percentage of soluble chromatin was determined from the A_{260} of the supernatants. Proteins contained in supernatants and pellets were analyzed by SDS gel electrophoresis [12]. Prior to electrophoretic analysis, proteins contained in supernatants were precipitated with 18% trichloroacetic acid.

3. Results

3.1. Solubility of chromatin fragments

The solubility of the chromatin fragments used in our experiments is shown in fig. 1. Chromatin core particles are soluble throughout the entire range of ionic strengths examined. The presence of lysine-rich histones H1 and H5 confers marked insolubility to chromatin. The maximum of insolubility is centered around 0.15 M NaCl for oligonucleosomal particles. In particular, the fraction containing from penta- to octanucleosomes ($\bar{v} = 5.75$) is completely insoluble at 0.15 M NaCl. In contrast, polynucleosomal particles containing on average more than 50 nucleosomes are less insoluble than shorter oligonucleosomes and, ad-

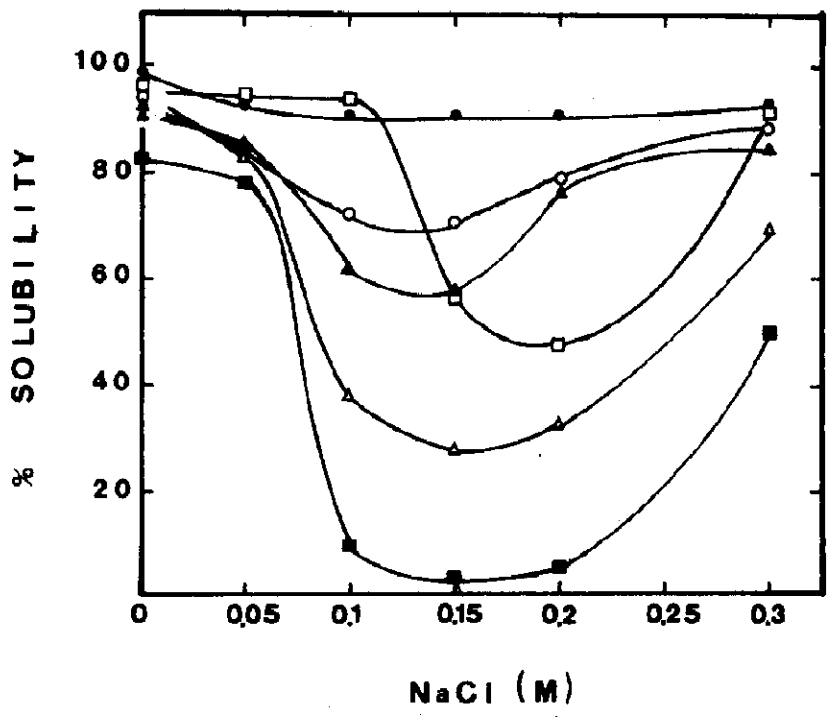

Fig. 1. Solubility of different chromatin fragments as a function of NaCl concentration. (●) Core particles, (○) mononucleosomes, (▲) dinucleosomes, (△) oligonucleosomes ($\bar{v} = 3.75$), (■) oligonucleosomes ($\bar{v} = 5.75$), (□) polynucleosomes ($\bar{v} > 50$). $\bar{v}$, average number of nucleosomes.

ditionally, the maximum of insolubility occurs at higher salt concentrations, around 0.20 M NaCl. This higher solubility is likely to be related with the organization of polynucleosomes into higher-order structures [9,13].

3.2. Addition of protein ϕ_0 to chromatin results in its insolubilization

The addition of increasing amounts of purified protein ϕ_0 to different chromatin particles results in their insolubilization. Fig. 2 shows the effect of adding increasing amounts of protein ϕ_0 on the solubility of different chromatin particles at three different NaCl concentrations, namely, 0, 0.05 and 0.15 M. In all cases, marked insolubilization is detected but the actual ϕ_0/nucleosome molar ratio at which precipitation occurs depends on both the ionic strength and complexity of the chromatin particle. Table 1 summarizes the results shown in fig. 2. In general, we have observed that for any chromatin fragment, the higher the salt concentration the lower is the molar ratio required for its insolubilization. Likewise, large oligonucleosomes are precipitated more easily than shorter fragments. For instance, at 0.05 M NaCl, complete precipitation of mononucleosomes occurs at a molar ratio of approx. 2.8 ϕ_0 molecules per nucleosome while oligonucleosomal samples containing 5.75 nucleosomes on average precipitate at a molar ratio of about 1.2 ϕ_0 molecules per nucleosome. However, a much larger excess of protein ϕ_0 is required to precipitate chromatin core particles which lack lysine-rich histones H1 and H5 as well as internucleosomal DNA. About 6 ϕ_0 molecules are necessary to achieve complete precipitation at 0.05 M NaCl.

On the other hand, polynucleosomal particles showed quite different behaviour. They are in general more soluble than shorter oligonucleosomal particles (fig. 1). Similarly, their complexes with protein ϕ_0 are also more soluble. At 0.05 M NaCl, complete insolubilization is achieved at a molar ratio of 3.5 ϕ_0 molecules per nucleosome. It is worth noting that the molar ratio required to insolubilize polynucleosomes at 0.15 M NaCl correlates with the amount of protein ϕ_0 present in mature spermatozoa. Since the amount required to insolubilize polynucleosomes at 0 M NaCl is similar to that required to precipitate shorter oligonucleosomes, it is likely that the higher solubility of the ϕ_0-polynucleosome complexes detected at 0.05 and 0.15 M NaCl might be related to the organization of polynucleosomes into thick 30-nm chromatin fibers which are known to occur at these NaCl concentrations [9,13].

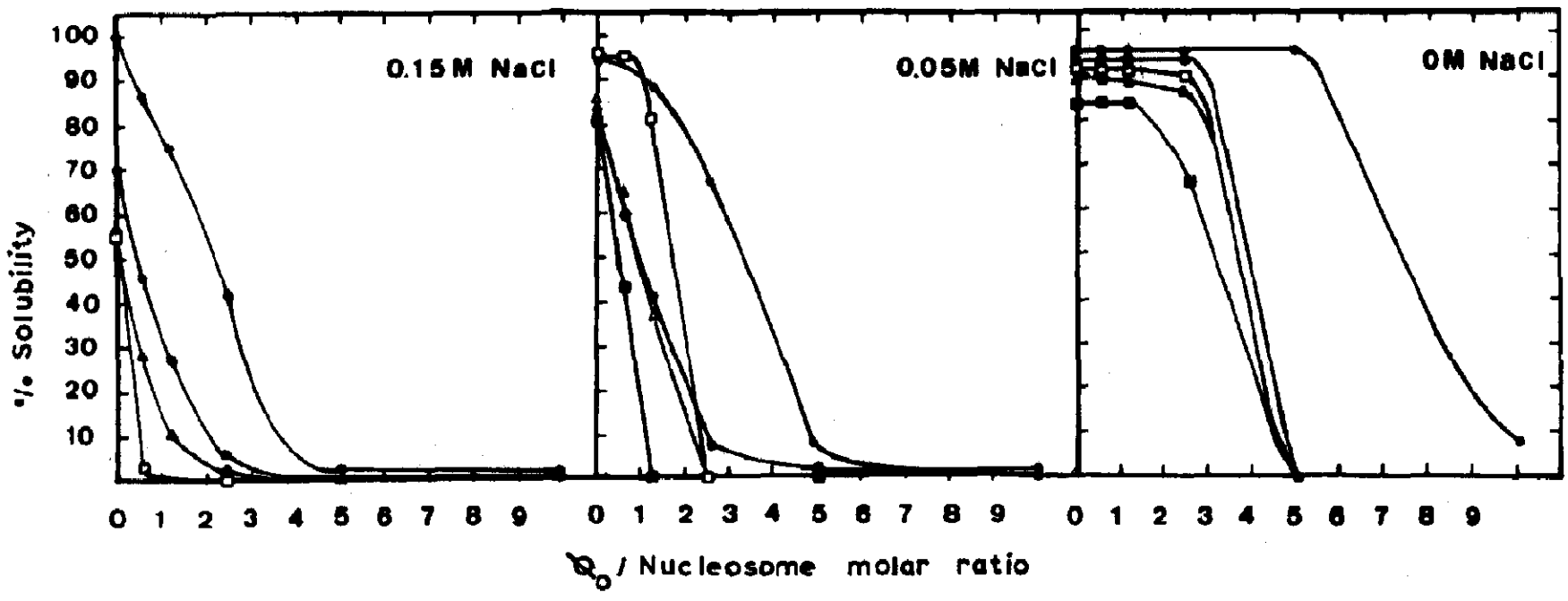

Fig. 2. Insolubilization of different chromatin fragments as a function of added protein ϕ_0 at three NaCl concentrations. (●) Core particles, (*) mononucleosomes, (▲) dinucleosomes, (△) oligonucleosomes ($\bar{v} = 3.75$), (■) oligonucleosomes ($\bar{v} = 5.75$), (□) polynucleosomes ($\bar{v} > 50$). $\bar{v}$, average number of nucleosomes.

Table 1

ϕ_0/nucleosome molar ratio at which 50% precipitation occurs for different chromatin particles at three NaCl concentrations

	NaCl concentration		
	0 M	0.05 M	0.15 M
Cores	6.7	3.2	2.3
Mononucleosomes	4.0	1.4	1.1
Dinucleosomes	–	–	0.6
Oligonucleosomes ($\bar{v} = 3.75$) [a]	3.7	1.0	0.4
Oligonucleosomes ($\bar{v} = 5.75$)	3.2	0.6	0
Polynucleosomes ($\bar{v} > 50$)	3.7	1.7	0.25

[a] $\bar{v}$, average number of nucleosomes.

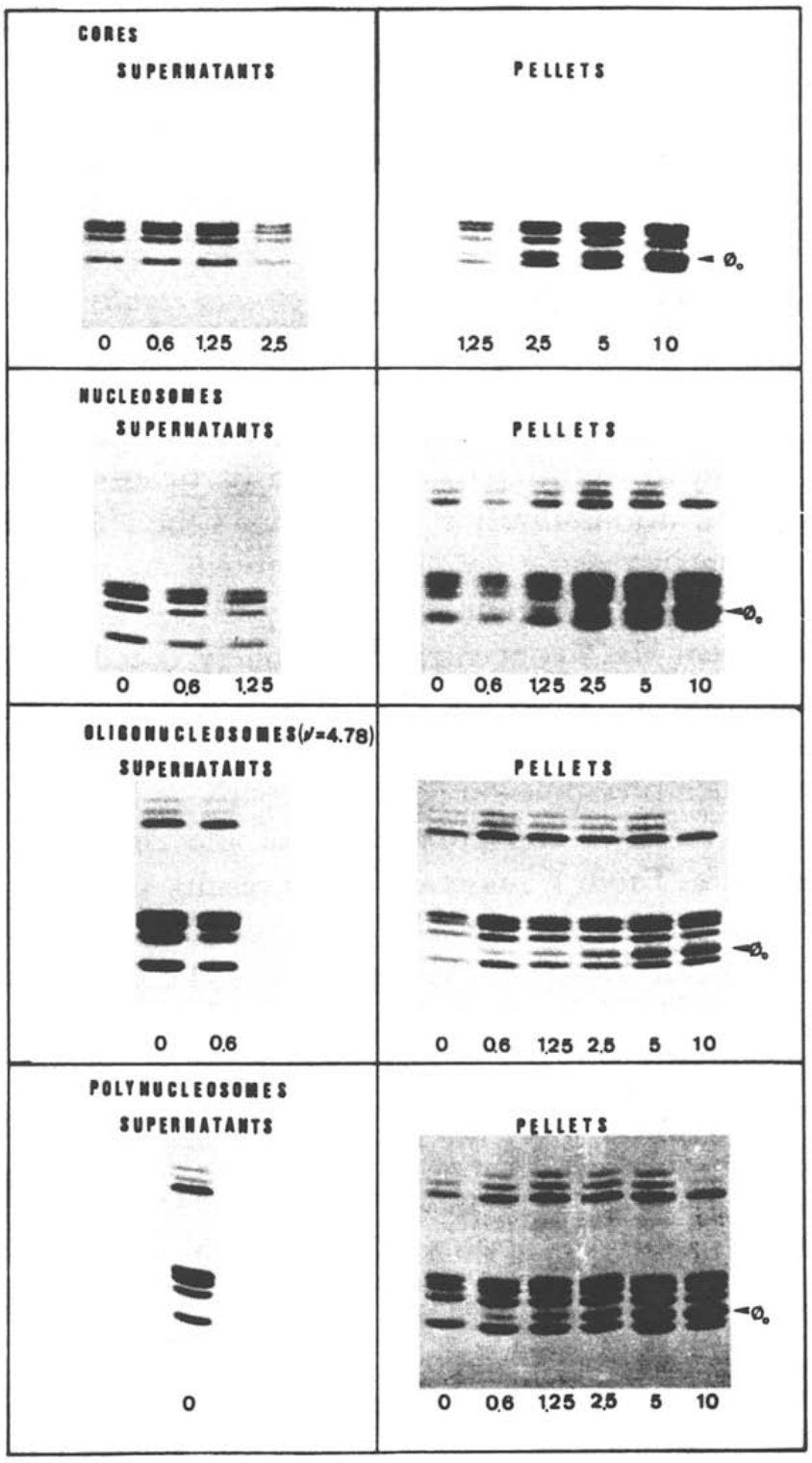

Fig. 3. SDS gel electrophoresis of proteins contained in the supernatants and pellets obtained after addition of increasing amounts of protein ϕ_0 to different chromatin particles when the interaction was carried out at 0.15 M NaCl. Values indicate the ϕ_0/nucleosome molar ratio at which the interaction took place.

3.3. *Interaction of protein ϕ_0 with chromatin is cooperative*

We have found that a given amount of ϕ_0 in the presence of more than its molecular equivalent of chromatin particles will bind extensively to some of these particles and only partially or not at all to the rest, indicating that the interaction of protein ϕ_0 with chromatin is cooperative. At NaCl concentrations of 0.15 or 0.05 M any ϕ_0-containing particle precipitates. Fig. 3 shows the SDS gel electrophoretic patterns of the supernatants and pellets obtained after addition of increasing amounts of protein ϕ_0 to different chromatin particles at 0.15 M NaCl. Protein ϕ_0 is always found in the precipitates (fig. 3, right-hand panels) so that it is never detected in the supernatants (fig. 3, left-hand panels). Similar results were obtained when the interaction was carried out at 0.05 M NaCl. From fig. 3 it appears that soluble mononucleosomal particles are deficient in lysine-rich histones H1 and H5. However, since the corresponding mononucleosomal pellets are not enriched in these proteins, we conclude that the apparent depletion of H1 and H5 in soluble mononucleosomes is mostly due to losses occurring during preparation of the samples prior to electrophoresis, as has been observed by others [15]. As shown in fig. 4, the ϕ_0/nucleosome molar ratio of the pellets is constant and independent of the input molar ratio until complete precipitation of chromatin occurs, indicating that protein ϕ_0 does not distribute itself evenly among all accessible particles but rather interacts in a cooperative fashion with chromatin. At an input ϕ_0/ nucleosome molar ratio of unity and assuming a random non-cooperative interaction, each mononucleosome should have received one ϕ_0 molecule. In contrast, at this input molar ratio we observed

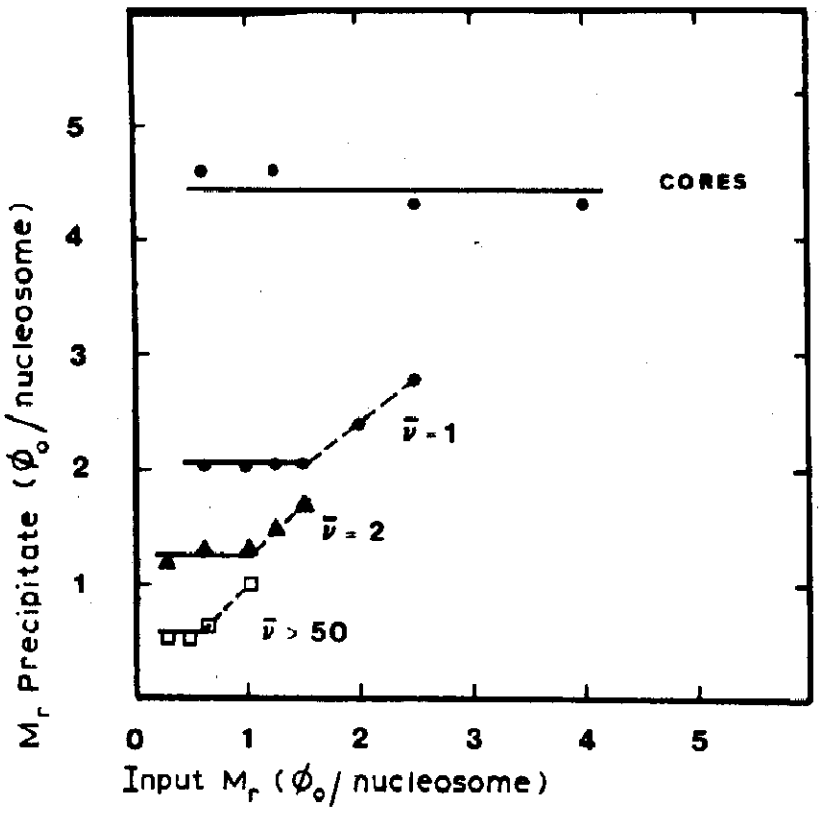

Fig. 4. ϕ_0/nucleosome molar ratios (M_r) of the precipitates as a function of the input molar ratio for different chromatin fragments. $\bar{\nu}$, average number of nucleosomes.

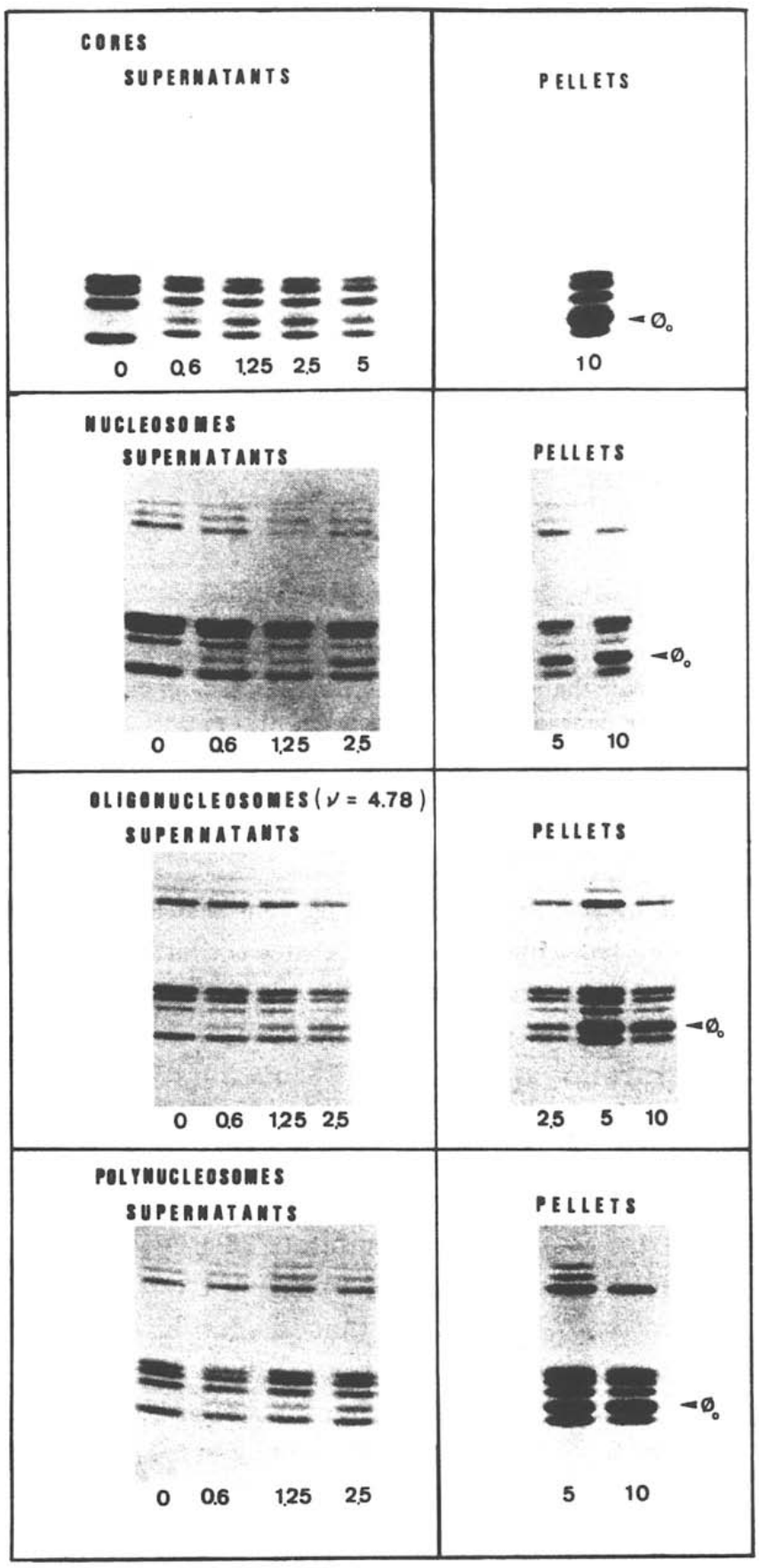

Fig. 5. SDS gel electrophoresis of proteins contained in the supernatants and pellets obtained after addition of increasing amounts of protein ϕ_0 to different chromatin particles when the interaction was carried out at 0 M NaCl. Values indicate the ϕ_0/nucleosome molar ratio at which interaction took place.

that half of the mononucleosomal particles contained two ϕ_0 molecules while the remaining 50% contained none.

When the interaction is carried out at 0 M NaCl the behaviour is quite different, since under these salt conditions protein ϕ_0 is found either in the supernatants, at low molar ratios, or in the pellets, at higher molar ratios (fig. 5). That, at low input molar ratios, protein ϕ_0 actually interacts with chromatin under these salt conditions is demonstrated by the fact that, following incubation with protein ϕ_0 at an input molar ratio of 0.6, this protein is found associated with mononucleosomes purified by sucrose gradient centrifugation. In order to establish whether the interaction is also cooperative at this ionic strength the following experiment was performed. Either core particles or mononucleosomes were incubated at 0 M NaCl with protein ϕ_0 at different molar ratios. Following incubation for 10 min, the salt concentration was raised to 0.15 M NaCl and the percentage of soluble particles determined. We obtained a similar value for the percentage of soluble particles as if the interaction had been performed directly at 0.15 M NaCl, suggesting that the interaction had also been cooperative at 0 M NaCl. These results might also be interpreted as being the consequence of redistribution of protein ϕ_0 at 0.15 M NaCl. However, when purified *H. tubulosa* sperm nuclei were incubated at 0.15 M

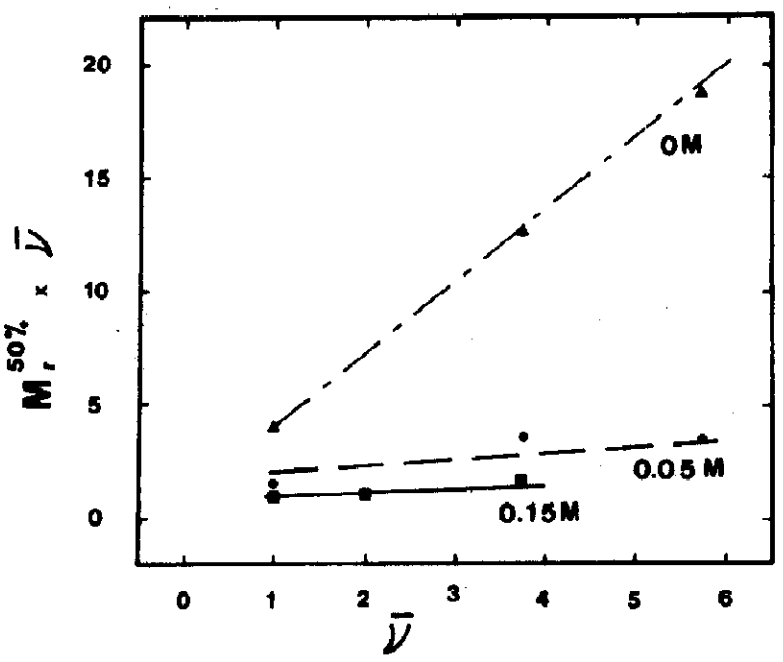

Fig. 6. The total number of protein ϕ_0 molecules required to precipitate 50% of the chromatin particles is represented as a function of the average number of nucleosomes in each particle. The interaction was carried out at: (■) 0.15 M, (●) 0.05 M and (▲) 0 M NaCl. $M_r^{50\%}$, molar ratio at which 50% precipitation occurs. $\bar{v}$, average number of nucleosomes.

NaCl in the presence of long chicken erythrocyte polynucleosomal fragments, no redistribution of protein ϕ_0 was detected [14].

The results given in table 1 indicate that the molar ratio at which 50% precipitation occurs decreases as the size of the chromatin particle increases. In fact, as shown in fig. 6, at 0.15 or 0.05 M NaCl, a similar total number of ϕ_0 molecules are required to precipitate oligonucleosomal particles of an average size ranging from 1 to 5.75 nucleosomes, suggesting that insolubilization of a single nucleosome with about 2 molecules of ϕ_0 is sufficient to precipitate oligonucleosomes containing up to 5.75 nucleosomes on average. At 0 M NaCl, the total number of protein ϕ_0 molecules required for precipitation increases with the size of the chromatin particle (fig. 6), again reflecting the higher solubility of ϕ_0-chromatin complexes at this salt concentration.

4. Discussion

As discussed in section 1, protein ϕ_0 can be considered as an H1-related protein which is smaller – lacking the central globular part – and richer in arginine than somatic H1. However, in contrast to what is found with other histone H1 variants, chromatin containing protein ϕ_0 appears to be highly insoluble, since no ϕ_0-containing particles can be solubilized by micrococcal nuclease digestion of purified nuclei or chromatin [2,3]. In addition, protein ϕ_0 appears to be capable of displacing core histones from nucleosomes, giving rise to anomalous nucleosomal particles [3].

In order to establish whether protein ϕ_0 is actually responsible for this very peculiar behaviour, we have studied the in vitro interaction of protein ϕ_0 with chicken erythrocyte chromatin. We have found that the addition of protein ϕ_0 actually results in marked chromatin insolubilization. It is known that in general, addition of histones to nucleosomal particles induces their precipitation [16]. Precipitation induced by core histones occurs when the amount added exceeds the in vivo core histone complement of native chromatin several fold. On the other hand, histones H1 and H5 induce chromatin precipitation at molar ratios which are close to the in vivo situation. In agreement with its similarity to histone H1, the insolubilization effect detected here for protein ϕ_0 occurs at physiological ionic strength and within a range of ϕ_0/nucleosome molar ratios which is similar to that shown by mature *H. tubulosa* spermatozoa.

We have also determined some of the features of the interaction of protein ϕ_0 with chromatin:

(i) The interaction appears to be cooperative at any salt concentration within the range 0–0.15 M NaCl.

(ii) The lower the ionic strength the higher is the solubility of the ϕ_0-chromatin complex. As shown in table 1 and fig. 6, when the interaction is carried out at either 0.15 or 0.05 M NaCl, insolubilization is achieved with a much lower quantity of ϕ_0 than that at 0 M NaCl. At 0.15 or 0.05 M NaCl, it appears that the amount of ϕ_0 required to precipitate an oligonucleosome is almost constant and does not depend on its size, in contrast with what is found at 0 M NaCl.

(iii) At physiological ionic strength, about 2 molecules of ϕ_0 are required to insolubilize a mononucleosome, whereas an excess of about 5 molecules of ϕ_0 is required to precipitate chromatin core particles lacking lysine-rich histones

and internucleosomal DNA.

(iv) Complexes formed with large polynucleosomal fragments are insoluble at a molar ratio of 0.5 molecules of ϕ_0 per nucleosome at 0.15 M NaCl, which is very similar to the in vivo ϕ_0 molar ratio of mature *H. tubulosa* spermatozoa.

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References

1 J.A. Subirana, Exp. Cell Res. 63 (1970) 253.
2 F. Azorin, C. Olivares, A. Jordan, L. Perez-Grau, L. Cornudella and J.A. Subirana, Exp. Cell Res. 148 (1983) 331.
3 F. Azorin, E. Rocha, L. Cornudella and J.A. Subirana, Eur. J. Biochem. 148 (1985) 529.
4 J.A. Subirana, S. Muñoz-Guerra, A.B. Martinez, L. Perez-Grau, X. Marcet and I. Fita, Chromosoma 83 (1981) 455.
5 L. Cornudella and E. Rocha, Biochemistry 18 (1979) 3724.
6 A. Jordan, Ph.D. Thesis, Faculty of Chemistry, University of Barcelona (1982).
7 M.T. Casas, Ph.D. Thesis, Faculty of Biology, University of Barcelona (1986).
8 A.L. Olins, R.D. Carlson, W.B. Wright and D.E. Olins, Nucleic Acids Res. 3 (1976) 3271.
9 F. Azorin, L. Perez-Grau and J.A. Subirana, Chromosoma 85 (1982) 251.
10 L. Perez-Grau, F. Azorin and J.A. Subirana, Chromosoma 87 (1982) 437.
11 P.S. Rasmussen, K. Murray and J.M. Luck, Biochemistry 1 (1962) 79.
12 U.K. Laemli, Nature 227 (1970) 680.
13 F. Thoma and T. Koller, J. Mol. Biol. 149 (1981) 709.
14 C. Olivares, Ph.D. Thesis, Facultad de Biologia, Universidad de Barcelona (1987).
15 D.L. Bates, P.J.G. Butler, E.C. Pearson and J.O. Thomas, Eur. J. Biochem. 119 (1981) 469.
16 G. Voordouw and H. Eisenberg, Nature 273 (1978) 446.