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A FURTHER ASSESSMENT OF THE SUB-NUCLEOSOMAL PRODUCT, "PEAK A", FROM PHYSARUM POLYCEPHALUM

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Nuclease digestion studies of Physarum polycephalum nuclei (1-3) and nucleoli (4) over the past few years have been centred on a number of modified nucleosomal products which have been related to active-gene regions of the genome. We have re-investigated one such particle, peak A, using the techniques of differential melting and polyacrylamide gel electrophoresis and show that this material is unlikely to be a specific histone:DNA complex as suggested by earlier authors. © 1985 Academic Press, Inc.

The continued interest, as shown by the recent publication of Czupryn and Toczko (3), in the structure-functional relationships of core particles and modified core particles from Physarum polycephalum has led us to closely re-examine (5) one of these entities.

It has been shown by Johnson et al (1,2) that treatment of Physarum nuclei with staphylococcal nuclease can give rise to sub-nucleosomal products (peak A) which have enhanced hybridisation potential for ribosomal RNA. The peak A product has a sedimentation coefficient of 5S and is quoted as having a maximum possible protein:DNA ratio of 1:1. This compares to the nucleosome core particle which has a histone:DNA ratio of 1.2:1 and a sedimentation coefficient of 11S.

We have prepared peak A material, and its initial characterisation (sedimentation coefficient, DNA PAGE and histone complement) is shown to be similar to that presented in the earlier publications. However a more detailed

ABBREVIATIONS: Tris, Tris-(hydroxymethyl)-amino methane; EDTA, Ethylene diamine tetra acetic acid; PMSF, Phenyl methyl sulphonyl fluoride; TBE, Tris, 90 mM, EDTA 2.5 mM adjusted to pH 8.3 with 0.48% boric acid; SDS, Sodium dodecyl sulphate; PAGE, Polyacrylamide gel electrophoresis; bp, Base pairs.

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physico-biochemical investigation has, in our hands, failed to show the presence of any structurally significant association between histone and DNA in peak A.

MATERIALS AND METHODS

All procedures for the isolation of nuclei and the production of nuclease digestion products were carried out at 4°C unless otherwise stated.

Isolation of Nuclei

Physarum polycephalum microplasmodia, strain M3C VI were grown at 26°C in liquid culture using the semi-defined medium as described by Daniel and Rusch (6). The microplasmodia were harvested in mid log-growth phase and after washing twice with ice-cold water, nuclei were released by homogenisation using a Waring Blender (260 v, half speed, 30s) in a medium comprising 250 mM sucrose, 10 mM Tris, 0.1% Triton X-100, 5 mM $MgCl_2$, pH 7.0-7.2.

After allowing to stand for ten minutes the suspended nuclei were syphoned through two layers of milk filters (Cresta) pelleted by centrifugation, 1325xg, 10 mins, washed and resuspended in digestion buffer (20 mM Tris, 15 mM NaCl, 1 mM $CaCl_2$, 1 mM PMSF, pH 7.8).

Nuclease Digestion and Isolation of Digestion Products

The digestion technique was that essentially described by Johnson et al (1), using a nuclease:DNA ratio of between 50-100 units of micrococcal nuclease per mg DNA for various time intervals at 37°C. The released digestion products were fractionated by centrifugation through a 5%-25% isokinetic sucrose gradient.

Preparation of Core Particles

Chicken erythrocyte core particles were prepared following the procedure of Sibbet and Carpenter (7) and Physarum core particles as described by Stone (5).

Melting Studies

Changes in absorbance at 260 nm in a 1 cm path length cell, as a function of temperature, were monitored using a Perkin-Elmer 572 spectrophotometer. The temperature was increased at a rate of one degree per minute and measured using a platinum resistance thermometer. The data was transferred via a low noise instrumental amplifier to a BBC Model B microcomputer and analysed using programs written in the Biophysics Laboratories, Portsmouth, by Dr A.J. Hunter.

Polyacrylamide Gel Electrophoresis

(i) Histones were characterised by employing a 1% sodium didecyl sulphate medium as described by Laemmli (8).

(ii) Non-denaturing gels were used to analyse intact DNA histone complexes and free double stranded DNA. They contained 7.7% acrylamide, 0.3% bisacrylamide in a TBE buffer containing 90 mM Tris 2.5 mM EDTA adjusted to pH 8.3 with 0.48% boric acid.

(iii) Single stranded DNA gels were prepared containing 11.6% acrylamide, 0.4% bisacrylamide in TBE buffer containing 7 M urea. Electrophoresis was carried out using TBE buffer without urea at a high current.

Visualisation of (i) was with Coomassie Blue; for (ii) and (iii) ethidium bromide was used.

RESULTS AND DISCUSSION

Figure 1 shows the sedimentation profile through a 5%-25% sucrose density gradient of the products obtained from a nuclease digest of Physarum nuclei carried out under conditions similar to those specified by Johnson et al (1). The material sedimenting at 5S was designated as "peak A" by the original authors.

If fractions through the gradient profile (Fig. 1) are subjected to SDS polyacrylamide gel electrophoresis it reveals that there are histones present in the peak A, monomer and dimer core particle fractions as well as in the slow migrating region prior to peak A. The histones in peak A are predominantly Band III (H2A) and Band IV (H2B and H3) together with some slower migrating non-histone proteins and possible degradation products. There is no visible presence of Band VI (H4). (The nomenclature of Mohberg and Rusch (9) was used for Band assignments). The results for total Physarum histone and the histone associated with monomer and dimer core particles migrating under similar conditions are also presented in Figure 1.

A comparison, in non-denaturing (nucleohistone) gels, of the electrophoretic migration pattern of peak A and the monomer and dimer core particles with the DNA extracted from these entities is highly revealing, (Fig 2). Whereas the DNA from mono and dimer core particles migrates much faster than the corresponding histone-DNA complexes, the peak A material and the monomer extracted DNA migrate in the same position. Further, although the monomer and dimer core particle bands stain for protein with Coomassie Blue, that for peak A does not. This observation shows that the DNA and protein from peak A are not complexed under the conditions prevailing for the electrophoresis experiment and implies either the initial interaction was extremely weak or there was no interaction at all. If there was no interaction originally

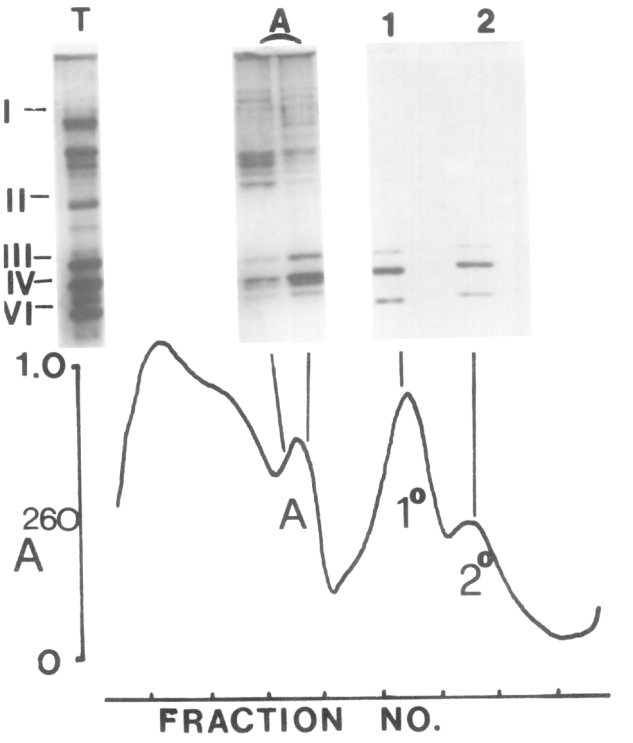

Figure 1

Separation of the nuclease digestion products of Physarum nuclei.

After pelleting the digested nuclei by centrifugation (1500xg, 10 min) the remaining supernatant was overlayered onto a 5%-25% isokinetic sucrose gradient containing 10 mM tris, 0.5 mM EDTA, 0.1% PMSF, 350 mM NaCl, pH 7.4. Centrifugation was carried out for 24 hours at 284,000xg or at 115,300xg for 36 hours. The gradient profiles were obtained by monitoring the absorbance at 254 nm or 280 nm after vertical displacement of the media by Maxidens (Nyegaard).

The histone profiles (after dissociation with SDS of pertinent fractions of the gradient) are displayed as their electrophoretic migration in SDS polyacrylamide gels.

(T) Total Physarum histone extract.

(A) Histones from Peak A.

(1) Histones from monomer core particle fraction.

(2) Histones from dimer core particle fraction.

present it is not unreasonable to postulate that the histones derived from the peak A region of the gradient are self-contained protein complexes which can sediment with low s-values. Such observations have been made by Thomas and Butler (10) who have reported that complexes of core particle histones from rat liver can sediment with s^{20}_{w} values of 4.8S.

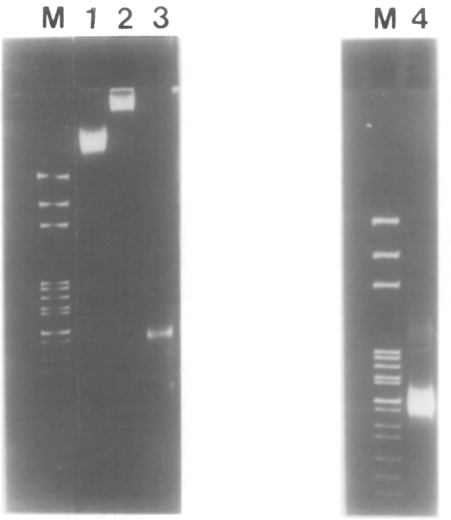

Figure 2

Non-denaturing nucleohistone gels. PAGE of the products from micrococcal digestion of Physarum nuclei.

(M) DNA marker fragments produced by the restriction of pAT 153 by Hpa II. (The assignment of the two bands migrating in the vicinity of core particle length DNA and peak A material are 160 bp and 147 bp respectively).

(1) Monomeric core particles.

(2) Dimeric core particles.

(3) DNA extracted from monomer core particles using chloroform/phenol (1:1).

(4) Peak A material, obtained directly from the sedimentation experiment.

Melting profiles of histone-nucleic acid complexes have been studied for many years (11), and it is well established that any stability introduced into the DNA by protein binding is made manifest by an increase in melting temperature compared to that of pure DNA. To ascertain if it was at all possible that the potential applied during electrophoresis could have brought about a dissociation of a peak A protein-DNA complex we considered the melting profile of peak A, comparing this to core particle length DNA from Physarum and from chicken erythrocytes as well as core particles from both sources.

Figure 3(C) shows the derivative melting profile in 0.25 mM Na_3 EDTA of Physarum core particles and the DNA extracted from these particles with phenol/chloroform. The Physarum core particles show a biphasic melt with the

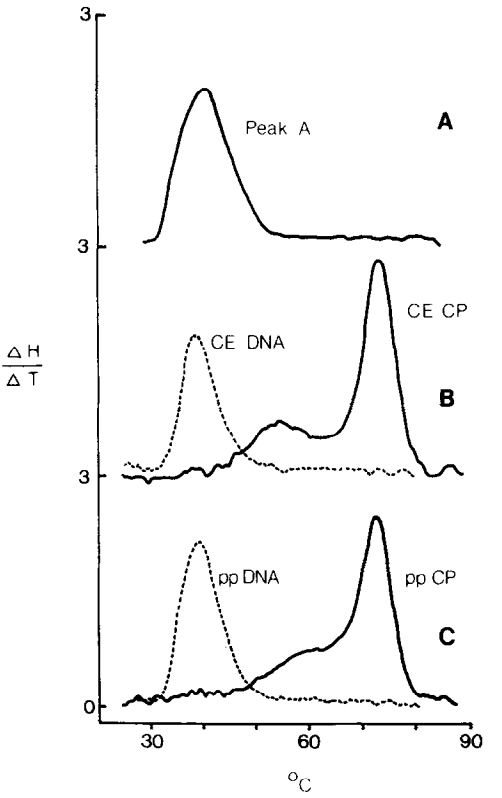

Figure 3

Derivative (change in hyperchromicity per unit temperature rise) melting profiles, in 0.25 Na_3 EDTA, of :

(A) Peak A from Physarum polycephalum.

(B) Core particle length DNA and core particles from chicken erythrocytes.

(C) Core particle length DNA and core particles from Physarum polycephalum.

principal maximum at ~72°C and a subsidiary maximum at ~59°C. These values and the general shape of the curve compare well to the melting profile of chicken erythrocyte core particles, Fig 3(B). The DNA from both Physarum and chicken erythrocyte core particles melts at much lower temperatures, 37.2±0.9°C and 38.2±0.8°C respectively; the curves in each case providing just one maximum. The melting profile of peak A is also monophasic, Fig 3(A), with a melting temperature of 39.8±1.1°C. The general features of both DNA and peak A melting curves are thus very similar, and differ from those of structural DNA complexes. Slight differences between the peak A and DNA curves can possibly be assigned to the presence of contaminating histones. The latter are expected to be

present in the sample, since after fractionation on the sucrose gradient no further sample purification was attempted apart from changing the sucrose gradient buffer for 0.25 mM Na_3 EDTA. The evidence thus indicates there is no enhancement of the stability of peak A DNA induced by the presence of Physarum histones. If there is any association of histones with DNA it must be nominal and of a non-structure inducing nature. We have also observed (5) that when attempting to purify Physarum core particles by previously published methods, there has always been a production of material sedimenting at 5S on secondary and subsequent recentrifugation of the core particle fraction.

Consideration of the electrophoretic patterns of the histones obtained from peak A as well as monomer and dimer core particles show a minor band migrating between Bands IV and VI. Although it has been suggested that this is a "fifth histone component" of Physarum core particles we believe it is a degradation product, possibly derived from H3. This postulate is substantiated when the histones extracted from a rapid preparation of Physarum core particles, and which show no indication of the "fifth" histone are compared to the profile of histones taken across the density gradient in figure 1. In the former preparation histones were kept in contact with solution for approximately three times longer than in the rapid core particle preparation.

The generation of a characteristic ten base pair ladder produced by the DNase I digestion of nucleosome core particles results from the enzyme's restricted access to the DNA due to (i) histone protection of the inside surface of the DNA, (ii) the shape of the DNA as it folds about the core histone (12), (iii) the protection by adjacent superhelical turns of DNA (13). Therefore if peak A contains protected or structural DNA regions some distinctive DNase 1 digest pattern would be expected. This (Fig 4) is not the case; the peak A material is digested in a similar way to that expected for histone-free DNA, with the products of the digestion being a random distribution of DNA lengths which appear as a smear on the gel. By comparison,

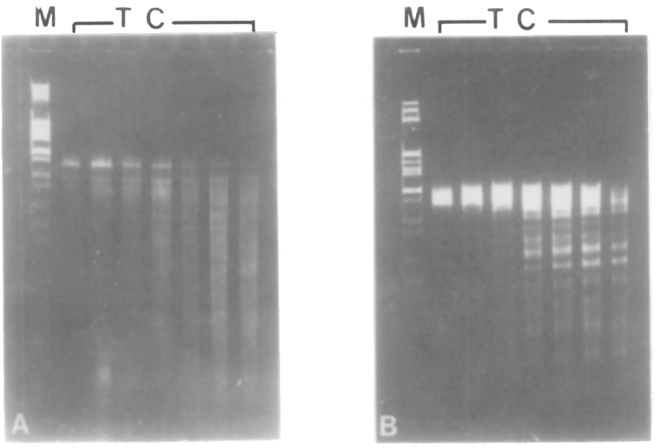

Figure 4

The course of DNase I digestion with time (TC) of (A), peak A material, (B), Physarum core particles as revealed by DNA single stranded PAGE. Times of digestion at a nuclease:DNA ratio of 4 units mg^{-1} were, for peak A (L→R) 0, 1, 3, 5, 10, 15 and 20 minutes for core particles (L→R) 0, 1, 3, 5, 10, 20 and 30 minutes.

the DNase I digest pattern of the Physarum core particles give the expected distinct ladder pattern.

Previous authors have shown that during micrococcal nuclease digestion of Physarum nuclei there is preferential excision of ribosomal DNA, with peak A being the corresponding subunits derived from these active genes. Structural changes in Physarum nucleoli have also been studied (4), where it is suggested that in actively transcribing chromatin the nucleosome core particle opens out to form an elongated arrangement of histone and DNA termed the lexosome. Congruency between these two entities appears yet to be established. A notable difference is that the lexosome contains a relatively large amount of two high molecular weight proteins (LP32 and LP30) which are different from the non-histone proteins associated with peak A.

The conclusion of this present communication is therefore that although modified structures do most likely occur in active genes the subunit structure described as peak A is not a discrete histone-DNA complex when isolated by centrifugation. The question then remains : are active regions of Physarum nuclear chromatin different from nucleolar chromatin? A more detailed

examination of the nucleolar subunits, together with a determination of the relative binding of the core histones in active and non-transcribing genes in Physarum polycephalum appears a most worthwhile study.

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