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SOME UNUSUAL FEATURES OF PHYSARUM POLYCEPHALUM
CHROMATIN ARE DUE TO THE PRESENCE OF SLIME*

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Chromatin of lower eukaryote Physarum polycephalum, while showing typical
nucleosomal organization, reveals upon digestion with micrococcal nuclease
certain features not found in chromatin of higher eukaryotes, the most
pronounced of which is the unusual pattern of degradation of core-size DNA,
without accumulation of subcore fragments. It has been shown that these
peculiarities are not due to intrinsic features of Physarum nucleohistone complex
but to the presence of a specific polysaccharide, the main component of
Physarum slime, contaminating chromatin preparations.

The nucleosomal structure of chromatin has been found in all eukaryotic
species so far examined (Kornberg, 1977; Felsenfeld, 1978). As a rule, the
same scheme of nucleosome is present both in higher and lower eukaryotes
except that the latter have a slightly smaller average length of nucleosomal
DNA (Lohr & Van Holde, 1975; Morris, 1976; Noll, 1976; Staroń et al.,
1977).

Prolonged digestion of nuclei with micrococcal nuclease yields nucleosomes
which become further converted into distinct subcore nucleoprotein fragments
containing DNA of between 140 and 30 base pairs (Axel, 1975; Compton
et al., 1976). These subcore nucleoproteins are less sensitive to micrococcal
nuclease than whole chromatin. This is the reason of about 50% limit
of digestion of DNA to acid-soluble products observed even upon very
long incubation with the enzyme (Sollner-Webb & Felsenfeld, 1975).

The situation is different when nuclei from plasmodia of Physarum
polycephalum, a primitive slime mould, are digested with micrococcal

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nuclease. Prolonged digestion does not result in a series of subcore DNA fragments but, instead, it is a core-size fragment which seems to be protected against further digestion. We have found that this peculiar feature of Physarum chromatin is due to the presence of an acidic polysaccharide from slime which has been reported to occur also in Physarum nuclei (Farr & Horisberger, 1978).

MATERIALS AND METHODS

Materials. The M3CIV strain of Physarum polycephalum was grown in submerged shaken cultures as described by Daniel & Baldwin (1964). To label DNA, 0.5 μCi/ml of [3H]thymidine was added to the growth medium. Cultures were harvested after two days of growth and used immediately.

Fresh calf thymus was cut into small pieces, immediately frozen and kept at −20°C until used.

Preparation of polysaccharide from Physarum slime. The polysaccharide was prepared from growth medium after prolonged (4 days) culturing of Physarum according to Farr et al. (1972). The crude polysaccharide preparation was digested with DNAase I, RNAase and pronase and then was deproteinized by the chloroform-isoamyl alcohol procedure (Stern, 1968).

Preparation of nuclei and digestion with nuclease. Both Physarum and calf thymus nuclei were prepared by the method described by Jockusch & Walker (1974) with 0.1% Triton X-100. When indicated the solution of polysaccharide (10 mg/ml) was slowly added to the suspension of calf thymus nuclei to the final concentration of 1 mg/ml. The reaction with micrococcal nuclease was carried out at 37°C and was stopped by adding Na-EDTA to the final concentration of 10 mM and chilling on ice. The suspension was then used for isolation of DNA by gel electrophoresis. When the percentage of DNA hydrolysis was to be determined, the enzymatic reaction was stopped by adding an equal amount of cold 0.8 M-perchloric acid in 0.8 M-NaCl. After 10 min at 0°C the suspension was centrifuged at 1000 g for 10 min and the pellet was used for the estimation of DNA by the method of Burton (1956) or the radioactivity was counted in the supernatant in a liquid scintillation counter.

In all experiments except that illustrated in Fig. 1, fresh solution of phenylmethylsulphonylfluoride (PMSF) was added to all buffers used for preparation and digestion of nuclei to the concentration of 1 mM.

Preparation of DNA and electrophoresis. DNA was isolated with the use of the chloroform-isoamyl alcohol procedure as described by Stern (1968). DNA fragments were analysed in 3.5% polyacrylamide gels as described earlier (Jerzmanowski et al., 1976) except that ethidium bromide was absent in the sample. Gels were stained with “Stains all” or with ethidium bromide (1 μg/ml).
Reagents. The reagents used were as follows: micrococcal nuclease, Worthington (N.J., U.S.A.), DNAase I and ethidium bromide, Calbiochem (Los Angeles, U.S.A.), RNAase and pronase, Koch-Light (Colnbrook, U.K.), phenylmethylsulphonylfluoride and "Stains all", Serva (Heidelberg, F.R.G.), [3H]thymidine (18 Ci/mmol), UVVVR (Prague, Czechoslovakia).

RESULTS

The limit of digestion of Physarum nuclear DNA with micrococcal nuclease is dependent on whether the action of endogenous proteases has been stopped or not. For nuclei isolated and digested without the protease inhibitor added the amount of acid-soluble products reached 90 - 100% of total DNA (Fig. 1). Addition of the protease inhibitor, PMSF, to the concentration of 1 mM to both isolation and incubation media resulted in lowering of the amount of acid-soluble products (Fig. 1). However, even in this case the digestion gave more than 50% of acid-soluble products, thus exceeding the limit usually observed for chromatin of higher eukaryotes.

The pattern of degradation of chromatin DNA in Physarum nuclei was not dependent on the presence of PMSF but was clearly different from the patterns observed for chromatin of higher eukaryotes. Prolonged digestion

![Fig. 1. Time-course of hydrolysis of DNA in Physarum nuclei during digestion with micrococcal nuclease. with no PMSF (△) and with 1 mM-PMSF (○) present during both isolation and incubation of nuclei. Micrococcal nuclease: 100 units/ml, DNA concentration: 100 μg/ml.](image-url)
of calf thymus nuclei resulted in a gradual shift of the bulk of core-size DNA towards lower-size fragments positioned directly below it (Fig. 2). In the case of *Physarum* nuclei the bulk of DNA visible on the gel remained in the position of the core fragment throughout the whole digestion time (Fig. 3). Although traces of subcore fragments could also be seen, they constituted an only negligible part of total DNA on the gel and there was no visible shift of the bulk of DNA towards their position. The intense diffuse band located in lower part of the gel on Fig. 3A did not originate from DNA but represented a polysaccharide compound which always accompanied DNA preparations from *Physarum* (Hall et al., 1975). On gels stained with “Stains all” it could be distinguished from DNA by different colour of staining. However, to make sure that no polysaccharide was taken for DNA and *vice versa* we stained a parallel gel with ethidium bromide (Fig. 3B).

Since a considerable amount of acidic polysaccharide was present in *Physarum* nuclei preparations, it seemed reasonable to suspect that it could be responsible for the unusual feature of *Physarum* chromatin revealed upon digestion with micrococcal nuclease. We analysed therefore the DNA products resulting from micrococcal nuclease digestion of calf thymus nuclei performed in the presence of the polysaccharide purified from *Physarum* slime. The DNA/polysaccharide ratio was made similar to that found in *Physarum* nuclei. As it can be seen from Fig. 4, the electrophoretic pattern of DNA digestion products differed from that normally obtained for calf thymus while resembling the pattern of *Physarum* digestion. The subcore fragments were no longer present, and instead the bulk of DNA remained in the core fragment. The appearance of an intense diffuse band in the lower part of the gel could be easily attributed to the presence of polysaccharide. Thus, it could be concluded that in the above experiment the acidic polysaccharide from *Physarum* was responsible for the lack of subcore fragments in the micrococcal nuclease digestion products.

Fig. 2. Polyacrylamide-gel electrophoresis in 3.5% gel of DNA isolated from calf thymus nuclei digested with micrococcal nuclease. The samples contained, from left to right: 0.5, 4, 7, 8, 13, 19, 25, 35, 36 and 37% of acid-soluble products (1, 2.5, 5, 10, 20, 30, 60, 90, 120 and 150 min of digestion, respectively).

Fig. 3. Polyacrylamide-gel electrophoresis in 3.5% gel of DNA isolated from *Physarum* nuclei digested with micrococcal nuclease. A, Stained with “Stains all”; B, stained with ethidium bromide. The samples contained from left to right for A and B: 5, 10, 27, 28, 41, 55, 69, 80 and 81% of acid-soluble products. Digestion time as in Fig. 2 up to 120 min.

Fig. 4. Polyacrylamide-gel electrophoresis in 3.5% gel of DNA isolated from calf thymus nuclei digested with micrococcal nuclease in the presence of the polysaccharide purified from *Physarum* slime (1 mg/ml). The samples were taken after, from left to right: 5, 10, 30, 60, 90 and 120 min, e.g. at times corresponding to points shown on the kinetic curve for digestion of the polysaccharide-containing preparation on Fig. 5.
The arrows denote core fragments

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Fig. 5. Time-course of hydrolysis of DNA in calf thymus nuclei during digestion with micrococcal nuclease (△), with no polysaccharide added, and (○) in the presence of 1 mg/ml of polysaccharide purified from Physarum slime. Micrococcal nuclease: 100 units/ml.

DNA concentration: 100 μg/ml.

Addition of the Physarum acidic polysaccharide to calf thymus nuclei not only altered the electrophoretic pattern of DNA digestion products but also modified the kinetics of digestion. Apart from increased limit of digestion there was a dramatic increase of the rate of digestion of nuclear DNA (Fig. 5).

DISCUSSION

The presence of polysaccharide-containing slime in Physarum plasmodia has been a reason of numerous difficulties from the very beginning of the career of Physarum as a model eukaryotic organism. For example, the preparations of Physarum DNA isolated by standard methods contained as much as seven times more polysaccharide than DNA (Hall et al., 1975). This was due to the chemical nature of this compound which appeared to be a polygalactan containing phosphate and sulphate groups (Farr & Horisberger, 1978), and has been shown to occur both in external slime and in nuclear preparations (Farr & Horisberger, 1978).

In the present work, using calf thymus nuclei to which the polysaccharide has been added, we have demonstrated that the presence of this contamination is the direct cause of the lack of stable subcore fragments in the micrococcal
nuclease digest of Physarum nuclei. This peculiar feature of Physarum chromatin was not previously linked with the occurrence of slime (Stalder & Braun, 1978; Jerzmanowski et al., 1979). Although the action of acidic polysaccharide on nucleoprotein is probably based mainly, if not entirely, on competition with DNA for histones, its effect is more complex than simple dissociation of DNA and histones. Since its presence is manifested in accumulation of core-size DNA, we conclude that apart from loosening of DNA-histone interactions it effectively protects the core particles against the enzyme. It is worth noting that both in the case of Physarum and calf thymus nuclei the presence of the polysaccharide during digestion resulted also in protection of a certain amount of dimer-size DNA (Figs. 3 and 4).

On the other hand, the rapid increase in digestion rate after addition of the polysaccharide to calf thymus nuclei strongly resembles the effect observed upon removal of histone H1 (Whitlock & Simpson, 1976). Such an effect, while clearly showing the weakening of DNA-histone interactions, does not necessarily reflect the exact situation in Physarum chromatin. First, because the contaminating polysaccharide may be localized differently, and secondly because Physarum H1 histone seems to be different from H1 of higher eukaryotes (Mohberg & Rusch, 1969). However, high sensitivity of Physarum chromatin to endogenous protease suggests that its DNA may be indeed less tightly bound to histones due to the presence of a polyanionic carbohydrate. The last suggestion finds support in an unusual pattern of degradation of Physarum nucleoprotein particles revealed by sucrose gradient analysis. It has been found by us that, in addition to the monosome and higher multimer peaks, a slower sedimenting peak, called peak A, appears in sucrose gradient (Staroń et al., 1977). This peak, however, as established by Johnson et al. (1978), does not contain subcore DNA fragments but a core-size DNA associated with a considerably decreased amount of histones as compared to monosome. Such a pattern of degradation points to the possibility that the interaction between DNA and histones could be considerably weakened allowing dissociation of some histones in a salt-containing medium (0.35 - 0.5 m-NaCl) used for sucrose gradients (Staroń et al., 1977; Johnson et al., 1978). On the other hand, the core-size fragment with more loosely bound histones apparently remains protected against further degradation. These facts could suggest that the appearance of peak A in the micrococcal nuclease digest of Physarum nuclei resulted not from the properties of nucleohistone but rather from the presence of the contaminating polysaccharide.

It is difficult to answer whether the acidic polysaccharide is natively present in Physarum nuclei or enters them during the isolation procedure. However, even if it is native for nuclei, the changes it induces in chromatin do not seem essential for Physarum life. It can be seen on electrophoretograms presented by Stalder & Braun (1978) that the DNA pattern obtained upon
digestion of chromatin from Physarum amoebae, which contain very, little, if any, slime, resembles the pattern observed in higher eukaryotes.

REFERENCES


Niektóre nietypowe właściwości chromatyny Physarum polycephalum wynikają z obecności śluzy

Streszczenie

Chromatyna należącego do niższych eukariotów śluzewca Physarum polycephalum posiada typową strukturę nukleosomową. Jednakże trawienie chromatyny Physarum nukleazą z Micrococcus pozwala zaobserwować pewne szczególne właściwości nie spotykane w chromatynie wyższych eukariotów, z których najwyraźniej zaznaczoną jest nietypowy przebieg degradacji fragmentu DNA wchodzącego w skład rdzenia nukleosomu. Szczegółowe właściwości chromatyny Physarum wynikają z obecności swoistego policukrowca, głównego składnika śluzy Physarum, który stanowi domieszkę w preparatach chromatyny.

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