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NUCLEOSOME ASSEMBLY CAPACITY OF NUCLEAR LYSATE AFTER NUCLEASE DIGESTION

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SUMMARY

Limited digestion of lymphocyte nuclei with micrococcal nuclease degrades the nuclear DNA and results in a resistant plateau of about 50% of the original DNA. During the course of the nuclease cleavage as more and more DNA becomes acid-soluble an increasing amount of core histone is released from the disintegrated chromatin indicating that a part of nucleosomal protected DNA is degraded. These free histones appeared not to be different from those arising from resistant chromatin fragments. The released histones are in a native state which allows the exogenous DNA to be converted into nucleoprotein complexes which appear to exhibit a typical nucleosomal structure as tested by several criteria.

INTRODUCTION

Micrococcal nuclease digestion degrades the chromosomal DNA in a manner which has been well characterized before (1). The resistant plateau of about 50% of the original value may be explained by the nucleosomal protection of nuclear DNA. It is known that the nucleosome linker DNA is degraded faster than "core" DNA during digestion and that under limited nuclease digestion conditions, the nucleosomal organisation protects the histone associated DNA(2). However, if more than 90% of the DNA is organized into nucleosome structure (1), the protected fraction should comprise about 75-80% of the total nuclear DNA. The observed value of 50% protection could be explained if, during the course of nuclease cleavage, an increasing amount of core

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histones is released from disintegrated chromatin. I will present data which indicate that during limited nuclease digestion of lymphocyte chromatin, core histones are released which rapidly convert exogenous DNA to a nucleosome-like structure.

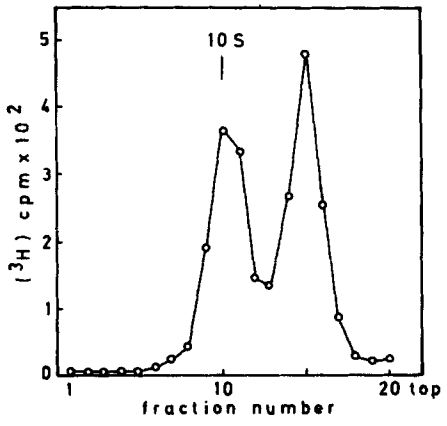
MATERIALS AND METHODS

Nuclei were prepared from Concanavalin A activated bovine lymphocytes (3). Nuclei ($2x10^7$) were digested at 37° with micrococcal nuclease (30 units, Worthington) in 0,5ml nuclease buffer (10mM Tris-HCl, pH 7,5, 1mM $CaCl_2$, 2mM $MgCl_2$, 10mM 2-mercaptoethanol). The reaction was terminated by transferring the treated nuclei into 0,3ml lysis buffer (5mM Tris-HCl, pH 7,5, 2,5mM EDTA, 25mM KCl, 10mM 2-mercaptoethanol). Salt-treated nuclear lysates were prepared by adding NaCl to 0,5M. Purified DNA (mononucleosomal DNA, 165±3 bp, isolated from a sucrose gradient (3), phage-SPP1-DNA, MW $25x10^6$ d (4) was added and incubated for 30-60min on ice. The assembly products were analyzed by layering the whole reaction mixture onto sucrose gradients (2mM EDTA, pH 7,5, 10mM 2-mercaptoethanol, 0,5M NaCl). Sedimentation was performed in a SW40 or SW27 rotor at 35000 rpm or 21000 rpm, respectively, at 4° for 16 hours .

DNA fragments obtained by nuclease treatment were incubated with proteinase K (Boehringer,Mannheim) and extracted twice with chloroform/isoamyl alcohol (24:1). The agarose gel system (5) was used to resolve native DNA fragments of different length. The gel was stained with ethidium bromide. Protein analysis was performed by using 15% polyacryamide gel according to Laemmli (6).

RESULTS AND DISCUSSION

During limited digestion of nuclei with micrococcal nuclease, core histones are released from disintegrated chromatin. This was be demonstrated by their ability to reassociate with exogenous mononucleosomal ^{3}H-DNA in the absence, as well as, in the presence of 0,5M NaCl (Fig.1) to form nucleoprotein complexes. A salt concentration of 0,5M was generally required to avoid precipitation of DNA by the histone H1 released during digestion. A large portion of the ^{3}H-DNA was recovered as a nucleoprotein complex which cosedimented with monomeric chromatin subunits at 10S. The unbound excess fraction of the ^{3}H-DNA sedi-



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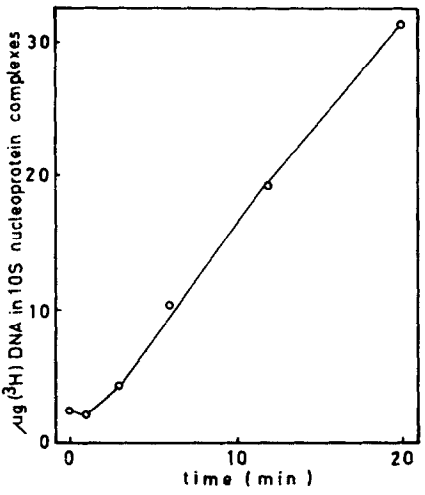

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Fig. 1. Sedimentation analysis of the ^{3}H-labeled nucleoprotein complexes formed in vitro.
Unlabeled nuclei were treated with micrococcal nuclease until 35-40% of the input DNA became acid-soluble. Nuclear lysates containing 0.5 M NaCl were incubated with ^{3}H-DNA fragments extracted from mononucleosomes (47μg). After 30min on ice, the reaction mixture was centrifuged through a 5-25% sucrose gradient containing 0.5 M salt and the distribution of the acid-precipitable radioactivity was determined.

Fig. 2. Relation between digestion time and the ability of nuclear lysates to form 10S nucleoprotein complexes.
Nuclei($12x10^7$) were digested for 0, 1, 3, 6, 12 and 20 min. Equal aliquots ($2x10^7$ nuclei) were removed, collected by brief centrifugation and lysed in 0.5M NaCl. ^{3}H-DNA(165 bp) was added in excess to each lysate: 10μg ^{3}H-DNA to the 0 and 1 min sample, 20μg to the 3 min sample, 40,50 and 64μg ^{3}H-DNA to the 6, 12 and 20 min samples,respectively. After 30 min at 0° the resulting nucleoprotein complexes were centrifuged as described in Fig.1. The amount of DNA in the 10S peak was determined by its specific radioactivity and plotted as a function of the nuclease digestion time.

mented at about 5S. The exogenously added DNA remained intact during the assembly procedure (not shown). The amount of ^{3}H-DNA found in 10S nucleoprotein complexes increased in nuclear lysates prepared at later nuclease digestion times when larger amounts of histones are expected to be available (Fig.2). There was an approximately linear relation between the amount of ^{3}H-10S complexes formed and the time of nuclease treatment (Fig.2). A small amount of DNA was also converted to nucleopro-

tein by proteins of an untreated chromatin sample (Fig.2), suggesting a small pool of available core histones. This assumption is supported by the observation that the extent of formation of ^{3}H-labeled nucleoprotein complexes was about 2-fold higher in an untreated S-phase nuclear lysate (3,65µg ^{3}H-DNA/2x10^{7}nuclei) than in a lysate from resting nuclei (1,8µg ^{3}H-DNA/2x10^{7} nuclei). Core histones released by nuclease treatment of isolated 10S nucleosomes, prepared in 0.5M NaCl, were also effective in formation of a nucleosome-like structure with monomeric DNA fragments. This study demonstrates that the formation of nucleosomes does not require any particular co-factors under the conditions of the assay. This confirms earlier results showing that the interaction of histones and DNA alone is sufficient to produce a nucleosome structure (7,8).

It appears unlikely that the observed nucleosome assembly is due to an exchange of core histone complexes between exogenously added DNA and endogenous nucleosomal DNA. Addition of an increasing amount of unlabeled monomeric DNA fragments to a nuclear lysates of nuclease-treated ^{3}H-thymidine labeled nuclei did not cause a release of an increasing amount of free ^{3}H-DNA (not shown).

In the following experiment, the assembly products of nuclear lysates with larger DNA molecules were analyzed. Using the SPP1-DNA (25x10^{6} d) it was possible to separate the formed nucleoprotein particles from the fraction of nuclease-resistant nucleosomes. As can be seen from the uv absorption curves (Fig.3), for a given amount of nuclear lysates, the sedimentation behaviour depended on the DNA concentration used. In the presence of 10µg of SPP1-DNA, the nucleoprotein complexes formed, were large and separated well from the nucleosome fraction derived

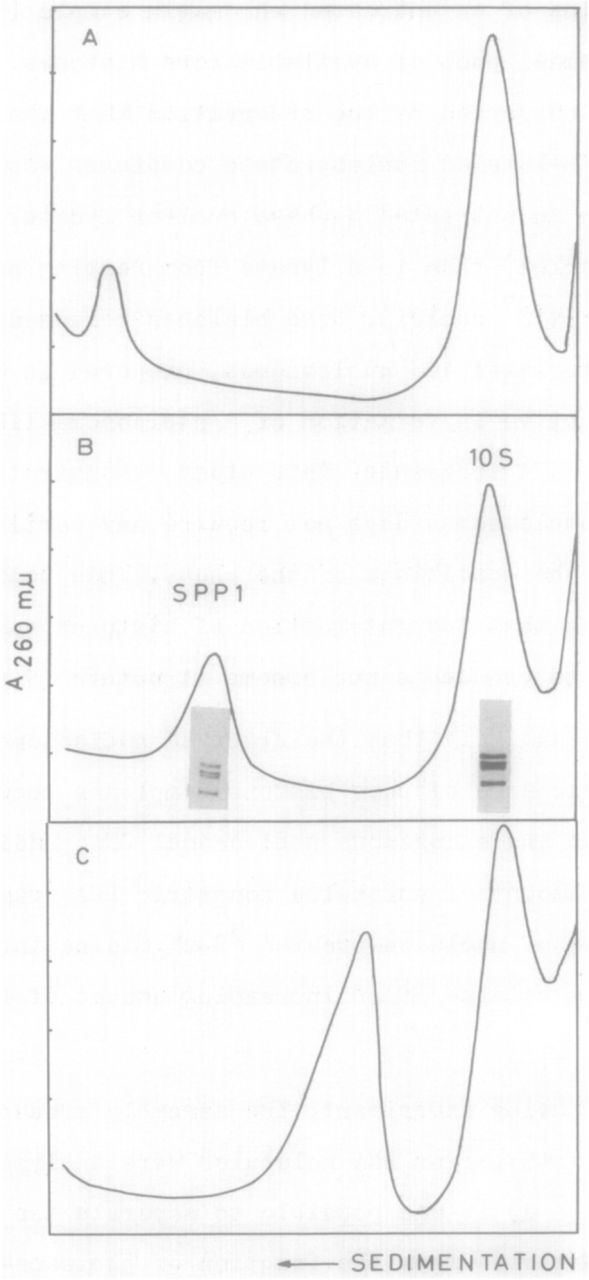

Fig.3 Reconstitution of SPP1-chromatin. Lysates of nuclease-treated nuclei ($2x10^7$) containing 0.5M NaCl were incubated with (A) 10μg, (B) 25μg and (C) 75μg of ^{3}H-labeled SPP1-DNA. After 1h incubation on ice , each sample was layered on the top of a sucrose gradient containing 0.5M salt and centrifuged in a SW 27 rotor.

from the degraded lymphocyte chromatin. The S-value of the SPP1-protein complexes decreased with increasing DNA concentration in the reaction mixture (Fig.3B,C). In the case of the lowest DNA concentration , the available core histones were probably sufficient for dense packaging of nucleosomes on the added DNA (Fig.3A). With larger amounts of SPP1-DNA, the histone complexes were randomly distributed among the DNA molecules present in the reaction mixture. The proteins associated with the separated SPP1-nucleoprotein complexes were analyzed on SDS-polyacrylamide gels and compared to the proteins of the 10S nucleosome fraction. As shown from the sucrose gradient in Fig.3B, all four core histones were present on the reconstituted nucleoproteins. The outer histone H1 was not detectable because in 0.5M salt H1 dissociates almost completely. Analysis on acid-urea polyacrylamide gels according to Paniym and Chalkley (9) did not reveal differences between SPP1-histones and histones from the resistant 10S fraction (not shown). It seems, therefore, that the core histones released during digestion are probably indistinguishable from histones which remained stably associated with the nuclease-resistant chromatin fraction.

Other evidence for the nucleosomal protection of the SPP1-DNA was obtained from the degradation kinetics of the reconstituted ^{3}H-SPP1-nucleoprotein complexes, as is shown in Fig.4A. After a fast degradation rate during the first 7 min, a resistant plateau of the SPP1-DNA was observed at about 25% of the original DNA. When the degradation products from a 7 min digestion were analyzed on a sucrose gradient, a large portion of the resistant SPP1-DNA sedimented at 16S and 11S like di- and mononucleosomes, respectively (Fig.4B). An additional peak of resistant ^{3}H-DNA was found at about 5S. These DNA fragments probably

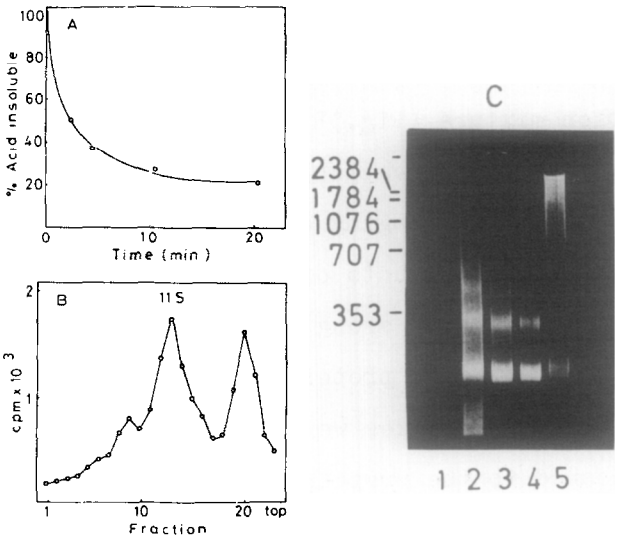

Fig.4 Nuclease digestion of the SPP1-nucleoprotein complexes (A) 3 ml of the peak fractions (sucrose gradient, Fig.3B) were diluted with 12 ml nuclease buffer and digested with micrococcal nuclease (3o units). At the times indicated the acid-solubility was determined. (B) Chromatin fragments obtained after 7 min digestion were centrifuged through a sucrose gradient containing low salt. (C) DNA fragments obtained after different digestion times were analyzed on a 1.6% agarose gel . Lane 1, col E1 HaeII fragments; 2, 3min; 3, 5 min; 4, 8 min digest; 5, digested lymphocyte chromatin.

arose from disintegrated DNA-histone complexes. The electrophoretic mobility of the DNA fragments generated during nuclease digestion was analyzed on an agarose gel (Fig.4C). The size of nuclease-resistant degradation products derived from SPP1-chromatin was considerably smaller than that found after digestion of lymphocyte chromatin. As calculated from marker fragments of known sizes, the DNA bound to a single nucleosome was 145±3 bp in length. The average size differences between successive multimers was also 145±3 bp. The repeat length of 145 bp of reconstituted SPP1-chromatin confirms earlier reports of other *in vitro* chromatin reconstitution systems (10,11,12) and is independent from the sedimentation value of the reconstituted nucleoprotein complexes shown in Fig.3.

Furthermore, nuclear lysates from digested nuclei introduce superhelical turns into originally relaxed covalently closed circular DNA (not shown), known for nucleosome assembly (13).

In summary : Nuclear lysates from nuclease-treated nuclei contain a significant amount of free core histones which can be used to form nucleoprotein complexes with exogenously added DNA fragments which resemble nucleosomes by several criteria (14).

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