Volume 318, number 1, 27–29 FEBS 12119 February 1993


# A novel nucleosome assembly procedure (with a little help from pectin)

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Received 5 November 1992; revised version received 30 December 1992

The acidic polysaccharide pectin (α-1,4-polygalacturonic acid) has been introduced as a nucleosome assembly facilitator as a substitute for polyglutamic acid. The pectin-assembled nucleosomes were indistinguishable from polyglutamic acid-assembled nucleosomes by thermal denaturation and DNAse I digestion. Pectin had two major advantages over polyglutamic acid – the yield of assembled cores was approximately 50% higher and the pectin could be easily removed after completion of the reassembly procedure by dialysis following pectinase cleavage.

Histone; Nucleosome; Assembly; Pectin; Core particle

## 1. INTRODUCTION

One approach to determining the function of histone variants and modified histones requires that the histone in question must first be incorporated into either core particles or reassembled chromatin [1–3]. The resulting preparations can then be analysed by a variety of techniques including thermal denaturation and enzymatic digestion of either the DNA or the protein component using the enzymes DNAse I and trypsin, respectively.

Core particle assembly from histone octamers and DNA has been carried out by a variety of methods including salt dialysis [4], urea/salt dialysis [5] and polyanion facilitated assembly [6] using, for example, polyglutamic/polyaspartic acid or tRNA. We have chosen to use this last method for the preparation of hybrid core particles since, in cases where the histone of choice cannot be directly reconstituted into histone octamers, the polyglutamic acid overcomes the barriers of histone association and allows the assembly of polycores on the DNA [1,3]. Two problems exist with the current methodology – the cost of the polyglutamic acid and the fact that the polyglutamic acid cannot be completely removed from the assembled core particle preparation even by sucrose gradient centrifugation [7]. We have therefore explored the possibility that the acidic polysaccharide pectin (α-1,4-polygalacturonic acid) could substitute for polyglutamic acid. This would overcome both the above problems since pectin is not only inexpensive but it can also be cleaved to monosaccharides by specific enzymes that recognise neither cleavage sites on the DNA nor on the histones. This manuscript reports that pectin facilitated nucleosome assembly produces particles which are indistinguishable form those assembled by polyglutamic acid mediation.

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## 2. MATERIALS AND METHODS

The methodology for reconstitution of chicken histone octamers [8] and hybrid histone octamers as well as the polyglutamic acid mediated assembly of (hybrid) core particles using long DNA has been published previously [1–3,9]. Long DNA (of length in excess of 1 kb) was prepared by digesting washed chicken erythrocyte nuclei with micrococcal nuclease (50 units/mg DNA for 10 mins at 20°C). The nuclei were extracted with 0.6 M NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 0.1 mM PMSF, pH 7.4, and the resulting stripped chromatin was fractionated on a 5–20% isokinetic sucrose gradient in this same buffer. The fraction containing penta-core particles and longer was pooled and the DNA purified by phenol extraction prior to storage in 1 mM EDTA, pH 8.0. Pectin (from oranges, Sigma) was dissolved in 10 mM Tris-HCl, pH 7.4 to a final concentration of 10 mg/ml. It was stable for several months at 4°C. Pectin-mediated assembly was carried out by adding 2.4 mg of pectin to 0.75 mg (7.14 nmol) of reconstituted histone octamers in 2 M NaCl, 10 mM Tris-HCl, pH 7.4. 1.3 ml DNA (1 mg/ml in 1 mM EDTA, pH 8.0) was then added and the entire solution was dialysed against 20 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 0.1 mM PMSF, pH 7.4, for 16 h at 4°C. A 500 μl aliquot of the assembled polycores was then digested with 20 U micrococcal nuclease at 37°C for various times (see Fig. 1) to determine the length of digestion required to release monomeric particles. For the preparation of the 167 bp core particles used for the experiments reported in Figs. 2 and 3, the remainder of the dialysed assembly mixture was digested for 30 s and the 167 bp core particles purified on a 5–20% isokinetic sucrose gradient in the same buffer. Pectinase digestions were carried out in 20 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4. Reducing sugars were determined using dinitrosalicylate [10].

Thermal denaturation [11] was carried out at a sample concentration of 25 μg/ml using a Hewlett-Packard 8450A diode array spectrophotometer equipped with an 89100A temperature controller. The absorption was monitored at 260 nm whilst heating the sample at 1°C per min. DNAse I digestion of the 167 bp core particles was carried out as described [12].

## 3. RESULTS AND DISCUSSION

Chicken histone octamers were assembled onto long

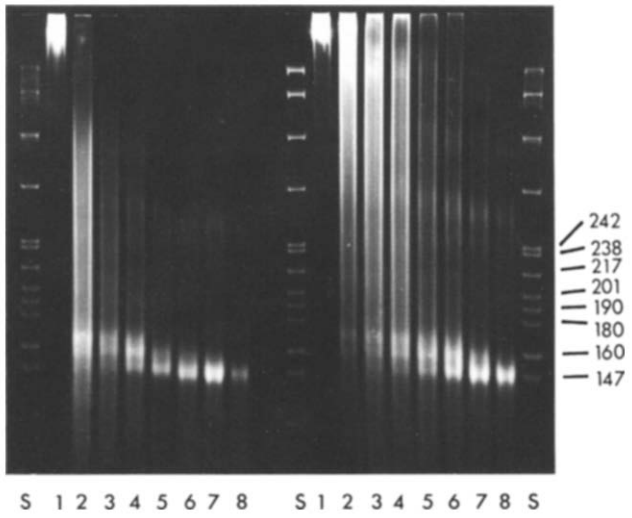


Fig. 1. PAGE using 6% gels of the MNase digestion in 10 mM Tris-HCl, 20 mM NaCl, 0.1 mM EDTA, pH 7.4, of the products of the polyglutamic acid (left) and polygalacturonic acid (right) assembly mixture of chicken octamers and long DNA. Digestion was stopped at 0, 0.25, 0.5, 1, 2, 5, 10 and 20 min (lanes 1–8). The standard is a *Hpa*II digest of pBR322. Four times less MNase was used for the digest containing polygalacturonic acid; the inhibition of MNase by polyglutamic acid has been described [6].

DNA using a 3-fold weight excess of pectin over histone octamers and the products of assembly were digested with micrococcal nuclease (MNase). The length of DNA associated with the monomeric particles decreased from 167 bp to 146 bp via a 156 bp intermediate as described previously both for stripped chicken erythrocyte chromatin [13] and for polyglutamic acid-assembled chicken core particles [2]. It would therefore appear that pectin could replace polyglutamic acid in the assembly of core particles.

We next determined whether the pectin could be removed by dialysis after pectinase treatment and whether pectinase interfered with core particle assembly. The MNase digestion products were therefore digested with pectinase (14 units/mg pectin, 10 mins, 37°C) and the reducing sugar content measured before and after dialysis against 10 mM Tris-HCl, 20 mM NaCl, 0.1 mM EDTA, pH 7.4. We found (results not shown) that no reducing sugar was present in the dialysed sample although both the undialysed and the original samples tested positive. Moreover, electrophoretic analysis of the histone and DNA content of the MNase digestion products showed that pectinase treatment had no effect on the assembled core particles. The MNase digestion products were therefore applied to a 5–20% sucrose gradient in 10 mM Tris-HCl, 20 mM NaCl, 0.1 mM EDTA, pH 7.4; after 16 h centrifugation the core particles were recovered and dialysed against this same buffer. Whereas polyglutamic acid-mediated assembly of core particles routinely resulted in approximately 21% of the input histone octamers being recovered in core particles, the yield with pectin mediated assembly was found to be approximately 32%. Dinitrosalicylate again failed to detect the presence of reducing sugars.

Thermal denaturation and DNAse I digestion have been shown to be sensitive indicators for altered histone:DNA interactions. The thermal denaturation behaviour of pectin assembled 167 bp core particles was therefore investigated. The biphasic transition (Fig. 2) was essentially the same as described previously for the native 146 bp core particle [10]. The pre-transition had a $T_m$ of 76.7 ± 0.1°C and displayed a hyperchromic contribution of approximately 25% to the total hyperchromicity observed; the main transition had a $T_m$ of 80.3 ± 0.1°C. Thermal denaturation of polyglutamic acid assembled 167 bp core particle displayed an almost identical melting profile except that the $T_m$ of the first transition occurred at 74.6 ± 0.1°C (results not shown).

The thermal denaturation behaviour of the pectin-assembled 167 bp core particles was essentially identical to the thermal denaturation behaviour observed with native and polyglutamic acid assembled core particles suggesting that identical histone:DNA interactions occurred in all these particles. This result was confirmed by subjecting these particles to DNAse I digestion after T4 kinase labelling of the 5′ end of the DNA with [$\gamma^{32}$P]ATP. The relative accessibility of the DNAse I to the DNA at specific positions on the core particle has been shown to display an asymmetrical pattern with the preferred cleavage towards the 5′ end of the strand [12]. Our results (Fig. 3) were identical to those shown previously for native 167 bp core particles [13] with infrequent cutting observed at sites 4, 7, 9 and 12. These sites correspond to sites 3, 6, 8 and 11 for the 146 bp core particle [12] and confirmed that the two-turn 167 bp core particle is very similar to the 146 bp particle as regards the asymmetric susceptibility of DNAse I digestion allowing for a 10 bp offset.

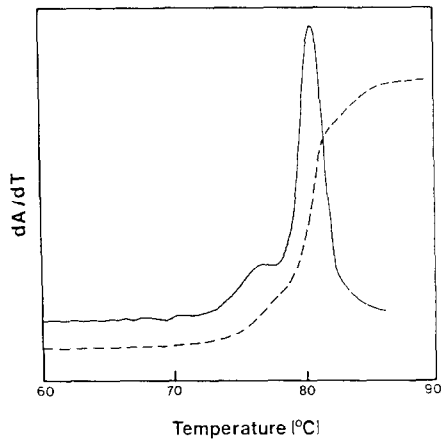


Fig. 2. Thermal denaturation in 10 mM Tris-HCl, 20 mM NaCl, 0.1 mM EDTA, pH 7.4, of 167 bp core particles. The change in absorption at 260 nm was monitored; the original data and the first derivative are shown.

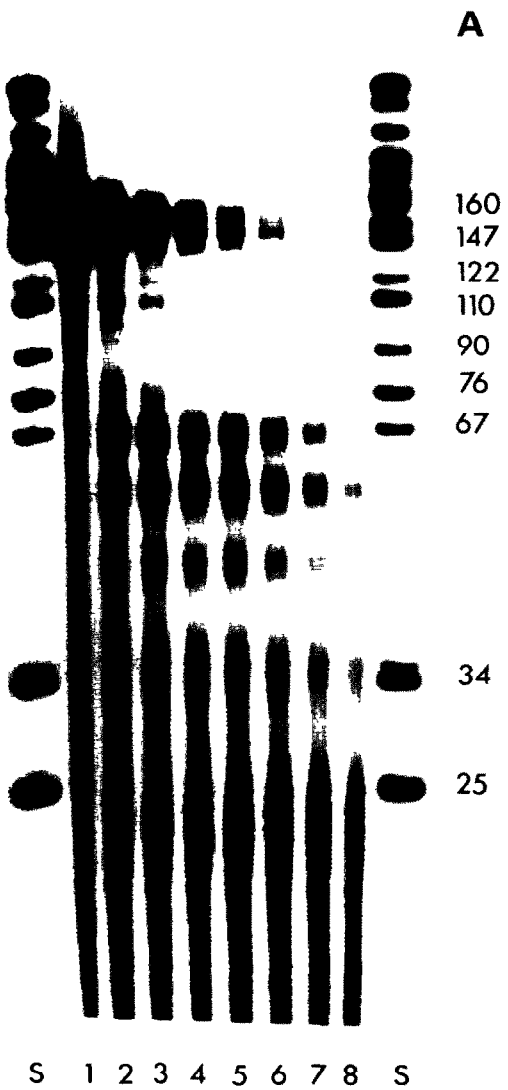


Fig. 3. (A) Autoradiograph of DNAse I digestion of 5'-$^{32}$P-end-labelled pectin-assembled 167 bp core particles. The reaction was stopped at 0, 0.25, 0.5, 1, 1.5, 2, 3 and 5 min (lanes 1–8). The standard was a *Hpa*II digest of pBR322 labelled by a 'fill-in' reaction with the Klenow fragment of *E. coli* DNA *Pol*I using [α-$^{32}$P]dGTP. (B) Densitometric scan of lane 2 of the autoradiograph shown in (A).

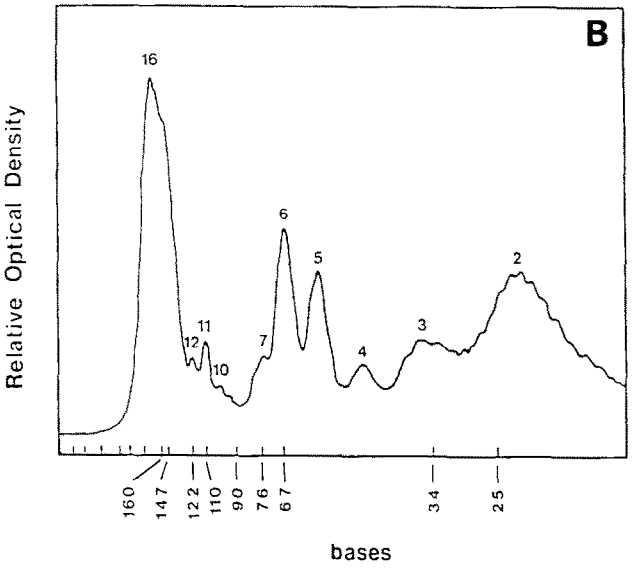


Fig. 3B

The results reported here show that the acidic polysaccharide pectin can successfully substitute for the polyamino acid, polyglutamic acid, in a nucleosome assembly system. Apart from the relative cost of the two compounds, the use of pectin has two major advantages compared with polyglutamic acid. Firstly, 50% more histone octamers were assembled into core particles, an important improvement in efficiency when working with small quantities of synthetic hybrid octamers. Secondly, the pectin could, unlike polyglutamic acid, be readily removed, after pectinase digestion to smaller polysaccharides, by sucrose gradient centrifugation and dialysis.

*Acknowledgements:* H.H.K. and G.G.L. would like to thank the Foundation for Research Development and the University of Cape Town Research fund for financial support.

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