CHROMATIN MONOMER: ABSENCE OF NON-HISTONE PROTEINS

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SUMMARY: Chromatin from a human colon carcinoma cell line has been digested with Staphylococcal nuclease (E.C.3.1.4.7) and the monomer subunit isolated by sucrose gradient centrifugation. Electrophoresis of the monomer proteins on sodium dodecyl sulfate-polyacrylamide gels revealed an absence of the nuclear non-histone proteins in the isolated monomer.

INTRODUCTION

Olins and Olins (1) first described a subunit structure of chromatin which consisted of spherical bodies about 70A° in diameter. These subunits can be isolated by limited nuclease digestion of intact chromatin or nuclei followed by sucrose gradient centrifugation (2-4). Kornberg (5) suggested that the chromatin subunit consisted of about 200 DNA base pairs and eight histone molecules (2 each of H2A, H2B, H3 and H4). We wish to present evidence here that in fact the nonhistone chromosomal proteins (NHCP) are not part of the subunit structure isolated from digested chromatin. We will present more extensive evidence elsewhere (manuscript in preparation) that the same is true of the purified chromatin subunit isolated by nuclease digestion of intact nuclei.

METHODS

Human colonic carcinoma cells (HT-29) were grown in monolayer culture as previously described (6) and were used in log phase. Cells were grown from the day of plating to the experiment in the presence of 0.01μCi/ml [^{14}C]-thymidine (57mCi/mmol, Amersham). Chromatin was isolated from detergent cleaned nuclei by the method of Bhorjee and Pederson (7) as described in detail elsewhere (6). The chromatin was dialyzed against 2 changes of 500 volumes of 5 mM sodium phosphate buffer, pH 6.8 plus 25 μM $CaCl_2$ and digested with 50μg of Staphylococcal nuclease (E.C. 3.1.4.7, 10,000 units/mg, Worthington) as described by Clark and Felsenfeld (8). The digestion was terminated by bringing the incubation mixture to 12 mM EDTA. Under these conditions, a maximum of 55% of the [^{14}C]-thymidine labeled DNA could be rendered acid soluble, as previously described (8).

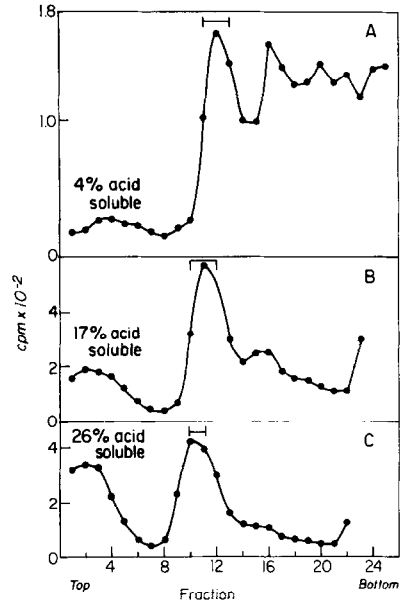

Fig. 1. Sucrose gradient centrifugation of chromatin digested with Staphylococcal nuclease. Isolated chromatin was digested for 2'(A), 10'(B), and 20'(C) and then centrifuged on 5-20% sucrose gradients in the Beckman SW41 rotor at 110,000 xg for 18 hours at 4°C.

Subunits were isolated by centrifugation of the chromatin digests on 5-20% sucrose gradients in 5 mM sodium phosphate, pH 7.4 plus 0.2 mM EDTA. An aliquot of each fraction was counted in Aquasol (New England Nuclear) containing 5% distilled water in an Intertechnique scintillation counter. Efficiency was monitored by external standard ratio. The position of the subunits could also be determined by measurement of absorption at 260 nm (not shown).

Proteins were analyzed in sodium dodecyl sulfate-polyacrylamide gels by the technique of Weber and Osborn (9). Protein and DNA were measured by the techniques of Lowry et al (10) and Burton (11), respectively.

RESULTS

Figure 1 presents the sucrose gradient fractionation of chromatin in which 4% (A), 17% (B) and 26% (C) of the ^{14}C-thymidine labeled DNA was rendered acid soluble by digestion for 2, 10, and 20 minutes, respectively. The monomer subunit sedimented near the middle of the gradient, with acid soluble material on top. Following brief digestion (Figure 1A), higher order structures were seen (dimers, trimers, etc.).

The protein/DNA ratio of chromatin was 1.99 (2.10, 1.89) while that of the

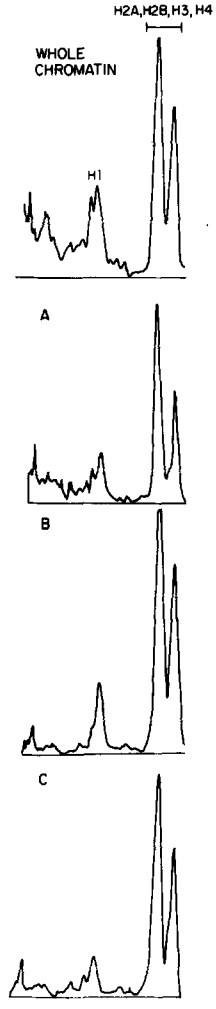

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of monomer proteins. The fractions indicated on the gradients of figure 1 were pooled and an aliquot run on gels as described in Methods.

monomer of samples digested as in 1B was 1.07 (1.20, 0.94). Figure 2 shows the electrophoretic profile of the proteins of the monomer fractions of Figure 1. It can be seen that the decrease in protein/DNA of monomer as compared to chromatin was due to a selective loss of most of the nonhistone chromosomal proteins. However, a single distinct nonhistone peak of high molecular weight was always seen in the monomer isolated from chromatin. It should also be noted that in the monomer, histone 1 was decreased in amount relative to the other histones.

DISCUSSION

We have found that the monomer subunit from chromatin is associated with very little nonhistone protein. We have obtained the same results using chromatin isolated by gentle lysis of the nuclei in distilled water, rather than by sonication (not shown). More importantly, similar results have been obtained with monomer isolated by Staphylococcal nuclease digestion of intact nuclei, although in this case the loss of nonhistone protein was related to both the extent of digestion and purification of the monomer (manuscript in preparation). Oudet et al. (12) have also reported that the isolated monomer contains only histone protein, but this was with chicken erythrocyte chromatin depleted of histone 1 as starting material, which has very little, if any, nonhistone protein to begin with.

We have also found that upon dissociation from the monomer during isolation, most of the nonhistones are found in the pellet of the sucrose gradient, probably because they form aggregates and/or are insoluble and therefore precipitate. At the completion of chromatin digestion, the limit digest also precipitates (4,8,13). This co-precipitation of the dissociated nonhistone protein and the monomer at the limit digest may be the reason Lacy and Axel (4) found all of the chromatin protein and undigested DNA in the precipitate of the limit digest.

These results may be interpreted in two ways; either the monomer subunit is not associated with nonhistone protein in situ, or the nonhistones become easily dissociable as the intact structure of chromatin is lost during digestion. Evidence will be presented elsewhere (manuscript in preparation) that the latter explanation is correct.

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