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Selective Removal of Histones from Calf-Thymus Nucleohistone with Sodium Dodecylsulfate

(sodium dodecylsulfate-gradient centrifugation/equilibrium gel filtration/histone F1-DNA complex/detergent binding/histone redistribution)

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ABSTRACT **Calf-thymus nucleohistone studied by a newly developed 'SDS gradient' centrifugation technique showed that histones dissociate sequentially when treated with increasing concentrations of sodium dodecylsulfate. Histones F2a1 and F2a2 were dissociated first at about 0.03% sodium dodecylsulfate, and F1 was removed lastly by the highest concentration of sodium dodecylsulfate (0.06% or more). A DNA-histone F1 complex, which consisted of DNA and all of the histone F1 and completely lacked other histones, was obtained by sedimenting nucleohistone through 0.05% sodium dodecylsulfate. Results of equilibrium gel filtrations in 0.05% sodium dodecylsulfate revealed that the binding of sodium dodecylsulfate to nucleohistone caused new binding sites to be available for the detergent which presumably was accompanied with dissociation of histones from DNA. This result indicates that no redistribution of histone F1 on DNA should occur in the presence of 0.05% sodium dodecylsulfate.**

Many workers have studied participation of various histone components in the structure and function of chromatin by analyzing selectively dehistonized chromatin (1–4). In some cases, selective dehistonization was accomplished by treating chromatin or nucleohistone with salts or acids (1–3), which resulted in the dissociation of histone F1 (F1) at the lowest concentration of the reagents. On further dissociation, however, the concentration range of dissociating agents needed for each histone component overlapped considerably with others, and efforts to obtain a complex of DNA and single species of histone have been unsuccessful. A combination of urea and sodium chloride was shown to dissociate all histones but F1 at an appropriate salt concentration (5), but in this case, like treatments with salts or acid, the possibility of redistribution of remaining histones on DNA was not excluded; hence, the resultant complex might be an artefact. Smart and Bonner (4) showed that when chromatin was treated with increasing concentrations of sodium deoxycholate F1 remained on DNA most tenaciously; however, in this case also, some of the other histones still remained on the DNA when F1 began to be dissociated. This report describes the mode of dissociation of histones in increasing concentrations of sodium dodecyl sulfate (SDS) and a method for the preparation of a pure F1–DNA complex.

MATERIALS AND METHODS

Nucleohistone. Calf-thymus nucleohistone was prepared by the method of Zubay and Doty (6), with slight modifications. Fresh or frozen tissues were minced, blended in 0.14 M NaCl–0.01 M ethylenediaminetetraacetic acid (pH 7.0), and centrifuged at 1500 × *g* for 10 min. The resultant pellet was repeatedly washed with the same solution until the supernatant was completely clear (16–20 times). The white precipitate thus obtained was briefly washed with water and dialized against 0.7 mM sodium phosphate buffer (pH 7.2) for 3 days; this was used as the nucleohistone. This material has a protein to DNA ratio of about 1.4, and its DNA moiety shows a $S_{20,w}$ value of 12 to 16 when analyzed by sucrose gradient centrifugation after complete deproteinization with excess SDS (1%). The nucleohistone had a histone sulfate:DNA ratio of 1.3 when the amount of histones was determined by weighing ethanol precipitates after extraction with sulfuric acid. Elementary analysis showed that, by weight, about 75% of the histone sulfate was histone base, which indicates that the histone base:DNA ratio was close to 1. Since histone sulfate shows an equivalent color yield to bovine-serum albumin (BSA) (10), the protein to DNA ratio of 1.4 as determined by the Lowry method implies that quite small amount of protein (<0.1 relative to DNA) is attributable to nonhistone protein, if any, in the nucleohistone used here. Several histone bands and an additional faint nonhistone protein band between histones F1 and F3 appeared when the total nucleohistone was fractionated by SDS gel electrophoresis, which also indicates that a majority of the protein in the nucleohistone preparation was attributable to histones.

SDS-Polyacrylamide Gel Electrophoresis. SDS-polyacrylamide gel electrophoresis was done essentially by the method of Weber and Osborn (7) in 10% gel. In most cases, nucleohistone or partially dehistonized nucleohistone was analyzed without removing DNA. Each histone band was identified by referring to fractionated histones which were donated by Dr. E. W. Johns.

SDS Gradient Centrifugation. A 30 ml, 5–20% linear sucrose gradient, which was also a gradient of 0.025–0.1% SDS, was made above 5 ml of 60% sucrose [all buffered with 0.7 mM sodium phosphate (pH 7.2)] in a cellulose nitrate tube for an SW 27 rotor (Beckman). A 2-ml sample of nucleohistone (A_{260} = 4) was overlayed and the gradient was centrifuged at 27,000 rpm for 15 hr. Fractions were collected from the top, and the A_{260} and protein content of each fraction were determined. The SDS concentration of each fraction was estimated from its sucrose concentration (determined refractometrically) on the assumption that the extent of diffusion of SDS and

Abbreviations: SDS; sodium dodecylsulfate, BSA; bovine-serum albumin; F1, histone F1.

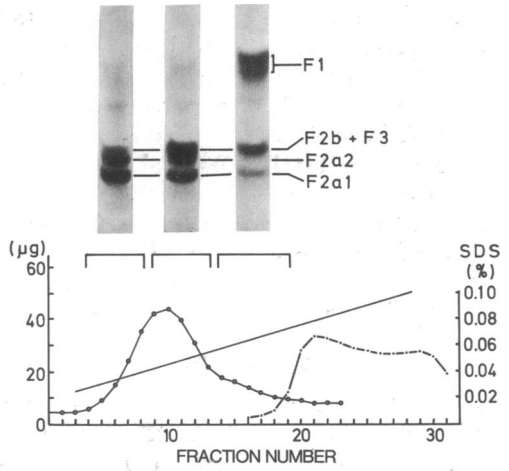

FIG. 1. Sedimentation profile of nucleohistone in an SDS gradient. Fractionation is from *top* to *bottom*. (— · —) DNA; (—O—) protein; (——) SDS. The *upper* photographs are the electrophoretograms of pooled fractions, as indicated.

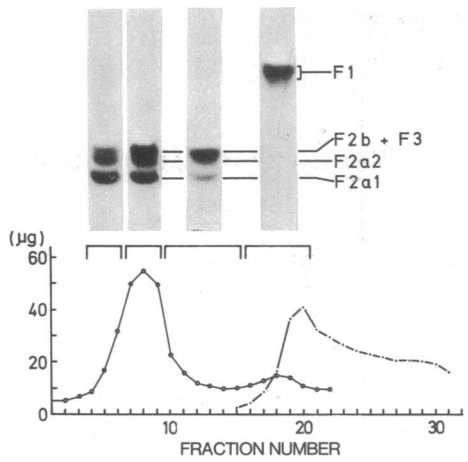

FIG 2 Sedimentation profile of nucleohistone in 0.05% SDS. (— · —) DNA; (—O—) protein. The *upper* photographs are the electrophoretograms of pooled fractions, as indicated.

sucrose does not differ significantly. Appropriate fractions were pooled, dialized against water, lyophilized, and subjected to SDS gel electrophoresis.

Preparation of F1-DNA Complex. The F1-DNA complex was prepared by sucrose gradient centrifugation under the conditions similar to those for SDS gradient centrifugation except that the concentration of SDS was kept at 0.05% throughout the gradient.

Equilibrium Gel Filtration. A column was filled with Sephadex G-25 (1 cm × 27 cm) and equilibrated with an eluting solvent which contained appropriate concentrations of SDS and 0.7 mM sodium phosphate (pH 7.2). A 1-ml sample of sonicated nucleohistone (A_{260} = 40), which contains the same concentration of SDS and sodium phosphate as the eluting solvent, was applied and eluted with the solvent at a flow rate of 4 ml/hr. Fractions (0.5 ml each) were collected, and the concentration of SDS in each fraction was determined.

DNA, Protein, and SDS Assays. The concentration of DNA was determined by measurement of A_{260}, on the assumption that A_{260} = 1.0 at 50 μg of DNA per ml. Protein concentration was determined by the method of Lowry *et al.* (8) using BSA (Armour) as a standard which in turn was determined by measurement of A_{279}, on the assumption that A_{279} = 6.67 at 1% BSA in water. SDS concentration was determined by a modified method of Mukerjee (9) which will be described in detail elsewhere.

RESULTS

Dissociation of histones from DNA in the presence of SDS was examined by sedimenting the nucleohistone in an SDS gradient (Fig. 1). It was expected that as the nucleohistone sedimented through increasing concentrations of SDS some histones would be removed from DNA at appropriate SDS concentrations, where dissociated histones (1–2S) remained, while DNA (12–16S) as well as bound histones would be moved further down to the region of higher SDS concentrations. Fig. 1 shows a typical sedimentation pattern in an SDS gradient. It is seen that when DNA has moved down to the 19th fraction or further, no protein has remained on the DNA and the dissociated proteins have been left behind between fractions 5 to 18 which corresponded to 0.03–0.07% SDS. Although the dissociated proteins banded as a single, wide peak, various histone fractions were distributed in ordered sequence in this peak. Thus, the electrophoretograms of pooled fractions (Fig. 1, photograph) clearly demonstrate that a leading fraction was rich with histone F2a1 and that the tailing fraction was with F1. The overall order of dissociation was histones F2a1 + F2a2 first, histones F2b + F3 second, and F1 third from lower to higher SDS concentrations. This result encouraged us to isolate a pure histone F1-DNA complex devoid of other histone components. Nucleohistone was sedimented through a gradient of sucrose containing 0.05% SDS, because all histone components but F1 should be removed from DNA at this concentration of SDS. Fig. 2 shows that the total proteins were separated into two peaks: one sedimented slowly as dissociated proteins and the other sedimented rapidly as associated with DNA. It should be noticed, however, that quantitative comparison of the two protein peaks is difficult because sucrose, especially at high concentrations, interferes considerably with protein determination by the Lowry method. Electrophoresis of appropriately pooled fractions revealed (Fig. 2, photograph) that the DNA-associated protein was attributable solely to F1, and no F1 was found in the dissociated protein fractions.

When the nucleohistone preparation was brought to 1% SDS beforehand to dissociate all the histones and sedimented through a sucrose gradient which contained 0.05% SDS, all the protein was found between fractions 1 to 6, while DNA was sedimented to fraction 12 or further. Electrophoresis of individual fractions revealed that all of the five histone components were contained in every protein fraction and no particular components seemed to be enriched in any particular fraction (data not shown). This means that the uneven distribution of histone components in Fig. 1 is really the reflection of sequential dissociation and that the cosedimentation of F1 and DNA in Fig. 2 is indicative of association of them but not of cosedimentation by chance after their dissociation. It was also observed (Fig. 2, photograph) that the sequential dissociation of histones occurred within the slow-sedimenting peak of Fig. 2, although the concentration of SDS was made at 0.05% throughout the gradient. This suggests that (*a*) a

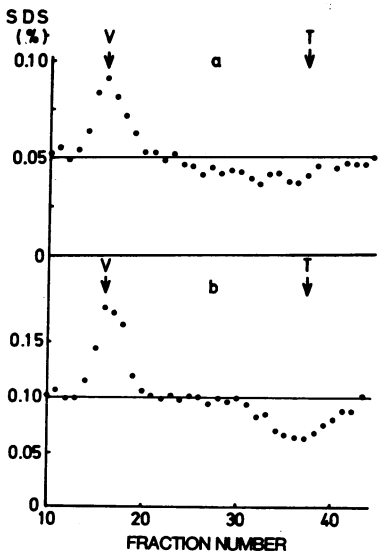

FIG. 3. Binding of SDS to nucleohistone in equilibrium gel filtration. The *arrows* with *V* and *T* indicates void volume and total volume, respectively. (*a*) in 0.05% SDS; (*b*) in 0.1% SDS. For experimental conditions, see *Materials and Methods*. DNA, nucleohistone, and dissociated histones are excluded from the gel particles; they are applied at total volume and outstrip toward void volume, where they are eluted, gathering SDS on their way. The total volume was determined beforehand as the elution volume of bromphenol blue. Since SDS is unlikely to bind to nucleic acid, all the SDS brought to total volume should be bound to histones.

significant amount of SDS had bound to histones so that reduction of effective concentration occurred, and a gradient of SDS was automatically formed, or that (*b*) there are differences in the rate of dissociation among the four histone components.

The experimental evidence supporting the binding of SDS to histones was obtained from equilibrium gel filtration (11) of nucleohistone in SDS (Fig. 3). In 0.1% SDS, where all histones were dissociated from DNA soon after elution was started, a monodisperse trough of SDS concentration was observed near the total volume (Fig. 3*b*). In contrast, when the nucleohistone was run down through 0.05% SDS, where the majority of histones except F1 are expected to dissociate during the run, the trough showed a polydisperse pattern with its bottom away from the total volume. This means that as the nucleohistone ran down through 0.05% SDS new binding sites became available for SDS, which probably is attributable to the dissociated histones. Furthermore, when the nucleohistone was treated with formalin to fix histones covalently on DNA (12) and examined by equilibrium gel filtration in 0.1% SDS, the amount of bound SDS was about one-third of the untreated nucleohistone (data not shown), which indicates that histones are poorer acceptors of SDS when associated with DNA than when dissociated. A direct verification that free histones have a higher affinity to SDS than DNA-bound histones was unsuccessful because free histones precipitate at low SDS concentrations, and equilibrium gel filtration of free histones gave irreproducible results.

The binding of SDS to proteins has been studied extensively by Reynolds and Tanford (13). According to their data, histones, including F1, are fully bound by SDS at the concentration of SDS used here (0.05% or 1.7 mM). Such a histone–SDS complex is highly anionic and impossible to bind to DNA. The apparent paradoxical phenomenon that F1 is still bound to DNA at 0.05% SDS suggests that the binding affinity of histones with SDS is different depending on whether they are associated with DNA, so that DNA-asscoiated histones are poorer acceptors for SDS. This in turn means that, under the experimental conditions employed here, histones, once dissociated from DNA, should accept saturated amounts of SDS and could not reassociate with DNA. This means that no redistribution of F1 on DNA was likely to occur in the presence of 0.05% SDS through dissociation and reassociation.

DISCUSSION

SDS has several advantages over other agents reported previously that dissociate histones from DNA because (*a*) SDS is effective at very low concentrations and possible disturbances caused by impurity in the agents can be minimized, (*b*) nucleohistone is soluble at all SDS concentrations, (*c*) SDS has no absorption in ultraviolet or visible light ranges at which protein or nucleic acids are usually measured and (*d*) SDS gel electrophoresis can be used without complicated procedures for determination of histones in microgram quantities.

In spite of these advantages, the attempt to elucidate the mode of dissociation of histones from DNA with SDS by batch-method yielded rather complicated results. Thus, the higher the concentration of the nucleohistone the more SDS was needed to achieve the same amount of histones removed per DNA. This difficulty was overcome by developing the SDS-gradient method. An experiment similar in principle was reported previously using a salt gradient to dissociate histones from DNA (2); however, the results were less informative than ours presented here because (*a*) appropriate techniques such as SDS gel electrophoresis for the identification of small quantities of histones were not available at that time and (*b*) nucleohistone tends to aggregate at low salt concentrations.

Extensive studies on the binding of SDS to proteins have been reported by several investigators (13–19). The results are summarized as follows: (*a*) only a small amount of SDS binds to protein at low SDS concentrations; (*b*) at increasing SDS concentrations, extensive unfolding of protein occurs so that binding sites for SDS become available; (*c*) at high SDS concentrations, a strongly anionic SDS–protein complex, fully covered with SDS, is formed. The SDS concentration that induces protein unfolding is identical (about 0.9 mM) regardless of the protein species, including various histones (13). The stability of histones against unfolding seems to increase significantly, however, when they are bound to DNA, as indicated by the observation that a much higher concentration of SDS is needed (more than 1.7 mM) to remove histones from DNA. The difference in critical SDS concentrations needed for dissociation of each histone component may be due to the difference in the stability of corresponding histone components or to the difference in each affinity to DNA. The fact that F1 is the most basic among histones, and is most tenaciously bound to DNA at increasing SDS concentrations, favors the latter possibility.

A large scale preparation of F1-DNA complex free from SDS is achieved by using gel filtration chromatography. Studies on the nature of this complex are in progress.

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