PHOTOAFFINITY LABELING OF CHROMATIN: NUCLEASE-SENSITIVE CHROMATIN SHOWS PREFERENTIAL LABELING OF HISTONE H_1

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1. Introduction

Active chromatin differs physically from bulk chromatin in that active genes are preferentially degraded by DNase I [1–4] and micrococcal nuclease [4]. Using a new photolabel for chromatin studies [5], I now demonstrate that the class of chromatin which is sensitive to DNase I and micrococcal nuclease exhibits a histone labeling pattern different from that of bulk chromatin.

2. Materials and methods

The labeling reagent (*N,N'*-bis-(9-acridinyl)-4-aza-4-(4-azidobenzoyl)-1,7-diaminoneptane, dihydrochloride) (fig.1) was synthesized as in [5]. Nuclei from mouse Ehrlich ascites tumor cells were prepared according to [6].

Photoaffinity labeling of the nuclei was performed as follows. To a nuclei suspension (20 A_{260}/ml in 0.34 M sucrose, 60 mM KCl, 1.5 mM NaCl, 1 mM $CaCl_2$, 10 mM Tris–HCl (pH 7.4), 0.5% Nonidet P40) the labeling dye was added (from a stock of 2 mg/ml in methanol) at ~0.5 $\mu g/A_{260}$ of nuclei. The mixture was stirred in ice and irradiated for 20 min with pyrex filtered light from an Osram Sp 200 super pressure mercury lamp (~3 × 10^{17} quanta/s . cm^2 at 300–450 nm).

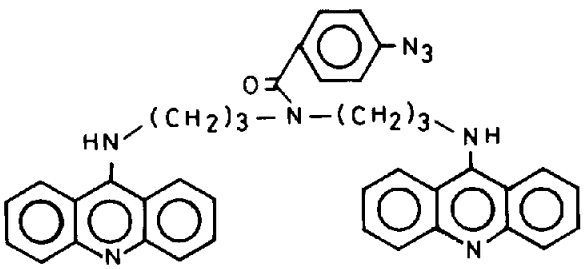

Fig.1. Structure of the photoaffinity labeling compound.

The photolabeled nuclei were digested in the above buffer with micrococcal nuclease (Boehringer Mannheim, 2 μg/ml, 10 min at 37°C) or DNase I (Worthington, 10 μg/ml, 10 min at 37°C) and the solubilized chromatin isolated by extraction of the spun down nuclei (2000 × g, 5 min) with 1 mM EDTA pH 8.0 (1 ml/A_{260}, 30 s on a Whirli mixer). The insoluble material was removed by centrifugation (10 000 × g, 10 min). Histones were extracted with 0.4 N H_2SO_4 and analyzed on acidic polyacrylamide slab gels [7], and histones photolabeled with the dye were identified by fluorescence: 2.5 mm gel slices were heated in 1.5 ml 5 N NaOH at 90°C for 60 min and analyzed for fluorescence (λ_{ex} 390 nm, λ_{em} 470 nm [5]).

3. Results

The histone labeling pattern is dependent on the physical state of the chromatin as shown by a photolabeling study [5].

Nuclei from Ehrlich ascites cells were photolabeled with this compound at 0.5 μg labeling reagent/A_{260} nuclei. The nuclei were then digested with nuclease and the liberated chromatin isolated by EDTA extraction (5–10% of the total A_{260} material was released). Liberated chromatin from micrococcal nuclease digestion showed the expected nucleosome pattern [8] when analyzed on a sucrose gradient (fig.2), and DNA extracted from nuclease-solubilized chromatin also showed the well known fragment sizes [8,9] when analyzed on polyacrylamide gels (fig.3).

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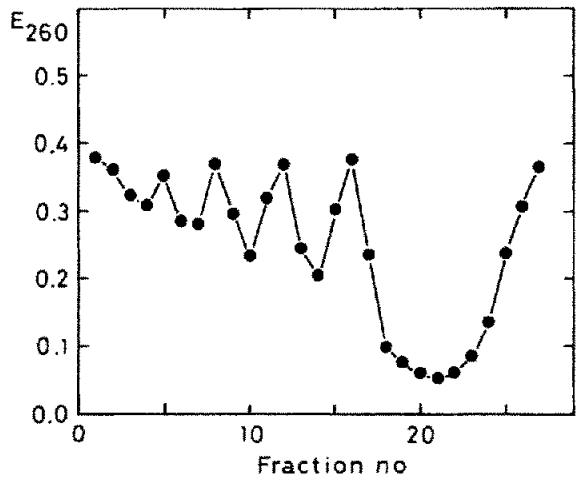

Fig.2. Sucrose gradient analysis of EDTA extract from photoaffinity labeled nuclei digested with micrococcal nuclease. The 5–25% gradient (20 mM NaCl, 0.2 mM EDTA pH 7.2) was run at 24 000 rev./min (SW 27) for 20 h, and collected from the bottom. Direction of centrifugation is from right to left.

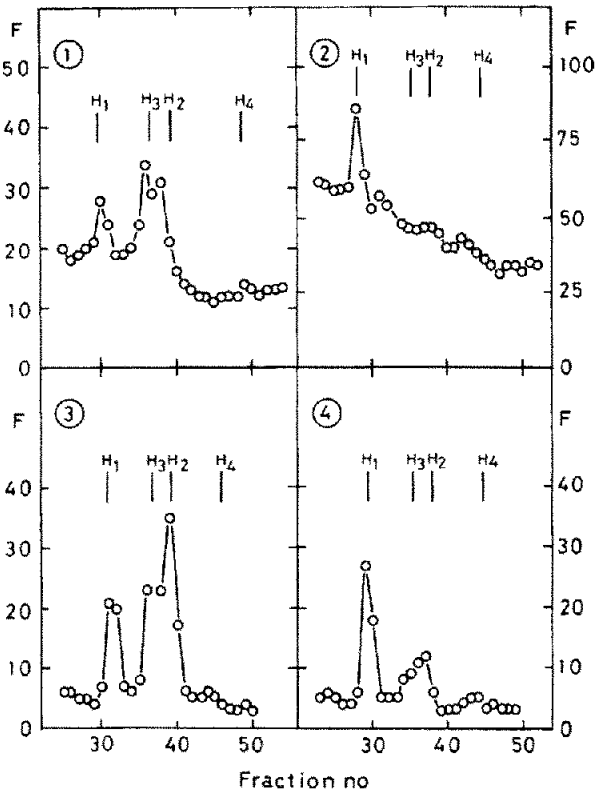

Fig.4. Nuclei (300 A_{260} units) were photoaffinity labeled as described and digested either with micrococcal nuclease [1,2] or DNase I [3,4]. Histones were extracted from both the EDTA extract [2,4] and the remaining nuclear pellet (1,3) and analyzed on polyacrylamide slab gels. One lane of each sample was treated for fluorescence analysis (and a reference lane was stained with Coomassie blue). The fluorescence units (F) are arbitrary.

Histones from both the EDTA extract and the 10 000 × g nuclear pellet were extracted and analyzed on gels. The histone patterns of all fractions were alike (representative gel, fig.3). Fluorescence analyses of the gels are shown in fig.4. While histones from the

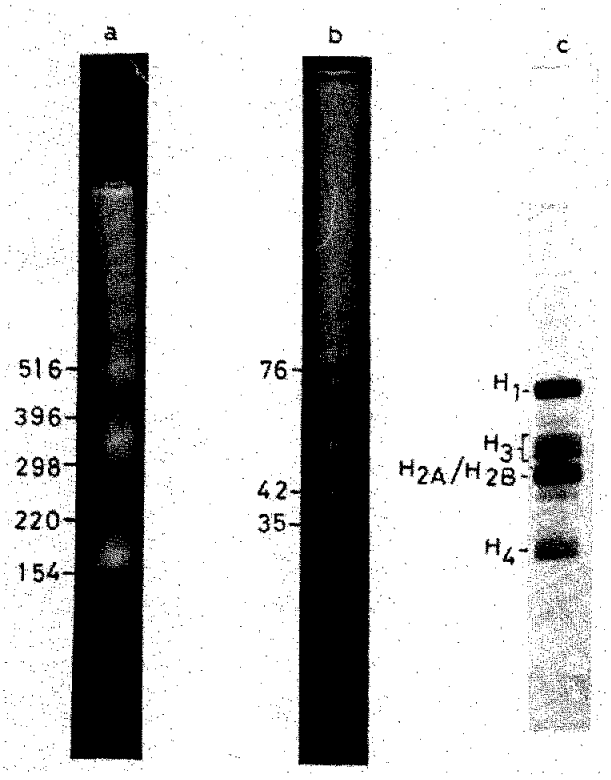

Fig.3. (a) Gel analysis (3% polyacrylamide, other conditions as in [13]) of DNA (phenol-extracted) from micrococcal nuclease liberated chromatin. The numbers designate the positions of *Hin*f fragments of pBR 322 DNA. (b) Gel analysis (8% polyacrylamide) of DNA from DNase I liberated chromatin. The numbers indicate the positions of S_1 fragments of yeast-tRNA^{Phe}. The DNA bands were visualized by ethidium bromide fluorescence staining. (c) Polyacrylamide gel of extracted histones (stained with Coomassie blue).

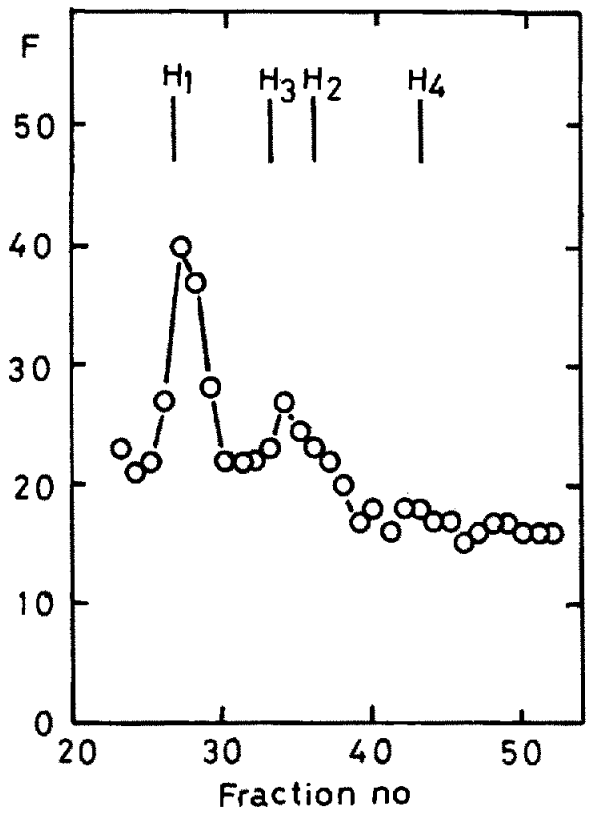

Fig.5. An oligonucleosome preparation obtained by EDTA extraction of micrococcal nuclease digested nuclei as in section 2, was photolabeled (1.2 μg dye/A_{260}) and the extracted histones were analyzed by polyacrylamide gel electrophoresis.

nuclear pellet gave a labeling pattern similar to that obtained from isolated chromatin [5], the nuclease-sensitive chromatin showed preferential labeling of histone H_1.

Furthermore, photolabeling of oligonucleosomes prepared by micrococcal nuclease digestion of nuclei also resulted in H_1 labeling (fig.5). If the nucleosomes were extensively digested with DNase I prior to photolabeling the histone labeling pattern (not shown) was indistinguishable from that of free histones [5], indicating that the histones themselves are not the cause of the difference in labeling.

The degree of specificity of the H_1-labeling has not been determined yet, since the weak labeling of H_2/H_3 (fig.4,5) were not always observed and may depend on both the degree of digestion and the dye concentration.

4. Discussion

These results indicate that the class of chromatin which is sensitive to both DNase I and micrococcal nuclease (probably the transcriptionally active part [4]) differs structurally from bulk chromatin as detected by photolabeling analysis. This property of the chromatin, which results in preferential labeling of histone H_1, is inherent in the oligonucleosome structure itself and not a consequence of higher order (solenoid [10]) structures of the chromatin.

On a molecular level several explanations are possible for the observed labeling patterns:

(i) The labeling reagent is bound to the DNA at the time of photolysis [5], and since the distance from the intercalated part of the reagent (the acridines) to the photolabile part (the azide) is very short (5–10 Å), the labeled histones must have been in close contact with the DNA at the time of reaction. Thus, one explanation for the weak labeling of the core histones H_2 and H_3 could be that the DNA is wrapped less tightly around the histone core in active chromatin [4], while H_1 is still strongly bound.

(ii) Two classes of binding sites for ethidium bromide exist in chromatin [11]. One may be in the linker region and the other confined to the nucleosome core. Therefore, only the binding sites in the linker region may be occupied by the labeling reagent in the nuclease-sensitive chromatin.

(iii) All labeling may be due to dye molecules bound at the internucleosomal linker. Thus, active chromatin has an extended conformation, thereby prohibiting dye molecules bound at the linker to reach the histone core, while bulk chromatin is more condensed, allowing contact between the linker and the nucleosome core. H_1 can be cross-linked to core histones in bulk chromatin [12].

Further experiments, including labeling with reagents having a longer distance between the intercalators and the photoactive group are underway to distinguish between (i)–(iii).

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