Koreeda, M., Moore, P. D., Yagi, H., Yeh, H. J. C., & Jerina, D. M. (1976) *J. Am. Chem. Soc. 98*, 6720–6722.
Lieberman, M. W., & Dipple A. (1972) *Cancer Res. 32*, 1855–1860.
Moschel, R. C., Baird, W. M., & Dipple, A. (1977) *Biochem. Biophys. Res. Commun. 76*, 1092–1098.
Moschel, R. C., Pigott, M. A., Costantino, N., & Dipple, A. (1983) *Carcinogenesis 4*, 1201–1204.
Osborne, M. R., Beland, F. A., Harvey, R. G., & Brookes, P. (1976) *Int. J. Cancer 18*, 362–368.
Rayman, M. P., & Dipple, A. (1973a) *Biochemistry 12*, 1202–1207.
Rayman, M. P., & Dipple, A. (1973b) *Biochemistry 12*, 1538–1542.
Sawicki, J. T., Moschel, R. C., & Dipple, A. (1983) *Cancer Res. 43*, 3212–3218.
Sims, P., Grover, P. L., Swaisland, A., Pal, K., & Hewer, A. (1974) *Nature (London) 252*, 326–328.
Sukumaran, K. B., & Harvey, R. G. (1979) *J. Am. Chem. Soc. 101*, 1353–1354.
Thakker, D. R., Yagi, H., Lu, A. Y. H., Levin, W., Conney, A. H., & Jerina, D. M. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 3381–3385.
Vigny, P., Duquesne, M., Coulomb, H., Tierney, B., Grover, P. L., & Sims, P. (1977) *FEBS Lett. 82*, 278–282.
Yang, S. K., McCourt, D. W., Roller, P. P., & Gelboin, H. V. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2594–2598.

Photoaffinity Labeling of Chromatin Using a Tritiated (Azidoaryl)bis(acridinyl)spermidine Photoprobe†

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ABSTRACT: The synthesis of a tritium-labeled photoactive bis(acridine), [1,8-^{3}H]-*N,N'*-bis(9-acridinyl)-4-aza-4-(4-azidobenzoyl)-1,8-diaminooctane, is described. This reagent may be used as a photoprobe for the study of chromatin structure. Photolabeling of nuclease-solubilized chromatin resulted in labeling of both the DNA and the proteins (1% and 5–10% yield, respectively, in terms of added reagent). Equal labeling of the histones, H_1, H_{2A}, and H_3, was observed while no significant labeling of H_{2B} and H_4 was detected. However, in the presence of 6 M urea a drastic increase in the labeling of the core histones including H_{2B} was seen. In the absence of DNA only H_3 was labeled in both cases. It is concluded that the reagent binds preferentially to the internucleosomal linker at low reagent to base pair ratios (~0.01). It is furthermore shown that qualitatively similar histone labeling is obtained with whole cells as well as with isolated nuclei and solubilized chromatin. These results indicate that the reagent may be useful as a probe for chromatin structure both in vitro and in situ.

The structure and function of eukaryotic chromatin are the subject of intense study [recently reviewed by Igo-Kemenes et al. (1982) and Weisbrod (1982)] and detailed knowledge of the architecture of the nucleosome core with regard to interrelationships between the individual histones as well as between the histones and the DNA has been obtained (Mirzabekov, 1980; Klug et al., 1980). However, our understanding of the mechanisms governing the function, e.g., the turning off and on of individual genes, is still fragmentary.

Recently, photoaffinity labeling was introduced as an additional method for studying protein–nucleic acid interactions by development of photosensitive derivatives of 9-(alkylamino)acridines (Nielsen, 1981, 1982, Nielsen et al., 1983). These compounds contain an aminoacridine moiety, which binds to double-stranded DNA by intercalation (Hansen et al., 1983), and in most cases an arylazido moiety which upon irradiation with long wavelength ultraviolet light is able to bind covalently to both proteins and nucleic acids with high efficiency (Nielsen, 1982; Nielsen et al., 1983). This photoreaction most probably takes place with nucleophilic groups of the macromolecules (Nielsen & Buchardt, 1982; Nielsen, 1982). Preliminary results indicated that the labeling pattern of the histones reflects the condition of the chromatin even though a detailed interpretation was not possible (Nielsen, 1981, 1982; Nielsen et al., 1983).

Initially a fluorescence detection system was employed for analysis of the photoaffinity labeling of chromatin (Nielsen, 1981, 1982). However, this technique prevented the use of sodium dodecyl sulfate (SDS)[1] containing gel systems, thereby resulting in a limited application for the technique, and furthermore, a higher sensitivity was warranted.

These problems have now been overcome by the synthesis of a tritium-labeled photoprobe: [1,8-^{3}H]-*N,N'*-bis(9-acridinyl)-4-aza-4-(4-azidobenzoyl)-1,8-diaminooctane ([^{3}H]ABA) (Figure 1). This paper describes the synthesis of this compound as well as results for a further evaluation of the potentials of photoaffinity labeling as a method for analyzing chromatin structure and function both in vitro and in situ.

Materials and Methods

Synthesis of [1,8-^{3}H]-N,N'-Bis(9-acridinyl)-4-aza-4-(4-azidobenzoyl)-1,8-diaminooctane. A total of 400 μCi of [1,8-^{3}H]-4-aza-1,8-diaminooctane ([^{3}H]spermidine) (22 Ci/mmol, 1 Ci/mL; New England Nuclear) was mixed with

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[1] Abbreviations: SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; bp, base pairs.

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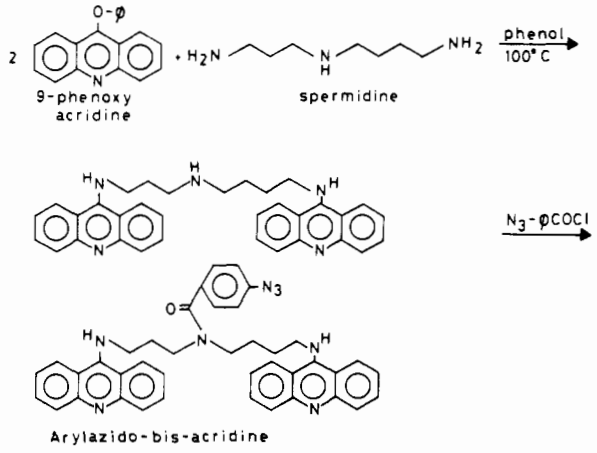

FIGURE 1: Synthetic scheme for the preparation of [1,8-^{3}H]-*N*,*N*′-bis(9-acridinyl)-4-aza-4-(4-azidobenzoyl)-1,8-diaminooctane [(azidoaryl)bis(acridine) = ABA].

200 μg (0.78 μmol) of spermidine trihydrochloride, evaporated to dryness in vacuo, and redissolved in 900 μL of phenol (Merck). A 1-mg sample of 9-phenoxyacridine (Dupre & Robinson, 1945) dissolved in 100 μL of phenol was added, and the mixture was heated at 90 °C for 60 min. After the mixture was cooled 2 mL of ether was added, and this solution was extracted with 2 mL of 10 mM HCl. The aqueous phase was extracted with 2 × 2 mL of ether in order to remove the phenol and excess 9-phenoxyacridine and was then lyophilized. The lyophilysate was redissolved in 400 μL of 5% triethylamine in dry ethanol, and three portions of 400 μg of 4-azidobenzoyl chloride (Nielsen & Buchardt, 1982) in 80 μL of dry dioxane were added over 30 min. The title compound was finally purified by thin-layer chromatography (silica gel, 1% triethylamine in ethanol–acetone, 1:1) and extracted from the silica by dimethyl sulfoxide (3 × 150 μL). Typically the isolated yield on the basis of radioactive spermidine was 30%.

Isolation of Chromatin. Chromatin was isolated from Ehrlich ascites tumor cells. The nuclei were isolated essentially according to Lerner & Steitz (1979), treated with micrococcal nuclease, and extracted with 1 mM EDTA, pH 8. Typically, nuclei (~1.5 mg of DNA equivalents) resuspended in 4 mL of buffer (0.34 M sucrose, 60 mM KCl, 15 mM NaCl, 1 mM $CaCl_2$, 10 mM Tris-HCl, pH 7.5 and 0.5% Nonidet P-40) were treated with 200 units of micrococcal nuclease (Boehringer) for 15 min at 37 °C. The nuclei were reisolated by centrifugation (1000*g*/5 min) and extracted with 5 mL of 1 mM EDTA, pH 8 at 0 °C. The supernatant (10000*g*/10 min) from this extraction was used as solubilized chromatin. The pellet was allowed to swell overnight in 5 mL of 1 mM EDTA (4 °C) and was termed the nonsolubilized chromatin fraction. The solubilized chromatin typically contained 50% of the total nuclear DNA, and the DNA isolated from this fraction ranged from 200 base pairs up to 20 kilobase pairs in size as previously reported (Matzuoka et al., 1984).

Photolabeling. In a typical experiment 10^6 cpm (1.3 μg) of [^{3}H]ABA dissolved in 100 μL 1 mM EDTA and 10 mM Tris-HCl, pH 7.4, was added to solubilized chromatin (100 μg of DNA equivalents) in 500 mL of the same buffer. Following a 10-min equilibration period (4 °C) the sample was irradiated for 15 min with Pyrex-filtered light from an Osram Sp 200 super pressure mercury lamp (69 J/cm^2) (Nielsen et al., 1983). During the irradn. the sample was stirred magnetically while kept in an ice bath.

Extraction Procedures. Histones were extracted in 0.2 M H_2SO_4 and precipitated in 66% ethanol. Loosely bound non-histone chromosomal proteins were extracted in 0.35 M NaCl and likewise precipitated. DNA was extracted with 1 volume of phenol after making the solution 0.5% in sodium dodecyl sulfate and 0.1 M in sodium acetate, pH 8. The aqueous phase was then extracted twice with ether and the DNA precipitated in 66% ethanol.

Gel Electrophoresis. Proteins were analyzed by SDS–polyacrylamide gel electrophoresis (18% gels) according to Thomas & Kornberg (1978) and visualized by staining with Coomassie brilliant blue R 250.

DNA was analyzed on 1% agarose gels run in TAE buffer (40 mM Tris–acetat, 2 mM EDTA, and 5 mM NaOAc, pH 8.0) or on 5% polyacrylamide gels using TBE buffer (0.09 M Tris–borate and 1 mM EDTA, ph 8.3). DNA bands were visualized by staining with ethidium bromide combined with fluorescence detection by photographing the UV-illuminated gel through a red filter.

Detection of Photolabeled Proteins and DNA. Photolabeled proteins and DNA were detected by slicing the gels and measuring the radioactivity in the gel slices by liquid scintillation counting (lipo-luma, luma solve from "Lumac").

Alternatively, photolabeled proteins were detected by fluorography using "EN^{3}HANCE" (New England Nuclear) and Kodak X-omat X-ray films. Exposure times were typically 1–4 weeks. Densitometric scannings were performed with a Shimadzu CS 930 instrument equipped with a DR-2 data recorder.

Isolation and Cleavage of H_1. Histone H_1 was extracted from photolabeled chromatin in 5% perchloric acid and isolated by precipitation with 3 volumes of acetone and 0.2 volume of concentrated HCl.

H_1 (10 μg) was cleaved with 0.03 μg of α-chymotrypsin in 15 μL of 0.2 M Tris-HCl, pH 8.0, at 26 °C for 10 min, and the reaction was stopped by heating the sample for 1 min at 100 °C.

Cleavage by *N*-bromosuccinimide (NBS) was performed by treating 10 μg of H_1 with 3 μg of NBS in 10 μL of 15 mM HCl for 10 min at 26 °C. The reaction was stopped by adding 50 μg of tryptophan. These procedures are essentially as described by Boulikas et al. (1980) and Singer & Singer (1976).

Preparation and Nuclease Digestion of H_1-Depleted Chromatin. To photolabeled chromatin (500 μg of DNA equivalents in 1 mL of 10 mM Tris-HCl and 1 mM EDTA pH 7.5) was added 1 volume of 1 M NaCl and 100 mg of cation-exchange resin AG 50 W-X2 (Bio-Rad) [washed as described by Bolund & John (1973)]. The mixture was stirred for 30 min at 0 °C, and the supernatant was then dialyzed overnight (4 °C) against 1 L of 1 mM EDTA. The H_1-depleted chromatin was treated with 5 units of micrococcal nuclease in 2 mL of 10 mM $CaCl_2$ and 10 mM Tris-HCl, pH 7.5 at 37 °C. At the times indicated in Figure 5 the DNA was isolated from 500-μL aliquots: 100 μL of 1.5 M Tris-HCl and 2 M NaCl, pH 8.8 and 50 μL of 5% sarcosyl was added, and the samples were extracted with 500 μL of (i) $CHCl_3$/octanol (9:1), (ii) phenol, and (iii) 2 × diethyl ether. The DNA was precipitated with 1 mL of ethanol and washed once with ethanol. The amount of DNA was determined spectrophotometrically (ϵ_{260} ~ 6600) and the amount of attached label by liquid scintillation counting.

RESULTS

Synthesis. The tritiated labeling reagent [[1,8-^{3}H]-*N*,*N*′-bis(9-acridinyl)-4-aza-4-(4-azidobenzoyl)-1,8-diaminooctane]([^{3}H]ABA) (Figure 1) was synthesized analogously to the procedure for the nonlabeled compound (Nielsen, 1982), with tritiated spermidine, except that 9-phenoxyacridine was

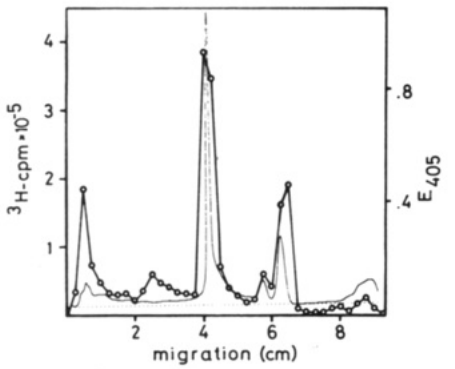

FIGURE 2: TLC analysis of the crude reaction product. An aliquot of the reaction mixture was analyzed by TLC (silica gel; ethanol/acetone/triethylamine, 50:50:1). The TLC plate was scanned at 405 nm (—) and subsequently analyzed for radioactivity by liquid scintillation counting of scrapeoffs from the plate (O). The peak at 4 cm is [^{3}H]ABA.

used instead of 9-chloroacridine and the reaction was carried out in phenol.

The commerically available [^{3}H]spermidine had a specific activity of 22 Ci/mmol ~ 80 μCi/μg but was diluted with unlabeled spermidine to a specific activity of 1 μCi/μg in order to obtain satisfactory yields. The reaction was probably disturbed by impurities originating from the hydrochloric acid solution in which the [^{3}H]spermidine was supplied (1 μCi/μL). Even under optimized conditions [varing the amount of 9-phenoxyacridine, incubation time at 90 °C, amount of *p*-azidobenzoyl chloride, and temperature (0 or 25 °C) of the acylation] a byproduct which has not been identified was always formed (Figure 2). The isolated radiochemical yield of [1,8-^{3}H]-*N*,*N*′-bis(9-acridinyl)-4-aza-4-(4-azidobenzoyl)-1,8-diaminooctane was typically 30%.

Photolabeling of Histones. The tritiated (azidobenzoyl)-spermidine bis(acridine) labeling reagent was first used to verify the results previously obtained by fluorometrical detection using the analogous norspermidine reagent (Nielsen, 1982). When SDS–polyacrylamide gel electrophoresis for protein separation and fluorography was employed for detection of labeled proteins, the histone-labeling patterns for solubulized chromatin as well as isolated histones (a natural mixture of all five major species obtained by acid extraction of chromatin) were analyzed. The results presented in Figure 3a show that, in chromatin, histones, H_1, H_{2A}, and H_3 are labeled to an equal extent while no labeling of H_{2B} or H_4 is taking place. In contrast, H_3 is predominantly labeled if no DNA is present (Figure 3b). These results agree well with the ones obtained previously by fluorometrical detection combined with urea/acetic acid/polyacrylamide gel electrophoresis (Nielsen, 1982).

In order to evaluate the usefulness of the histone-labeling pattern for probing of the chromatin structure/conformation, the effect of urea, which causes unfolding of the nucleosomal structure [see, e.g., Olins et al. (1977)], was investigated. These results are discussed in full in a separate paper (Nielsen et al., 1985), but as seen from Figure 3c, the histone-labeling pattern changes drastically in the presence of urea. A significant increase in the relative, as well as the absolute, labeling intensity of the core histones is observed, and furthermore, histone H_{2B} is being labeled above 3 M urea.

The effect of changes in the reagent/base pair ratio is shown in Figure 4. At low ratios equal labeling of H_1, H_{2A}, and H_3 is seen, but with increasing ratios the relative labeling of the linker histone, H_1, decreases while the relative labeling of H_3 increases.

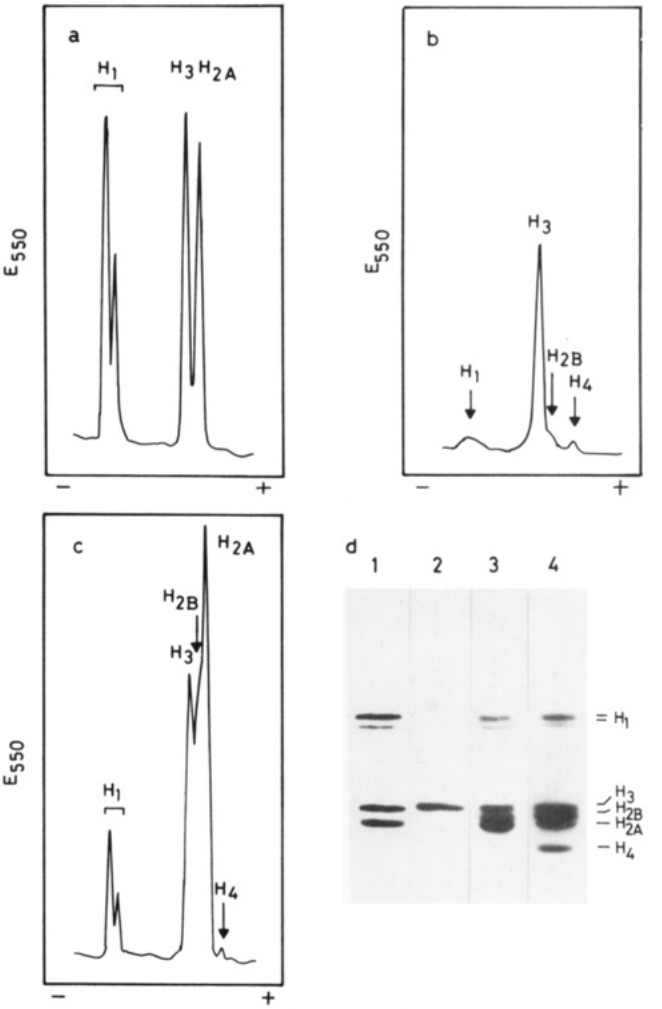

FIGURE 3: Histone-labeling patterns. The figure shows the densitometric scans of autoradiograms. (a) Solubilized chromatin (100 μg of DNA equivalents) was photolabeled with 1.3 μg of [^{3}H]ABA, and the histones were extracted and analyzed by SDS–polyacrylamide gel electrophoresis. (b) Histones (100 μg) were photolabeled with 1.3 μg of [^{3}H]ABA and analyzed. (c) As in (a) except for the presence of 6 M urea during the photolabeling. In all experiments histone and molecular weight markers [bovine serum albumin (M_r 68K), ovalbumin (M_r 45K), and lysozyme (M_r 13.5K)], were always run alongside the labeled histones, and the latter were identified on the basis of their relative migration. In (d) the autoradiograms (lanes 1–3) which were scanned to obtain (a)–(c) are shown as well as the histone marker run in parallel and stained with Coomassie blue (lane 4).

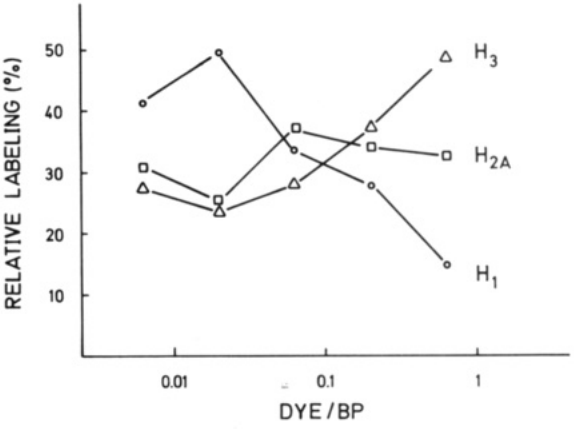

FIGURE 4: Dependency of the histone labeling pattern of chromatin on the reagent/base pair ratio. A 1.3-μg sample of [^{3}H]ABA was used in all experiments while the concentration of chromatin was varied.

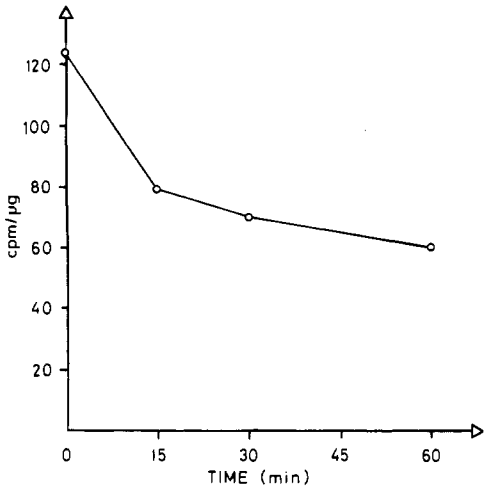

FIGURE 5: Specific activity of DNA isolated from photolabeled chromatin. Solubilized chromatin (125 μg of DNA equivalents) was photolabeled with [^{3}H]ABA (1.5 μg). The chromatin was depleated of H_1 and digested with micrococcal nuclease for the times indicated. The DNA was finally extracted, and the specific radioactivity was determined. The isolated DNA was also analyzed on a 6% polyacrylamide gel by using a *Hae*III restriction enzyme digest of plasmid pBR 322 as the size marker. This analysis showed that the DNA from the undigested sample (O′) consisted of mono- (~190 bp), di- (~330 bp), tri- (~510 bp) and higher oligonucleosomal DNA, while the digested samples consisted of 90% core particle DNA (150 bp) and a small amount (10%) of DNA from linker-free dinucleosomes (290 bp).

Furthermore, it was found that the size of the chromatin ranging from 2 to ~100 nucleosomes (mean size) does not significantly influence the histone labeling pattern (results not shown).

The labeling results presented in Figures 3–5 were all obtained with solubilized chromatin, i.e., the chromatin fraction extractable in 1 mM EDTA from nuclei treated with micrococcal nuclease. Photoaffinity labeling of the nonsolubilized fraction resulted in an ~4-fold lower labeling yield and also lower relative labeling of H_1. typically the labeling yields of histones in solubilized chromatin were 3–5% relative to the amount of reagent.

Photolabeling of DNA. Photolabeling of oligonucleosomes also resulted in labeling of the DNA. Approximately 1% of the ^{3}H label was recovered in the DNA fraction upon phenol extraction. Micrococcal nuclease digestion of photolabeled, H_1-depleted chromatin furthermore indicated preferential labeling of the linker DNA (Figure 5).

The linker preference is probably even more pronounced than the data in the figure suggests, since an analyses by gel electrophoresis showed that the radioactivity in the 15-min sample was mainly located as a peak coinciding with the DNA peak (150 base pairs) while no distinct peaks of radioactivity were detected in the 60-min sample, indicating that the specific activity of the core DNA in this sample was significantly lower than that of the total DNA which is presented in Figure 5.

Distribution of Label within H_1. In order to determine if any specificity of the labeling within the linker histone H_1, could be detected, H_1 was extracted from photolabeled chromatin with perchloric acid and cleaved with either α-chymotrypsin or *N*-bromosuccinimide (NBS). The results presented in Figure 6 indicate that the label is distributed equally between all the fragments produced by the NBS cleavage, while a slight abundance of label is found in the larger fragment obtained by cleavage with α-chymotrypsin (peak 1 vs. peak 3).

Photolabeling of Non-Histone Chromosomal (nhc) Proteins. The non-histone chromosomal proteins of the chromatin were also photolabeled by the reagent as can be seen from Figure 7e,f, but this protein fraction has not been analyzed in any detail yet.

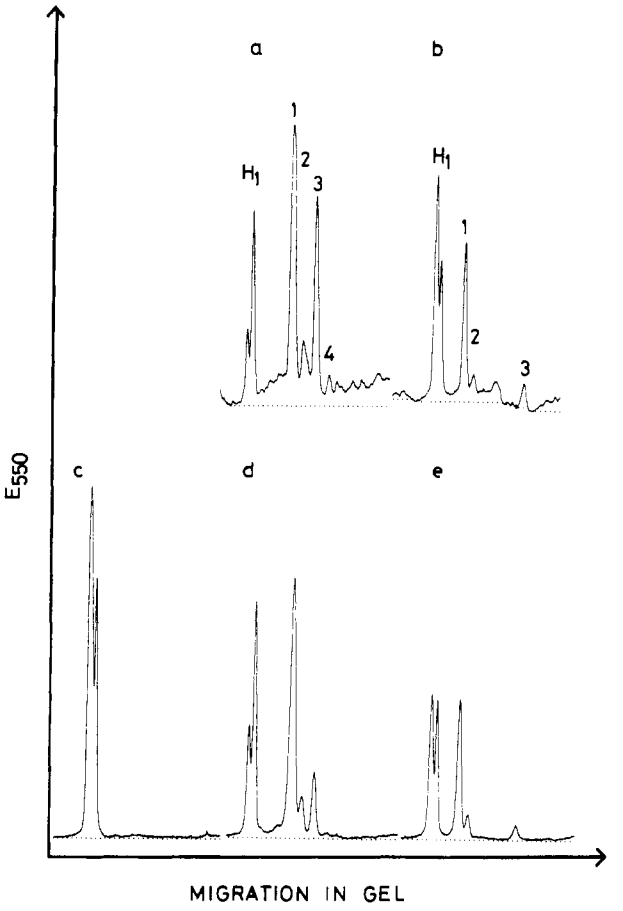

FIGURE 6: Cleavage of H_1 extracted from photolabeled chromatin. Histone H_1 was extracted from photolabeled chromatin, treated with α-chymotrypsin or NBS and analyzed by SDS gel electrophoresis. Parts a and b show the densitometric scans of the stained gel while parts c–e show the densitometric scans of the autoradiograms. (c) No treatment; (a, d) cleaved with α-chymotrypsin; (b, e) cleaved with NBS. The fragments were tentatively assigned as follows: a_1, ~107–212; a_3 ~1–106 (b_1, ~73–212; b_2, ~1–72). All samples were analyzed side by side on the same slab gel, and a size marker of 13.5 kilodaltons migrated between peaks 3 and 4 of the α-chymotrypsin digest (a).

Photolabeling of Nuclei and Whole Cells. One of the major advantages of the photoaffinity labeling technique is the potential possibility to study macromolecular systems in situ, i.e., without the manipulation of an isolation procedure. In order to evaluate this aspect of the present compound, photoaffinity labeling experiments were performed on isolated nuclei as well as on intact cells (Figure 7). Results qualitatively identical with the ones obtained with isolated chromatin were obtained in terms of the histone labeling, but relative to chromatin an increased labeling yield (~4-fold) was found for nucleic and a decreased (~40%) yield for whole cells. Quantitative differences in the relative labeling of the various histones, H_1, H_{2A}, and H_3, were also observed but have not yet been analyzed in detail. Furthermore, significant labeling of a 45K protein was detected with nuclei but not with chromatin. The molecular weight of this labeled protein coincides with that of actin.

Discussion

In order to exploit a photochemical probe for studying chromatin structure and function, it is essential to understand

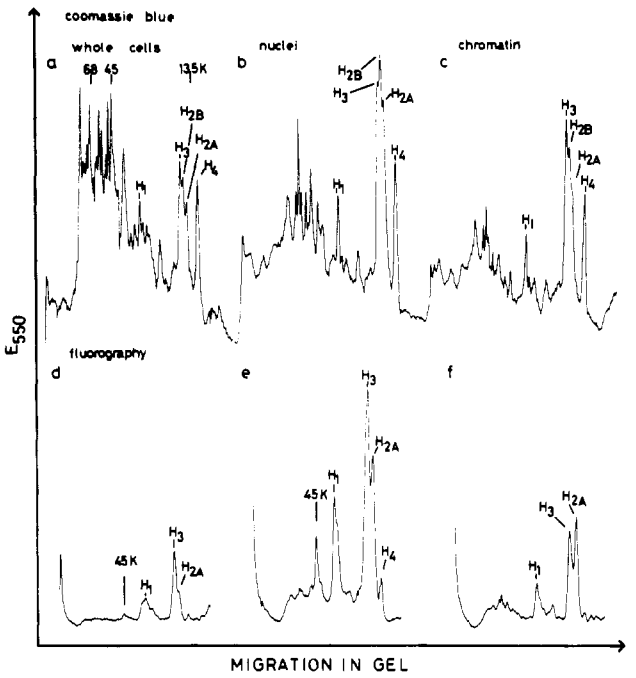

FIGURE 7: Photolabeling of proteins in intact cells, nuclei, and isolated chromatin. A total (a, d) of 5 mg (wet weight) of cells was photolabeled with 1.3 μg of [^{3}H]ABA in 500 μL of 0.15 M NaCl and 10 mM Tris-HCl, pH 7.5. The cells were washed twice, resuspended in the SDS application buffer (100 °C, 5 min), and analyzed by SDS gel electrophoresis. (b, e) Nuclei (100 μg of DNA equivalents) were photolabeled in 500 μL of sucrose buffer (0.34 M sucrose, 60 mM KCl, 15 mM NaCl, 1 mM $CaCl_2$, 10 mM Tris-HCl, pH 7.5, and 0.5% Nonidet P-40) with 1.3 μg of [^{3}H]ABA, washed twice in this buffer, and analyzed by SDS gel electrophoresis. (c, f) Chromatin was prepared from nuclei (100 μg of DNA equivalents) by lysis in 1 mM EDTA, pH 8.0. The chromatin was washed in 1 mM EDTA photolabeled with 1.3 μg of [^{3}H]ABA and analyzed. Parts a–c show densitomertric scans of the stained gels while parts d–f show scans of the autoradiograms.

the basic interactions of this probe with the macromolecular complex in a state where the complex is well characterized. At low ionic strength the nucleosomes are organized in a "zigzag" configuration along the chromatin fiber (Thoma et al., 1979; Worcel et al., 1981) which is thus in an extended conformation. Furthermore, the approximate positions of the histones, H_{2A}, H_{2B}, H_3, and H_4 in the nucleosome core have been described (Mirzabekov et al., 1978; Mirzabekov, 1980; Richmond et al., 1984). Histone H_1 is believed to be associated with the linker DNA at the point of entry/exit from the nucleosome (Thoma et al., 1979; Klug et al., 1980; Richmond et al., 1984), thereby stabilizing the zigzag conformation and also being responsible for the characteristic salt-induced condensation into the 30-nm fiber (Thoma et al., 1979).

Several studies have concluded that intercalators like ethidium bromide, methyl green, and psoralens bind preferentially to the linker DNA (Genest et al., 1981; Cech & Pardue, 1977; Härd, Kubista, Nielsen, and Nordén, unpublished results). The (azidoaryl)bis(acridine) used in this study is expected to behave similarly due to the intercalative properties of the aminoacridine moieties of the molecule. Upon irradiation the azidoaryl part of the probe is thus able to photoreact with the DNA itself or with proteins which are closely associated with the DNA (Nielsen, 1982) within an estimated distance of 5–10 Å. The data of Figure 5 indicate that the photoprobe preferentially associates with the linker DNA at the dye/base pair ratios ($r \sim 0.015$) used in most of the experiments in the present study. Photolabeling of H_1 is thus to be expected, as observed (Figure 3a). The labeling of H_3 and H_{2A} may be taken as support for the nucleosome models suggested by Mirzabekov (1980), Klug et al. (1980) and Richmond et al. (1984) since these models position H_{2A} and H_3 at the entry/exit of the DNA from the nucleosome. Thus, they are in close contact with the linker DNA which harbors the probe. Consequently, the labeling of H_{2B} in the presence of urea (Figure 3c; Nielsen et al., 1984) indicates an unfolding of the nucleosome core, which has also been suggested by using a variety of other techniques [e.g., see Olins et al. (1977) and Zama et al. (1978)]. Furthermore, the drastic changes in the histone labeling pattern in the presence of urea clearly demonstrate that the probe can be used to monitor structural changes of the chromatin. The changes of the labeling pattern as a function of the probe/base pair ratio most likely reflect the changes in the chromatin structure induced by the probe itself. At low ratios ($r \sim 0.01$) only small changes are observed on the pattern, indicating only minor perturbation of the structure, while a significant relative decrease in the labeling of H_1 accompanied by a relative increase in the labeling of H_3 is apparent as the probe/base pair ratio is increased ($r = 0.1$–1). This change is attributed to a partial unfolding of the nucleosome structure, a phenomenon previously described for the interaction between ethidium bromide and chromatin (Jerzmanovski et al., 1978; Paoletti, 1979).

Since the probe preferentially associates with the linker DNA, it was of interest to examine if some part of histone H_1 was specifically labeled. H_1 may be divided into three domains: a globular central region with two basic tails which contain most of the basic amino acid residues (Hartman et al., 1977; Isenberg, 1979). It has been shown that histone H_1 from calf thymus is cleaved by α-chymotrypsin near residue 106 in the COOH-terminal tail of the molecule (Bradbury et al., 1975; Singer & Singer, 1976) while initial cleavage with NBS is taking place near residue 72 in the globular region (Bustin & Cole, 1969). Although a rigorous identification of the fragments obtained by NBS or α-chymotrypsin cleavage of H_1 from Ehrlich ascites cells has not been performed, a comparison with the results from calf thymus H_1 allows for a tentative assignment (Figure 6) of at least the fragments from the cleavage with α-chymotrypsin, while an assignment of the fragments from the NBS digestion is much more ambiguous. α-Chymotrypsin treatment of H_1 generates two major fragments (fragments 1 and 3 in Figure 6a) which are thus assigned to the COOH-terminal tail (fragment 1) and the globular region plus the NH_2-terminal tail (fragment 3). The results therefor indicate a preference for labeling of the COOH-terminal tail. This may be explained on the basis of the sequence of H_1 (Isenberg, 1979) since a 2-fold abundance of lysine is found in this half of the molecule and the labeling reagent most likely preferentially reacts with amino groups (Nielsen, 1982; Nielsen & Buchardt, 1982). However, it could also be that more binding sites for the labeling reagent are in proximity to the COOH tail of H_1. Anyway, it may be concluded that photolabeling is taking place to both tails of the H_1 molecule, indicating that both tails are DNA associated. This binding mode is also included in the models for the association of H_1 with chromatin (Boulikas et al., 1980).

Although it is still premature to draw firm conclusions concerning the chromatin structure on the basis of photoaffinity labeling experiments such as the ones described here, this technique appears to have great potential as a means to monitor structural changes within the chromatin, particularly since the labeling reagent appears to possess a specific affinity toward chromatin as indicated by the preferential labeling of the histones in intact cells (Figure 7a,d). Thus, by this

technique it should be possible to analyze chromatin structure in situ in whole cells. It is thus interesting that very similar labeling patterns are obtained with nuclei (Figure 7e) and whole cells (Figure 7d) while a slightly but still distinctly different pattern is obtained if solubilized chromatin is analyzed (Figure 7f).

Registry No. [^{3}H]ABA, 95514-66-2; [^{3}H]spermidine, 95514-67-3; 9-phenoxyacridine, 2148-14-3; [1,8-^{3}H]-*N,N'*-bis(9-acridinyl)-4-aza-1,8-diaminooctane, 95514-68-4; 4-azidobenzoyl chloride, 14848-01-2.

References

Bolund, E. W., & John, C. (1973) *Eur. J. Biochem. 35*, 546–553.

Boulikas, T., Wiseman, J. M., & Garrard, W. T. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 127–131.

Bradbury, E. M., Chapman, G. E., Danby, S. E., Hartman, P. G., & Riches, P. L. (1975) *Eur. J. Biochem. 57*, 521–528.

Bustin, M., & Cole, D. (1969) *J. Biol. Chem. 244*, 5291–5294.

Cech, T., & Pardue, M. L. (1977) *Cell (Cambridge Mass.) 11*, 631–640.

Duprē, D. J., & Robinson, F. A. (1945) *J. Chem. Soc.*, 549–551.

Genest, D., Sabeur, G., Wahl, Ph., & Aubel-Sadron, G. (1981) *Biophys. Chem. 13*, 89–96.

Hansen, J. B., Koch, T., Buchardt, O., Nielsen, P. E., Wirth, M., & Nordēn, B. (1983) *Biochemistry 22*, 4878–4886.

Hartman, P. G., Chapman, G. E., Moss, T., & Bradbury, E. M. (1977) *Eur. J. Biochem. 77*, 45–51.

Igo-Kemenes, T., Hörz, W., & Zachau, H. G. (1982) *Annu. Rev. Biochem. 51*, 89–121.

Isenberg, I. (1979) *Annu. Rev. Biochem. 48*, 159–191.

Jerzmanovski, A., Staron, K., Thyniec, B., & Toczko, K. (1978) *Biochim. Biophys. Acta 521*, 493–501.

Klug, A., Rhodes, D., Smith, J., Finch, J. T., & Thomas, J. O. (1980) *Nature (London) 287*, 509–516.

Lerner, M. R., & Steitz, J. A. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 5495–5499.

Matsuoka, Y., Nielsen, P. E., & Nordēn, B. J. F. (1984) *FEBS Lett. 169*, 309–312.

Mirzabekov, A. D. (1980) *Q. Rev. Biophys. 13*, 255–295.

Mirzabekov, A. D., Shick, V. V., Belyavsky, A. V., & Bavykin, S. G. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4184–4188.

Nielsen, P. E. (1981) *FEBS Lett. 135*, 175–176.

Nielsen, P. E. (1982) *Eur. J. Biochem. 122*, 283–289.

Nielsen, P. E., & Buchardt, O. (1982) *Photochem. Photobiol. 35*, 317–323.

Nielsen, P. E., Hansen, J. B., Thomsen, T., & Buchardt. O. (1983) *Experientia 39*, 1063–1072.

Nielsen, P. E., Matsuoka, Y., & Nordēn, B. J. F. (1985) *Eur. J. Biochem. 147*, 65–68.

Olins, D. E., Bryan, P. N., Harrington, R. E., Hill, W. E., & Olins, A. L. (1977) *Nucleic Acids Res. 4*, 1911–1931.

Paoletti, J. (1979) *Eur. J. Biochem. 100*, 531–539.

Richmond, T. J., Finch, J. T., Rushton, B., Rhodes, D., & Klug, A. (1984) *Nature (London) 311*, 532–537.

Singer, D. S., & Singer, M. F. (1976) *Nucleic Acids Res. 3*, 2531–2547.

Thoma, F., Koller, Th., & Klug, A. (1979) *J. Cell Biol. 83*, 403–427.

Thomas, J. O., & Kornberg, R. D. (1978) *Methods Cell Biol. 18*, 429–440.

Weisbrod, S. (1982) *Nature (London) 297*, 289–295.

Worcel, A., & Strogatz, S., & Riley, D. (1981) *Proc. Natl. Acad. Sci U.S.A. 78*, 1461–1465.

Zama, M., Olins, D. E., Wilkinson-Singley, E., & Olins, A. L. (1978) *Biochem. Biophys. Res. Commun. 85*, 1446–1452.