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STUDIES OF THE EFFECTS *IN VITRO* OF β-PROPIOLACTONE AND β-PROPIOLACT[^{14}C]ONE ON WHOLE MOUSE SKIN CHROMATIN*

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Abstract Whole mouse skin chromatin was reacted with β-propiolactone (BPL) *in vitro*. The alkylated chromatin preparation was isolated essentially intact. The transition midpoint (Tm) of the alkylated chromatin preparation was reduced by 24°, its template activity for RNA synthesis was virtually abolished, its chromosomal RNA (cRNA) content was reduced and lysine-rich histones F1 and 1 could no longer be detected on polyacrylamide gels. DNA from this alkylated chromatin showed a similar decrease in Tm and template activity. The histones in alkylated chromatin stabilized DNA against heat denaturation to a similar extent compared to untreated chromatin. Mouse skin DNA alkylated with BPL had properties similar to those of DNA from alkylated chromatin. Buoyant density studies revealed that DNA isolated from alkylated chromatin exhibited peaks corresponding to alkylated and normal DNA. Mouse skin DNA alkylated under identical conditions exhibited a single peak for alkylated DNA. Thus portions of DNA in mouse skin chromatin appeared to be protected against alkylation *in vitro* with BPL. This was confirmed in studies using β-propiolact[^{14}C]one (BPL-^{14}C). The alkylation reactions were repeated using reduced quantities of BPL-^{14}C. DNA isolated from BPL-^{14}C-alkylated whole mouse skin chromatin contained 29 per cent of the bound radioactivity found in mouse liver DNA alkylated under identical conditions. Acidic proteins and histones as well as DNA were shown to bind BPL-^{14}C. Among the histone classes, F1 and 1 contained the greatest amount of bound BPL-^{14}C.

β-PROPIOLACTONE (BPL)† is carcinogenic to the skin of mice[1] and subcutaneously at the site of administration in mice.[2] BPL is an initiator of tumorigenesis in mouse skin[3] and is mutagenic in *Neurospora crassa*.[4]

BPL reacts with RNA and various guanine derivatives *in vitro*[5,6] and with DNA and RNA in mouse skin[7] to form 7-(2-carboxyethyl)guanine. The reaction of BPL with DNA *in vitro* resulted in a lowering of transition midpoint (Tm), buoyant density and priming activity with RNA polymerase of the alkylated DNA.[8]

In eukaryotic cells, DNA does not exist in a free form but is part of the chromatin complex which consists of DNA, histones, acidic proteins and a small quantity of chromosomal RNA (cRNA).[9] Some of the properties of these components are different from that of their behavior in the free form.[9–12] The object of the present investigations was to study the effects of BPL and BPL-^{14}C on whole mouse skin chromatin *in vitro*. The isolation and characterization of whole mouse skin chromatin

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† The preferred *Chemical Abstracts* name for BPL is 2-oxytanone.

in our laboratory have been reported.[13] Preliminary reports concerning this research have appeared in the literature.[14,15]

EXPERIMENTAL

Materials. Female ICR/Ha Swiss mice were obtained from Millerton Research Farms, Millerton, N.Y. They were vaccinated against ectromelia and used after age 6 weeks.

BPL (96–98 per cent purity) was obtained from Eastman Organic Chemicals, Rochester, N.Y., and stored at 0–4°. β-Propiolact[^{14}C]one (BPL-^{14}C) (95 per cent purity, 561 μCi/m-mole), labeled at the carbonyl carbon, was obtained from New England Nuclear, Boston, Mass. Sufficient BPL was added to give a specific activity of 103 μCi/m-mole. BPL-^{14}C was stored at 0–4°. β-Hydroxypropionic acid was obtained from K & K Laboratories, Plainview, N.Y. Cesium chloride, O.D. 0·02 max; was purchased from Gallard Schlesinger, Carle Place, N.Y. Bac-T-Flex membrane filters, type B-6, 25 mm, were purchased from Carl Schleicher & Schuell, Keane, N.H. Uridine-5-^{3}H(*N*)5-triphosphate, tetrasodium salt (23·6 Ci/m-mole), was obtained from New England Nuclear. RNA polymerase (2000 units/mg) isolated from *Escherichia coli*, B, was purchased from the Biopolymers Lab of General Biochemicals, Dover, N.J. Pronase (45,000 PUK units/g) was purchased from Calbiochem, Los Angeles, Calif. Buffer systems used were 0·2 M phosphate buffer (PB), pH 7·5;[5] dilute saline citrate buffer (DSC), 0·015 M NaCl and 0·0015 M Na citrate, pH 8·0; and Tris-HCl, 0·01 M, pH 8·0. The liquid scintillation solution, Diatol, contained 10 mg dimethyl-POPOP,* 7·6 g 2,5-diphenyloxazole and 52 g naphthalene dissolved in 150 ml methanol and 250 ml each of dioxane and toluene. The liquid scintillation solution, Aquasol, was obtained from New England Nuclear.

Instrumentation. Ultraviolet spectra of whole mouse skin chromatin and mouse DNA preparations were recorded in Tris-HCl in 1 cm cells in a Hitachi Perkin Elmer model 139 UV-Vis spectrophotometer. The Tm values of chromatin and DNA preparations were determined in DSC in a Beckman DK-2A u.v. spectrophotometer with attached Beckman temperature-controlled cuvette holder. Microdensitometer tracings of stained histone bands in polyacrylamide gels were obtained in a Joyce Loebl Chromoscan. Centrifugations were conducted in a Sorvall SS-1 centrifuge with fixed angle rotor at 0–4° unless otherwise noted.

Counting of radioactive samples. Scintillation solutions were counted in glass vials in a Nuclear-Chicago Mark I liquid scintillation spectrometer at 0–4°. All DNA and protein samples were dissolved in Tris-HCl and added to Diatol prior to counting. Radioactivity in histone bands contained in polyacrylamide gels was determined in the following manner. Gels were sliced into 1 mm thick discs with a Canalco lateral gel slicer. Discs were then placed in scintillation vials, dissolved in hydrogen peroxide and counted in Aquasol according to the method of Benjamin.[16] BPL-^{14}C counted at approximately 89 per cent efficiency in Diatol or Aquasol at the ranges recorded in these experiments.

Preparation of whole mouse skin chromatin and histones from whole mouse skin chromatin. The preparation of whole mouse skin chromatin, the determination of its histone, acidic protein and cRNA content, and the isolation of histones have been de-

* POPOP = 1,4-bis-2-(4-methyl 5-phenyloxazolyl)benzene.

scribed.[9,13] All solvents used in the isolation and storage of chromatin contained 0·05 M $NaHSO_3$ to prevent proteolytic degradation of histones.[12]

Polyacrylamide gel electrophoresis of histones. Gel electrophoresis was performed in polyacrylamide gels of pH 3·2.[13,17] The gels were stained with Amido Schwarz and then destained electrophoretically.

Preparation of DNA from whole mouse skin and mouse liver chromatin. The isolation of DNA from whole mouse skin chromatin was by sedimentation of the DNA in CsCl according to the method of Huang and Bonner.[18] Whole mouse skin chromatin (not more than 1·5 mg/ml of DNA) was dissolved in 5·0 ml of 4 M CsCl in Tris-HCl. The sample was centrifuged in a Beckman L-2 ultracentrifuge at 35,000 rev/min using a Spinco SW-50 rotor (rotor temperature 4°) for 18 hr. The DNA pellet was then dissolved in the desired buffer and dialyzed against the same buffer for 16 hr at 0–4°. DNA thus prepared contained less than 1 per cent protein as determined by the method of Lowry *et al.*[19] DNA was isolated from mouse liver chromatin by the procedure of Marmur.[20] DNA was further deproteinized by treatment with pronase[21] and contained less than 1 per cent protein.

Determination of the template activity of whole mouse skin chromatin and DNA. The procedures used were adapted from Bonner *et al.*[9] The mixture for RNA synthesis contained per 0·5 ml: 20 μmoles Tris-HCl; 2 μmoles each of $MgCl_2$ and $MnCl_2$; 0·32 μmole dithiothreitol; 0·2 μmole each of ATP, CTP and GTP; 0·1 μmole UTP-^{3}H; 0·05 to 2·0 μg MSC or DNA; and 0·2 μg RNA polymerase. The mixture was incubated for 30 min at 37° and the reaction was stopped by the addition of 1 ml of 0·1 N sodium pyrophosphate and 1·5 ml of cold 10% trichloroacetic acid. The acid-insoluble material was collected by filtration through a Bac-T-Flex membrane filter and washed three times with 5-ml portions of 6% trichloroacetic acid. Filters were air dried and counted in 20 ml Diatol.

Buoyant density determinations of DNA. The method of Schildkraut *et al.*[22] was adopted for buoyant density determinations. To 1·50 ml of an aqueous solution of CsCl (density 1·91 g/ml), neutralized to pH 7·4 with CsOH, was added with stirring 0·50 ml Tris-HCl containing 2–3 μg of mouse skin DNA isolated from whole mouse skin chromatin. The solution was centrifuged in a Beckmann model E ultracentrifuge at 44,770 rev/min for 20 hr at 25°. Ultraviolet absorption photographs were taken and analyzed in a Joyce Loebl Chromoscan. *Micrococcus lysodeikticus* DNA was used as a density marker.

Reactions of BPL in vitro with whole mouse skin chromatin and mouse liver DNA. In a typical reaction, 500 μg of whole mouse skin chromatin (as DNA) or DNA was dissolved in 1·0 ml PB.[5,8] BPL (57 mg, 800 μmoles) was added and the reaction mixture kept at 0–4° for 20 hr. Chromatin solutions were then dialyzed against Tris-HCl for 16 hr at 0–4° and then pelleted from the same solvent by centrifugation at 10,000 *g* for 20 min; the chromatin pellet was then redissolved in Tris-HCl. Chromatin so treated was designated BPL-alkylated whole mouse skin chromatin. DNA was isolated from BPL-alkylated whole mouse skin chromatin by the method of Huang and Bonner[18] as described. Mouse skin DNA, after the alkylation reaction, was dialyzed against Tris-HCl and then pelleted from 4 M CsCl as described.[18] DNA thus obtained was designated BPL-alkylated mouse skin DNA. In another reaction, mouse skin chromatin was incubated in PB as described, except that BPL was omitted from the reaction. Chromatin isolated in this manner was designated control

whole mouse skin chromatin. DNA isolated from control whole mouse skin chromatin as described[18] was designated mouse skin DNA.

A portion of mouse skin DNA was alkylated with BPL in the manner described. Both alkylated and unreacted DNA were stored in Tris-HCl at −20° for 5 weeks without significant changes in Tm or hyperchromicity. BPL-alkylated and unreacted mouse skin DNA were also stored in Tris-HCl at 0–4° for 5 days without changes in Tm, hyperchromicity or buoyant density (see below). The stability of alkylated DNA preparations under these storage conditions indicated an absence of decomposition (i.e. depurination).[8]

Reactions of BPL-^{14}C in vitro with whole mouse skin chromatin and mouse liver DNA. The alkylation reactions with whole mouse skin chromatin were run in the manner described above, except that the quantity of BPL-^{14}C used per 500 μg of chromatin (as DNA) was reduced to 5·7 mg (80 μmoles, 8·1 μCi, $16{\cdot}3 \times 10^6$ cpm). After 20 hr, the alkylated chromatin was pelleted by centrifugation at 10,000 *g* for 15 min. The pellet was resuspended in 10 ml Tris-HCl and was repelleted. This was continued (10–15 times) until the supernatant contained less than 50 cpm/ml. Chromatin thus obtained was designated BPL-^{14}C-alkylated whole mouse skin chromatin. Histones were isolated from BPL-^{14}C-alkylated whole mouse skin chromatin by extraction with 1 N H_2SO_4 as described.[9,13] The acidic protein DNA complex remaining after acid extraction of chromatin was isolated from the acid extract by centrifugation at 17,000 *g* for 30 min. The acidic protein DNA complex was then subjected to density gradient centrifugation in 4 M CsCl as described.[18] This yielded a denatured DNA pellet and an acidic protein pellicle. Native DNA was obtained from BPL-^{14}C-alkylated chromatin by density gradient centrifugation of the chromatin in 4 M CsCl.[18] Mouse liver DNA after alkylation with BPL-^{14}C was dialyzed against repeated changes of Tris-HCl (100 ml each) until the diffusate contained about 100 cpm/ml. Alkylated DNA was then precipitated by the addition of ethanol to a final concentration of 70 per cent and the precipitation was washed repeatedly with 70% ethanol. The DNA was further purified by density gradient centrifugation in 4 M CsCl.[18] Mouse liver DNA thus obtained was designated BPL-^{14}C-alkylated mouse liver DNA.

RESULTS

The average histone and acidic protein content relative to DNA determined in our laboratory on 16 whole mouse skin chromatin preparations was 1·00 ± 0·10 (S.D.) and 0·67 ± 0·080 (S.D.). Eight of the chromatin preparations were analyzed for cRNA. The average value was 0·033 ± 0·0065 (S.D.). Table 1 lists the chemical composition and Tm values of individual chromatin preparations both before and after alkylation with BPL.

The complete set of values obtained for acidic proteins, cRNA and histones for which average values are listed in Table 1 are as follows: control whole mouse skin chromatin (preparation 1), acidic protein (0·63, 0·54, 0·68); cRNA (0·043, 0·035); histone (0·96, 0·94, 0·83); BPL-alkylated whole mouse skin chromatin, acidic protein (0·55, 0·64, 0·62); cRNA (0·017, 0·021); histone (0·87, 0·87, 0·82); control whole mouse skin chromatin (preparation 2), acidic protein (0·75, 0·88, 0·81); histone (0·92, 0·84, 0·94); BPL-^{14}C-alkylated whole mouse skin chromatin, acidic protein (0·64, 0·66, 0·69); histone (0·83, 0·96, 0·84).

TABLE 1. CHEMICAL COMPOSITION OF WHOLE MOUSE SKIN CHROMATIN AND Tm VALUES

	Content relative to DNA					
	DNA	Histone*	Acidic protein*	cRNA†	Tm‡ (°)	Tm of DNA isolated from chromatin‡ (°)
Control whole mouse skin chromatin (preparation 1)	1	0·91	0·62	0·039	81	74
BPL-alkylated whole mouse skin chromatin§	1	0·85	0·60	0·019	57	50
BPL-alkylated mouse skin DNA§					56	
Control whole mouse skin chromatin (preparation 2)	1	0·90	0·81		82	73
BPL-^{14}C-alkylated whole mouse skin chromatin¶	1	0·88	0·66		81	70
Mouse liver DNA					74	
BPL-^{14}C-alkylated mouse liver DNA					67, 68	

* Average value obtained from analysis of three chromatin aliquots.

† Average value obtained from analysis of two chromatin aliquots (500 μg each as DNA).

‡ Hyperchromicities of chromatin and DNA preparations ranged between 23 and 35 per cent.

§ Reaction conditions: control whole mouse skin chromatin (preparation 1) (500 μg as DNA) or DNA (500 μg) in 1·0 ml PB (pH 7·5) was stirred for 20 hr at 0–4° with 57 mg (800 μmole) of BPL.

Reaction conditions: control whole mouse skin chromatin (preparation 2) (500 μg as DNA) or DNA (500 μg) in 1·0 ml PB (pH 7·5) was stirred for 20 hr at 0–4° with 5·7 mg (80 μmole) of BPL-^{14}C.

Reactions of BPL with mouse skin DNA. Mouse skin DNA incubated with BPL exhibited a decrease in Tm from 74 to 56° (Table 1) and a reduction of about 95 per cent in RNA polymerase activity. When 29 mg BPL was used in the alkylation reaction (reduced from 57 mg), the Tm of alkylated mouse skin DNA was 63°; when the quantity was further reduced to 12 mg, the Tm was 68°. The reduction in template activities of the alkylated DNA's was approximately 50 and 25 per cent, indicating a close association between reduction in Tm and decrease in template activity. A study of the decrease in Tm with time was conducted using mouse liver DNA (Tm 74°, hyperchromicity 35 per cent). At 1, 7, 14 and 20 hr, the Tm values were 67, 61, 57 and 55°. Hyperchromicities were greater than 28 per cent. When β-hydroxypropionic acid, the hydrolysis product of BPL, was substituted for BPL in the alkylation reaction, no change was observed in the Tm and hyperchromicity of mouse liver DNA. The pH levels of the reaction media were 4·4 and 4·2 at the end of the reactions with BPL and β-hydroxypropionic acid. pH values did not differ when mouse liver DNA was omitted from the reaction.

Reactions of BPL with control whole mouse skin chromatin. Control whole mouse skin chromatin (preparation 1) was essentially identical in chemical composition and Tm (Table 1), and in u.v. spectra and polyacrylamide gel electrophoresis of histones (Fig. 1A) to a whole mouse skin chromatin preparation previously isolated.[13] The histone and acidic protein composition and u.v. spectrum of BPL-alkylated whole

mouse skin chromatin did not differ markedly from those of control whole mouse skin chromatin. The cRNA content of BPL-alkylated whole mouse skin chromatin decreased from 3·9 to 1·9 per cent and the Tm was decreased to 57 from 81 . The template activity of BPL-alkylated whole mouse skin chromatin was reduced by about 75 per cent to control whole mouse skin chromatin (Fig. 2). The template activity of control whole mouse skin chromatin was approximately 20 per cent of its purified DNA, an order of repression reported for chromatin compared to its purified DNA.[23] The template activity of BPL-alkylated whole mouse skin chromatin was thus reduced by about 95 per cent compared to mouse skin DNA.

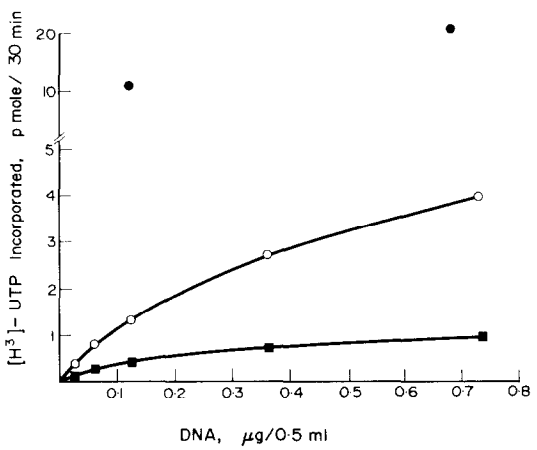

FIG. 2. Template activity for RNA synthesis of whole mouse skin chromatin preparations. ○, Control whole mouse skin chromatin; ■, BPL-alkylated whole mouse skin chromatin; ●, DNA from control whole mouse skin chromatin.

The polyacrylamide gel electrophoresis pattern of histones from control mouse skin chromatin revealed the presence of histones F1, 1 , F3, F2b, $F2a_2$ and $F2a_1$ (Fig. 1A). Except for 1 , histone designations are those of Johns.[24] Histone 1° (lysine-rich) was reported to be present in small amounts (~ 1 per cent of total histone) in chromatin from slowly dividing cells.[25] Histone designations were confirmed by comparison of electrophoretic mobilities in polyacrylamide gels with authentic samples of calf thymus F1, mouse lung F1 and 1 , and mouse liver $F2a_2$ and $F2a_1$. Polyacrylamide gel electrophoresis of histones from alkylated chromatin revealed the absence of histones F1 and 1 (Fig. 1B). The gel electrophoresis pattern was essentially identical for histones isolated from two BPL-alkylated whole mouse skin chromatin preparations. In addition, histone band F2b had a more diffuse appearance when compared to the corresponding histone band from control whole mouse skin chromatin. The intensities of histone bands $F2a_2$ and $F2a_1$ from BPL-alkylated whole mouse skin chromatin were reduced by about 40 per cent relative to F2b compared to similar bands from control whole mouse skin chromatin as determined by microdensitometer tracings. When β-hydroxypropionic acid was used in place of BPL in the alkylation reaction, chemical composition and Tm of the isolated mouse skin chro-

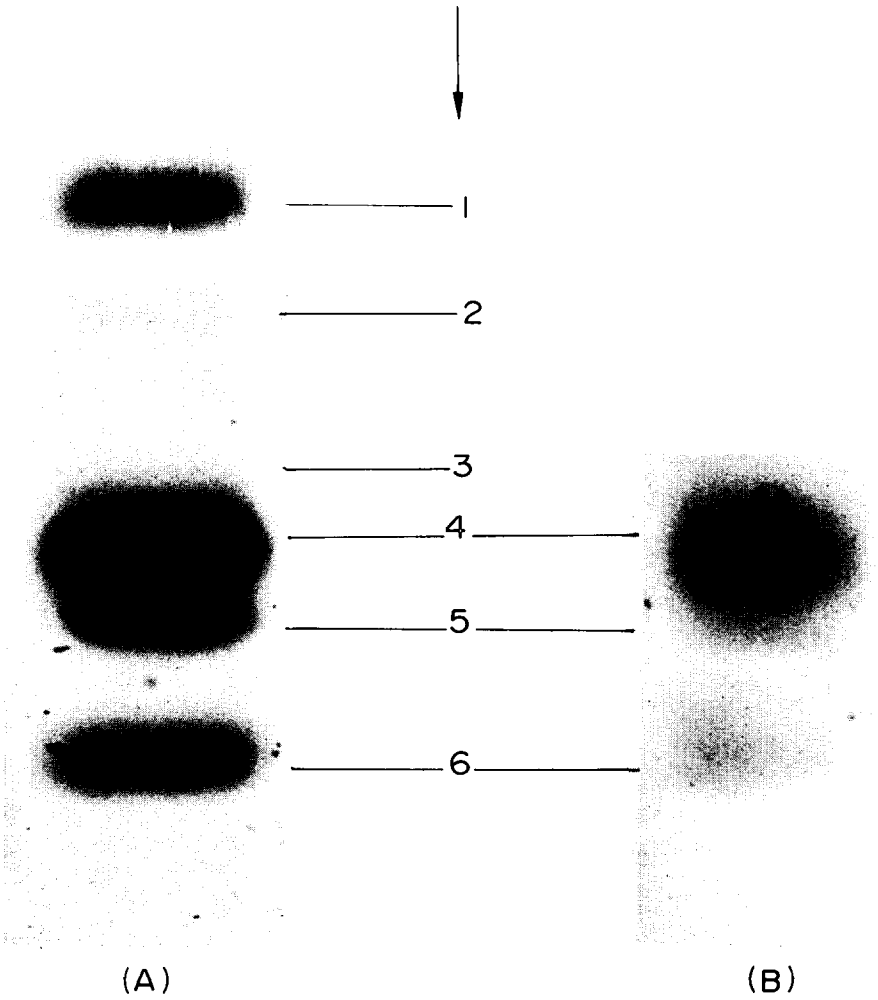

FIG. 1. Polyacrylamide gel electrophoresis of histones isolated from whole mouse skin chromatin preparation: (A) from control whole mouse skin chromatin; (B) from BPL-alkylated whole mouse skin chromatin. Bands 1–6 are histone classes F1, 1°, F3, F2b, $F2a_2$ and $F2a_1$. Arrow indicates direction of migration of histones; 30 μg histones was applied to each gel.

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matin were essentially identical to those of control whole mouse skin chromatin (preparation 1) as were histone patterns in polyacrylamide gels.

DNA isolated from BPL-alkylated whole mouse skin chromatin had a Tm of 50°, reduced from the 74° found for DNA isolated from control whole mouse skin chromatin (preparation 1). Its template activity was similar to that of BPL-alkylated whole mouse skin chromatin and BPL-alkylated mouse skin DNA (~5 per cent of mouse skin DNA).

Buoyant density studies. Mouse skin DNA isolated from whole mouse skin chromatin was resolved into two components by density gradient centrifugation in CsCl: main band DNA with buoyant density of 1·704 and satellite DNA with a density of 1·691 (Fig. 3A). The densities and relative amounts of main band and satellite DNA's correspond with those reported in the literature for mouse liver total DNA.[26] BPL-alkylated mouse skin DNA contained a single band of density 1·684 (Fig. 3B). A satellite band was not observed. DNA isolated from BPL-alkylated whole mouse skin chromatin exhibited a main band with a density of 1·686 and a smaller heavier band of density 1·701 (Fig. 3C).

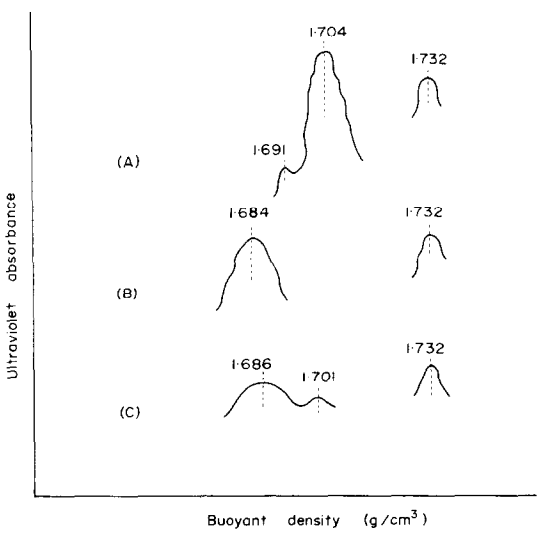

FIG. 3. Buoyant densities of whole mouse skin DNA preparations: (A) mouse skin DNA; (B) BPL-alkylated mouse skin DNA; (C) DNA isolated from BPL-alkylated whole mouse skin chromatin. *Micrococcus lysodeikticus* DNA, used as a density marker, has a density of 1·732.

Reactions of BPL-^{14}C with control whole mouse skin chromatin. Control whole mouse skin chromatin (preparation 2, Table 1) was used for these reactions. BPL-^{14}C-alkylated whole mouse skin chromatin was unchanged from control whole mouse skin chromatin (preparation 2) in chemical composition, u.v. spectra and histone patterns in polyacrylamide gels (Fig. 1A). The cRNA content of control whole mouse skin chromatin (preparation 2) was not determined. The Tm of BPL-^{14}C-alkylated whole mouse skin chromatin was 81° compared to 82° for untreated control chromatin. The alkylation reaction was run twice. The chemical composition and Tm value listed in Table 1 for BPL-^{14}C-alkylated whole mouse skin chromatin are

from the product isolated from reaction 1. The BPL-^{14}C-alkylated chromatin preparation isolated from reaction 2 had a Tm of 81° and was not subjected to chemical analysis. Native (Tm 70°) and denatured DNA isolated from BPL-^{14}C-alkylated chromatin (reaction 1) contained 15 and 12 cpm/μg. Denatured DNA isolated from BPL-^{14}C-alkylated chromatin (reaction 2) contained 16 cpm/μg (the average of 14 cpm/μg corresponds to 1 mole BPL-^{14}C/9·6 moles of guanine). BPL-^{14}C-alkylated mouse liver DNA from two alkylation reactions (reaction 1, Tm 67°; reaction 2, Tm 68°) contained 43 and 60 cpm/μg (the average of 52 cpm/μg corresponds to 1 mole BPL-^{14}C/2·6 moles of guanine). Acidic proteins isolated from BPL-^{14}C-alkylated whole mouse skin chromatin contained 67 cpm (reaction 1) and 73 cpm/μg (reaction 2). Histones contained 27 cpm (reaction 1) and 23 cpm/μg (reaction 2). Histones from reaction 2 were applied to a polyacrylamide gel (100 μg, 2300 cpm) and electrophorezed. The results in cpm/μg were 55, 32, 7·8, 10 and 5·6 for histones F1, 1°, F3 + F2b, $F2a_2$ and $F2a_1$. Histones F3 and F2b were not sufficiently resolved for separate counting. For histones F1, $F2a_2$ and $F2a_1$, the results correspond to 5·7, 0·74 and 0·31 moles BPL-^{14}C per mole of histone. The molecular weight of 1° has not been determined. Approximately 57 per cent of the applied radioactivity was recovered in the histone bands. The relative percentages of histones in control whole mouse skin chromatin determined from microdensitometer tracings was 12, 2, 46, 19 and 21 per cent for histones F1, 1°, F3 + F2b, $F2a_2$ and $F2a_1$.

DISCUSSION

Previous studies *in vitro* concerning the binding of carcinogenic alkylating agents to biological macromolecules have invariably studied the interaction of such agents with DNA or RNA. This appears to be the first study which shows that a carcinogenic alkylating agent may bind to all the major components of chromatin with the chromatin complex remaining essentially intact and that within a given class of molecules (histones) binding occurs in a fairly selective manner.

The protein content of BPL-alkylated whole mouse skin chromatin remained virtually unchanged compared to control. Histones are primarily responsible for stabilizing DNA against heat denaturation[27] and the stabilizing effect of histones on the Tm of DNA was similar in control and BPL-alkylated whole mouse skin chromatin. A surprising observation was that the Tm of DNA isolated from BPL-alkylated whole mouse skin chromatin was 6° lower than the Tm of BPL-alkylated mouse skin DNA. One would have expected DNA in chromatin to have been protected against alkylation either by proteins, which cover a large part of the DNA,[28] or by supercoiling, which could possibly make portions of DNA inaccessible to alkylation.[10,11] Interpretation of these results is further complicated by our buoyant density studies (see below) which show that DNA from BPL-alkylated whole mouse skin chromatin is alkylated to a lesser extent than is BPL-alkylated mouse skin DNA. The buoyant density results were supported by alkylation studies using BPL-^{14}C in which DNA from BPL-^{14}C-alkylated whole mouse skin chromatin contained about 29 per cent of the radioactivity of BPL-^{14}C-alkylated mouse liver DNA.

The template activities of BPL-alkylated whole mouse skin chromatin, its purified DNA, and BPL-alkylated mouse skin DNA were nearly abolished (~5 per cent of control mouse skin DNA). The alkylation of DNA in chromatin appears to be the main factor in the further repression of template activity.

The profile of the buoyant density scan of mouse skin DNA closely resembles that of mouse liver total DNA rather than heterochromatin or euchromatin DNA.[26] The reason that the satellite DNA peak was not detected in the scan of BPL-alkylated mouse skin DNA (Fig. 3B) may be due to the fact that main band DNA is much richer in GC content than satellite DNA[26] and is alkylated and thus lightened to a greater extent than satellite DNA.

The buoyant density scan of DNA isolated from BPL-alkylated whole mouse skin chromatin shows a major peak with a density close to that found for BPL-alkylated mouse skin DNA and a minor peak close to the density of main band DNA. Some of the DNA in chromatin thus appears to be protected against alkylation by BPL and, as discussed, these results were supported by our alkylation studies using BPL-^{14}C.

It is not known why the lysine-rich histones F1 and 1^{c} are absent in polyacrylamide gels of histones from BPL-alkylated whole mouse skin chromatin. The histones may be separated from chromatin after binding of BPL. A weakening of the interaction between DNA and histones could be due to alkylation (carboxyethylation) and/or acylation of the ε-amino groups in these lysine-rich histones. Both alkylation and acylation of secondary amino groups have been shown to result from reaction with BPL.[29] Alkylation and/or acylation of histones could be effective in reducing the net positive charge of the histones and consequently their affinity for negatively charged DNA. On the other hand, a reduction in the net charge of histones, while not causing a separation from DNA, could prevent migration of histones in polyacrylamide gels under the conditions used. A third possibility is that binding of BPL to histones could block binding sites of Amido Schwarz dye.

The results of polyacrylamide gel electrophoresis suggested that histones F1 and 1^{c} bound BPL to a greater extent than did other histones in chromatin. This was confirmed by analysis of radioactivity bound to histones isolated from BPL-^{14}C-alkylated whole mouse skin chromatin. The reason for the selective binding of BPL-^{14}C to histones F1 and 1^{c} is not clear at the present time, although the results suggest that binding of BPL-^{14}C to histones is at least partly lysine dependent. However, histone F1 may be particularly susceptible to alkylation (or acylation), as it is the histone most exposed to the aqueous environment.[30]

The dissociation of small amounts of histones from chromatin in Chinese hamster cells following X-irradiation has been reported.[31]

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