Isolation and Characterization of Nonhistone Chromosomal Protein C-14 Which Stimulates RNA Synthesis†

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ABSTRACT: The nonhistone chromatin protein, C-14, was extracted from chromatin of Novikoff hepatoma ascites cells and isolated in high purity as shown by its migration as a single dense spot on two-dimensional polyacrylamide gels. Its mobility on sodium dodecyl sulfate gels is consistent with a molecular weight of approximately 70 000. The amino acid composition shows that protein C-14 has an acidic:basic amino acid ratio of 1.8. Its amino terminal amino acid is lysine. Protein C-14 stimulated the incorporation of [^{3}H]UMP into RNA by approximately 30% when added to naked DNA and homologous RNA polymerase I. A 30% stimulation of [^{3}H]UMP incorporation into RNA was also found when protein C-14 was added to an *E. coli* RNA polymerase system containing either *E. coli* or Novikoff hepatoma DNA.

Nonhistone chromosomal proteins are now considered to play a role in the specification of gene products in eukaryotes (Simpson, 1973; Elgin and Weintraub, 1975; Paul and Gilmour, 1968; Olson et al., 1974a,b). This is in contrast to histones which had been suggested to be repressors of transcription of chromosomal DNA but now are considered to be structural components of nucleosomes or "ν bodies" (Olins and Olins, 1974; Van Holde et al., 1974). A number of recent reports have shown that nonhistone chromosomal proteins can activate the in vitro transcription of DNA-histone and chromatin (Kostraba and Wang, 1972; Kostraba et al., 1975; Teng et al., 1971).

The present study was designed to examine the properties of protein C-14, a nonhistone chromosomal protein from the chromatin of Novikoff hepatoma ascites cell nuclei which is one of the higher molecular weight proteins as shown by its migration in the C region of the two-dimensional polyacrylamide gel electrophoresis system (Orrick et al., 1973; Busch et al., 1974). Protein C-14 was of particular interest because it was one of the few nuclear proteins that decreased relatively in amount during liver hypertrophy produced by administration of thioacetamide to rats or following partial hepatectomy (Ballal et al., 1974, 1975). In addition, protein C-14 has been shown to have a high affinity for DNA (Prestayko et al., 1976). It is one of the nonhistone proteins that rapidly reassociated with DNA and histones after dissociation of chromatin in 5 M NaCl-7 M urea followed by stepwise dialysis to 0.15 M NaCl (Ballal et al., 1975). Protein C-14 was also shown to bind tightly to DNA-acrylamide columns since it required 2 M KCl for elution (Prestayko et al., 1976).

Using methods developed earlier, protein C-14 was purified by salt fractionation, exclusion chromatography, and slab gel electrophoresis (Taylor et al., 1973; Busch and Smetana, 1970; Marushige and Bonner, 1966; Goldknopf et al., 1975). The homogeneity of the product was demonstrated by its migration as a single spot on two-dimensional polyacrylamide gel electrophoresis and identification of a single amino terminal amino acid. A stimulatory effect of protein C-14 on DNA-dependent RNA transcription is described.

Materials and Methods

Preparation of Nuclei and Nucleoli. Novikoff hepatoma ascites cells were collected and washed as previously described (Taylor et al., 1973). Cells were swollen in 10 volumes of ice-cold buffer I[1] (10 mM NaCl-1.5 mM $MgCl_2$-0.1 mM $PhCH_2SO_2F$-10 mM Tris-HCl, pH 7.5). Cells were lysed by the addition of Nonidet P-40 (Particle Data Laboratories Ltd., Elmhurst, Ill.) to a final concentration of 0.3%. After stirring for 10 min, the suspension was centrifuged at 1000*g* for 10 min. The pellet was dispersed with a glass Teflon homogenizer in buffer I, treated with 0.2% Nonidet P-40, and centrifuged for 10 min at 1000*g*. The resulting pellet was homogenized in 0.34 M sucrose (special enzyme grade from Schwarz/Mann)-10 mM $MgCl_2$-0.1 mM $PhCH_2SO_2F$-10 mM Tris-HCl, pH 7.8; the nuclei were collected by centrifugation at 1500*g* for 20 min. This nuclear preparation had high RNA polymerase activity in contrast to citric acid nuclei (Taylor et al., 1973). Nucleoli were obtained from Novikoff hepatoma cells by sonication in sucrose containing 12 mM Mg^{2+} as previously described (Busch and Smetana, 1970).

Preparation and Extraction of Chromatin. Chromatin was prepared by the method of Marushige and Bonner (1966). The resulting chromatin was pelleted at 35 000*g* for 15 min and washed twice with 10 mM Tris-HCl, pH 8, in 0.1 mM $PhCH_2SO_2F$.

Proteins were extracted from chromatin three times by gentle homogenization in ice-cold buffer A (0.35 M NaCl-10 mM Tris-HCl (pH 8.0)-0.1 mM $PhCH_2SO_2F$). The supernatants were combined and centrifuged for 18 h at 10 000*g* to remove the small amount of nucleic acids present. The resulting

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[1] Abbreviations used: $PhCH_2SO_2F$, phenylmethanesulfonyl flouride; buffer I, 10 mM NaCl-1.5 mM $MgCl_2$-0.1 mM $PhCH_2SO_2F$-10 mM Tris (pH 7.5); buffer A, 0.35 M NaCl-10 mM Tris-HCl (pH 8.0)-0.1 mM $PhCH_2SO_2F$; buffer B, 0.6 M NaCl-10 mM Tris-HCl (pH 8)-0.1 mM $PhCH_2SO_2F$; NHP, nonhistone chromatin protein; $NaDodSO_4$, sodium dodecyl sulfate; dansyl, 5-dimethylaminonaphthalene-1-sulfonyl; Tos-PheCH_2Cl, L-1-tosylamido-2-phenylethyl chloromethyl ketone; PPO, 2,5-diphenyloxazole.

supernatant was then concentrated in an Amicon apparatus using a UM2 membrane to a protein concentration of 10 mg/mL.

Purification of the C-14 Protein. Proteins were precipitated and collected in three divisions: 0–30%, 30–60%, and 60–95% fractional saturations with ammonium sulfate. Protein solutions (10 mg/mL) were stirred at 0 °C and the resulting suspensions were kept in ice for 30 min before centrifugation at 21 000*g* for 1 h. Precipitated proteins were taken up in small volumes of ice-cold buffer A.

The 30–60% ammonium sulfate fraction was applied to a 2.5 × 140 cm Sephadex G-75 column equilibrated in buffer A. The column was eluted with buffer A and the effluent was monitored at 280 nm.

The pooled and concentrated column fractions were dialyzed against 0.9 N acetic acid–10 M urea–1% 2-mercaptoethanol at room temperature and applied to 6% acrylamide slab gels (0.3 × 7.5 × 10 cm) prepared with 0.9 N acetic acid and 10 M urea (Busch et al., 1974). Purified protein C-14 was eluted from the gel slices and dialyzed at 4 °C against buffer A (Goldknopf et al., 1975).

Two-Dimensional Polyacrylamide Gel Electrophoresis. Purification of C-14 was monitored by analytical two-dimensional gel electrophoresis. The acrylamide concentration in the first dimension was 6 or 10%, while the second dimension was 8 or 12%, respectively, as previously described (Orrick et al., 1973; Busch et al., 1974).

Amino Acid Analysis. Analyses were performed with a Beckman Model 121 amino acid analyzer according to the method of Spackman et al. (1958). Samples were hydrolyzed in sealed evacuated tubes containing 3 N mercaptoethanesulfonic acid for 22 h at 110 °C (Penke et al., 1974). Half-cystine content was measured as cysteic acid after performic acid oxidation by the method of Moore (1963). The oxidized protein was hydrolyzed with 6 N HCl at 110 °C for 22 h.

Molecular Weight Analysis. Determination of the molecular weight by polyacrylamide gel electrophoresis in sodium dodecyl sulfate was performed by the method of Shapiro et al. (1967) with the modifications of Weber and Osborn (1969).

NH_2-Terminal Analysis. For identification of the amino terminal amino acid, the dansyl method was carried out as described by Weiner et al. (1972). Dansyl amino acids were solubilized in acetone–acetic acid (3:2) and chromatographed on 7.5 × 7.5 cm polyamide sheets.

Peptide Mapping. The lysine residues of protein C-14 were carbamoylated as described by James and Noltmann (1973). Cysteine and cystine were oxidized to cysteic acid by the performic acid oxidation procedure of Hirs (1967). Modified and oxidized protein C-14 (250 μg) was heated to 80 °C in 0.1 M *N*-ethylmorpholine acetate, pH 8, for 3 min prior to digestion. Digestion twice with 1% (w/w) Tos-PheCH_2Cl-trypsin (Worthington Biochemical Corp., Freehold, N.J.) was completed in 4 h at 37 °C. Digestion was terminated by freezing and the products of digestion were freeze-dried.

Peptides were dissolved in 400 μL of 0.2 N $NaHCO_3$, pH 9, and mixed with 1.0 mCi of [^{3}H]dansyl chloride (specific activity, 24 Ci/mmol; Amersham/Searle, Arlington Heights, Ill.) in 400 μL of acetone. The coupling reaction was completed in 2 h at 37 °C. After addition of 0.15 mL of 3 M KOH to hydrolyze any unreacted dansyl chloride, the pH was adjusted to pH 3.5 with glacial acetic acid. The sample was loaded on a 0.5 × 3 cm AG-50W-X2 column equilibrated with 0.01 M acetic acid (Walker et al., 1976). [^{3}H]Dansyl peptides were eluted with H_2O–acetone–0.88 M NH_4OH (8:2:8). The eluent was dried under a stream of nitrogen.

The product was dissolved in 200 μL of acetone–H_2O (1:1) and a 1-μL aliquot spotted on a 15 × 15 cm polyamide sheet (Gallard-Schlesinger Chem. Mfg. Corp., Carle Place, N.Y.). The solvent systems employed were 5% formic acid in the first dimension and benzene–acetic acid (25:1) in the second dimension (Tichy, 1975). Peptides were detected by treating polyamide chromatograms with 6% PPO (w/v) in diethyl ether and autoradiography on RP-Royal X-Omat x-ray film at −80 °C (Randerath, 1970).

Assay of RNA Polymerase I Activity. The procedure of Roeder and Rutter (1970) was used to purify and assay RNA polymerase I from Novikoff hepatoma nucleoli. One unit of enzyme incorporated 1 pmol of [^{3}H]UMP into RNA (μg of DNA)$^{-1}$ min^{-1} (Blatti et al., 1970). Protein-free DNA (double stranded, average mol wt 10 × 10^6) from Novikoff hepatoma nucleoli was prepared as described by Sitz et al., 1973). *E. coli* RNA polymerase (395 units/mg) and *E. coli* DNA were obtained from Sigma (St. Louis, Mo.). The DNA (5 μg per sample) was dissolved in 10 mM Tris-HCl, pH 7.9; protein C-14 and other added proteins were in buffer A; polymerases were in 50% glycerol–10 mM $MgCl_2$–100 mM KCl–1.0 mM dithiothreitol–0.2 mM EDTA–20 mM Tris-HCl, pH 7.9. Buffer or protein in buffer, DNA, and polymerase were added sequentially to a test tube kept on ice. After incubation at 37 °C for 1 min, a 150-μL aliquot of reaction mixture containing [^{3}H]UTP was added; at specified times, 50-μL aliquots were pipetted onto Whatman DE 81 filter paper discs, washed, dried, and assayed for radioactivity (Blatti et al., 1970).

Other Analytical Methods. DNA was determined with the modified diphenylamine reaction (Richards, 1974). Protein was determined by the method of Lowry et al. (1951); crystalline bovine serum albumin was used as the standard (Miles Research Labs., Kankakee, Ill.). Protease activity was monitored by the azocoll method (Moore, 1969).

Results

Extraction of Chromatin Proteins. The protein to DNA ratio of the purified chromatin was 2.3:1. Three successive extractions of this chromatin with buffer A removed 32, 8, and 7%, or a total of 47%, of the chromatin proteins.[2] Two-dimensional gel electrophoresis (Figure 1) of the proteins (Busch et al., 1974; Prestayko et al., 1976), extracted with buffer A, showed a large distinct spot of protein C-14. Three additional extractions with buffer B removed an additional 26% of the chromatin proteins (Kostraba et al., 1972), but the concentration of protein C-14 in these fractions was low and they were not used.

Ammonium Sulfate Fractionation. The 30–60% ammonium sulfate fraction of proteins extracted with buffer A contained C-14 as a predominant spot that separated well from the other proteins of this fraction (Figure 2). The 0–30% and 60–95% ammonium sulfate fractions contained relatively little protein C-14.

Gel Filtration and Slab Gel Electrophoresis. Exclusion chromatography of the 30–60% ammonium sulfate fraction on Sephadex G-75 was employed to separate the larger proteins, including protein C-14, from the smaller proteins (unshaded) eluting later (Figure 3). The proteins of the shaded portion were separated further by slab gel electrophoresis

[2] Virtually no histones were found in this extract as shown by gel electrophoresis.

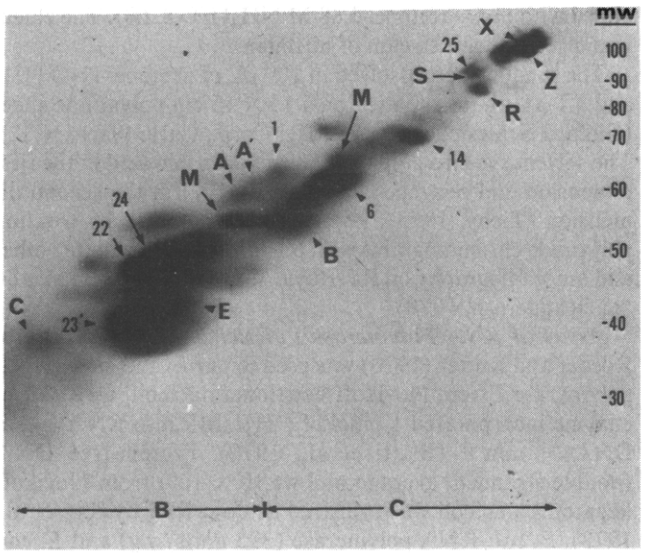

FIGURE 1: Two-dimensional polyacrylamide gel electrophoresis (Busch et al., 1974) of proteins extracted by the 0.35 M NaCl buffer from Novikoff nuclear chromatin. The first dimension (6% polyacrylamide) was run from right to left and the second (8%) from top to bottom; protein spots were stained with Coomassie blue. The values on the right margin indicate approximate molecular weights.

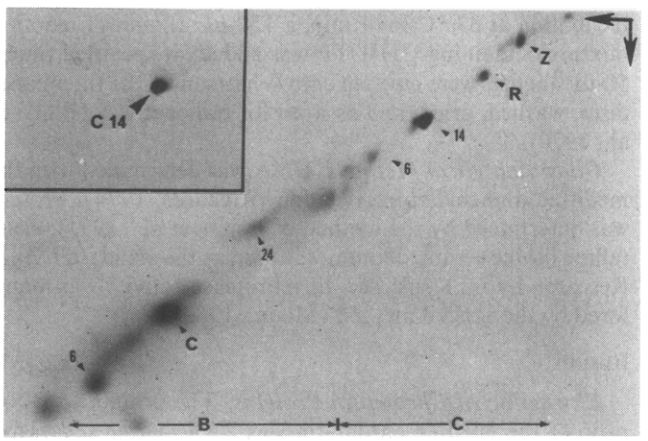

FIGURE 2: Same as Figure 1, except the proteins were obtained from the 30–60% ammonium sulfate precipitate of the 0.35 M NaCl extract. The inset in the upper left corner shows the migration of electrophoretically isolated protein C-14 as a single spot on a two-dimensional polyacrylamide gel.

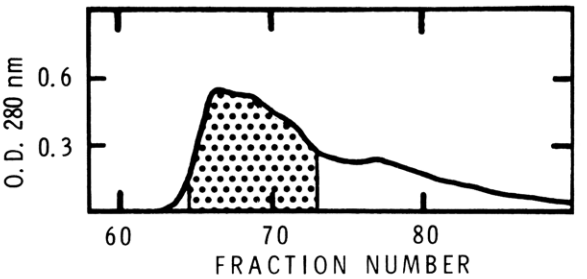

FIGURE 3: Elution profile of the 30–60% ammonium sulfate precipitated proteins redissolved in buffer A and applied to a 2.5 × 140 cm Sephadex G-75 column. The first 63 fractions (315 mL) constituted the void volume. Fractions within the shaded area were pooled and concentrated and used for subsequent experiments.

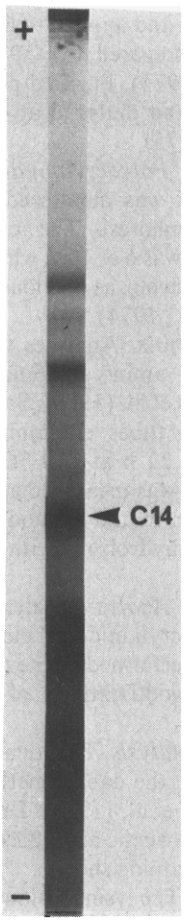

FIGURE 4: A stained strip from a slab gel after electrophoresis of the sample pooled from the Sephadex column, run on a 6% slab gel and stained with Coomassie blue. The arrow indicates protein C-14. These strips were used as guides for isolation of protein C-14 from the unstained portions of the slab gel (Goldknopf et al., 1975).

(Figure 4). A stained side-strip from a one-dimensional slab gel shows the position of protein C-14 (arrow, Figure 4). The band corresponding to protein C-14 was cut from the slab; protein C-14 was eluted by electrophoresis (Goldknopf et al., 1975). The product was a single protein spot by analytical two-dimensional polyacrylamide gel electrophoresis as shown in the inset to Figure 2. The amount of purified protein C-14 recovered was approximately 0.3 mg from 150 to 200 g of Novikoff hepatoma cells.

Properties of Protein C-14. Molecular Weight Studies. The molecular weight of protein C-14 is approximately 70 000 as determined by gel electrophoresis in 0.1% sodium dodecyl sulfate (Shapiro et al., 1967; Weber and Osborn, 1969) with marker proteins of known molecular weight.

Composition and Amino Terminal Analysis. Table I shows the amino acid composition of protein C-14. The ratio of acidic to basic amino acids was approximately 1.8. Tryptophan was not detected under conditions in which satisfactory analyses were obtained with proteins known to contain tryptophan. Lysine was found to be the amino-terminal amino acid of protein C-14 by the dansyl method (Weiner et al., 1972). By comparison with the major dansyllysine spot, only trace quantities of dansylalanine were detected. No other dansyl amino acids were found.

Peptide Map. The fluororadiogram of the [^{3}H]dansyl peptides produced from typtic digestion of carbamoylated and oxidized protein C-14 is shown in Figure 5. The lysyl residues of protein C-14 were carbamoylated with potassium cyanate to simplify peptide mapping of a protein of this size. After

TABLE I: Amino Acid Analysis of Protein C-14.

Amino acid	Mol %	Residues per molecule[a]
Lys	7.9	50
His	1.3	8
Arg	4.6	29
Trp[b]	0	0
Asx	11.5	76
Thr	6.8	44
Ser	7.2	50
Glx	13.5	89
Pro	4.1	27
Gly	8.7	59
Ala	8.0	50
½-Cystine[c]	1.5	9
Val	5.1	25
Met	2.1	14
Ile	4.7	28
Leu	7.4	48
Tyr	2.2	13
Phe	3.3	21
A/B[d]	1.8	
NH_2 terminal	Lys	

[a] Based on a molecular weight of 70 000 for protein C-14. [b] Determined after hydrolysis in mercaptoethanesulfonic acid; see text. [c] Determined as cysteic acid by the method of Moore (1963). Bovine serum albumin served as a standard protein containing half-cystine. [d] Ratio of (Glx + Asx)/(Lys + His + Arg).

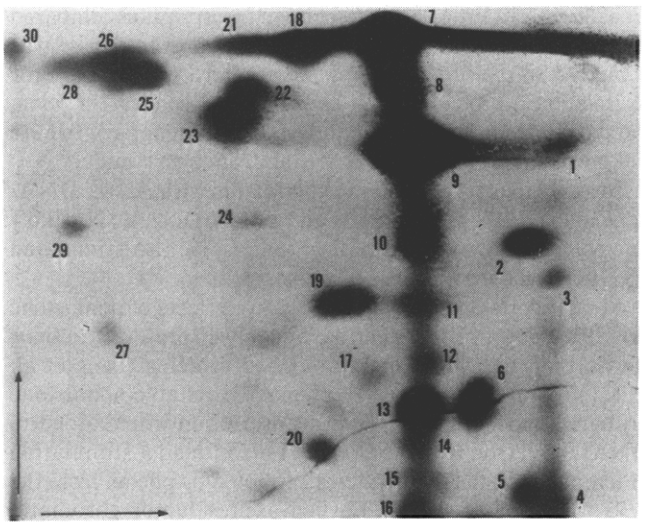

FIGURE 5: Map of [^{3}H] dansyl peptides from a tryptic digest of carbamoylated protein C-14 (James and Noltmann, 1973). The peptides were labeled with [^{3}H]dansyl chloride, dissolved in acetone–H_2O (1:1), and applied to a polyamide sheet, and the chromatogram was developed (Tichy, 1975) as described in Materials and Methods. Spots were detected by fluorography (Randerath, 1970).

performic acid oxidation and hydrolysis of carbamoylated protein C-14 with trypsin, the [^{3}H]dansylated peptides were separated on polyamide sheets. The 30 spots counted are in good agreement with the predicted number of peptides based on the molecular weight and the number of arginine residues.

Effect of Protein C-14 on the RNA Polymerase I System. In studies on the effects of protein C-14 on RNA synthesis, an increased incorporation of [^{3}H]UMP into RNA was found

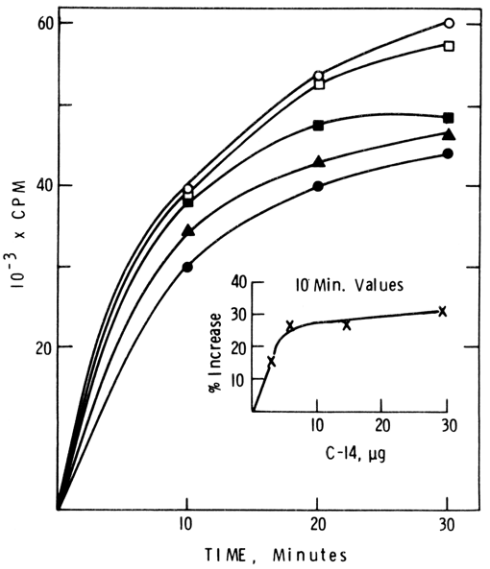

FIGURE 6: Stimulation of incorporation of [^{3}H]UMP by protein C-14 in an RNA polymerase I system with Novikoff nucleolar DNA as template. The values are averages of two experiments. Each sample contained 5 µg of DNA which was rate limiting for the reaction. (●—●) Control without protein C-14; (▲—▲) 3 µg; (■—■) 6 µg; (□—□) 15 µg; (○—○) 30 µg of protein C-14. The insert shows the stimulatory effect of increasing amounts of protein C-14 (see Table II).

TABLE II: Protein Stimulation of [^{3}H]UMP Incorporation into RNA in the Presence of Novikoff Polymerase I.[a]

Protein added	µg	Increase in [^{3}H]UMP incorp (%)
None[b,c]		
Protein C-14	2.5	17
	5	24
	10	29
Albumin	5	6
Hemoglobin	5	6
Apotransferrin	5	1
Apotransferrin[d]	5	1

[a] Polymerization of nucleotides into RNA was assayed using [^{3}H]UTP and 5 µg of Novikoff nucleolar DNA as described for Figure 6. Values given are the average of three separate experiments with incubation times of 15 min. Separate preparations of protein C-14 were employed in the repeated experiments. Stimulation or the increase in [^{3}H]UMP incorporation was calculated as the percent increase in radioactivity in RNA relative to controls which did not contain protein C-14, bovine serum albumin, bovine hemoglobin, or human apotransferrin. [b] No protein added to the system other than that in the polymerase present in all samples. [c] All transcriptional assays contained 5 µg of DNA. [d] Human apotransferrin subjected to and recovered from slab gel electrophoresis exactly as in the last step of purification of protein C-14.

when the DNA was preincubated with protein C-14 (Figure 6). Protein C-14 itself had no polymerase activity. The inset to Figure 6 shows the percentage increase in template capacity as a function of the amount of protein C-14 added. The increase was essentially linear to 6 µg of protein/5 µg of DNA.

Table II shows that with 5 µg of protein/5 µg of DNA, hemoglobin and albumin produced one-fourth the stimulation observed for protein C-14. Human apotransferrin that was isolated from slab gels by the same procedure employed for

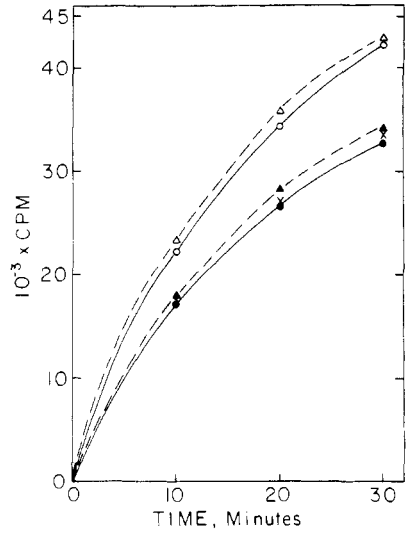

FIGURE 7: Stimulation of incorporation of [^{3}H]UMP by protein C-14 in an *E. coli* DNA-dependent RNA polymerase system with either Novikoff nucleolar DNA or *E. coli* DNA as template; 5 μg of protein C-14 was added. The other conditions were the same as those of Figure 5. (Solid symbols) No protein C-14; (open symbols) 5 μg of protein C-14 present. (●—● and ○—○) Novikoff nucleolar DNA; (X) same as ●—● with 5 μg of bovine serum albumin added; (▲—▲ and △—△) *E. coli* DNA.

protein C-14 had essentially no effect on the template capacity of the assay system (Table II).

Effect of Protein C-14 on the E. coli RNA Polymerase Assay. Additional experiments were performed with *E. coli* DNA-dependent RNA polymerase and *E. coli* or Novikoff hepatoma DNA as the template. Figure 7 shows that protein C-14 stimulated incorporation of [^{3}H]UMP into RNA in this system. Bovine serum albumin, however, had little effect.

To rule out the possibility that protein C-14 was stimulating RNA synthesis by nicking the template, alkaline sucrose gradients were run on [^{3}H]DNA that had been incubated with protein C-14. At a protein/DNA ratio of 2, which stimulated RNA synthesis by 30% (Figure 6), no DNA digestion or strand breakage was detected by comparison of alkaline sucrose gradients of [^{3}H]DNA before and after treatment with protein C-14. Moreover, no hyperchromicity was detected at the same protein/DNA ratio, and, accordingly, no unwinding was apparent.

Discussion

In this study, protein C-14, a nonhistone protein from Novikoff hepatoma nuclear chromatin, was isolated and partially characterized. Interest in protein C-14 was initially provided by the demonstration that it (Orrick et al., 1973; Busch et al., 1974) rapidly reassociated with DNA and histones in a reconstitution system (Ballal et al., 1975). Interestingly, it was found earlier (Prestayko et al., 1976) that protein C-14 was not eluted from DNA–matrix columns by 0.15 M KCl but required 2.0 M KCl for its elution. It was not found to be markedly phosphorylated following injection of [^{32}P]orthophosphate into Novikoff hepatoma bearing rats (Olson et al., 1974a,b, 1975). Like protein A-24 (Ballal et al., 1974; Goldknopf et al., 1975), protein C-14 is found in the nucleolus but is not present in ribosomes or ribonucleoprotein particles. It decreased relative to other proteins in nucleoli during the hypertrophy that follows thioacetamide treatment of rats or following partial hepatectomy (Ballal et al., 1974).[3] Since C-14 has a similar molecular weight to the 69 000 subunit of RNA polymerase Ia from Novikoff ascites cells (Froehner and Bonner, 1973) and stimulates transcription, this protein might be a subunit of the RNA polymerase or serve as an accessory factor. However, the fact that protein C-14 stimulates both *E. coli* polymerase and Novikoff hepatoma RNA polymerase I reactions suggests that its effects are probably on DNA.

There are few chemical data on isolated nonhistone proteins and even less on those of molecular weight greater than 30 000. Other nonhistone proteins studied have been considerably smaller in molecular weight (Elgin and Weintraub, 1975) than protein C-14, which has a molecular weight of 70 000. The ratio of acidic to basic amino acids after acid hydrolysis of protein C-14 is 1.8; other nonhistone proteins or groups of nonhistone proteins have been reported with ratios from 1.0 to 2.7 (Busch, 1965) and higher (Hacha and Fredericq, 1975). Protein C-14 does not contain tryptophan.

Up to 80 tryptic peptides would theoretically be expected from unmodified protein C-14 since one molecule contains about 50 lysyl and 29 arginyl residues. To simplify peptide mapping, the lysyl residues of C-14 were carbamoylated with potassium cyanate to form trypsin-resistant homocitrullyl residues. After performic acid oxidation and hydrolysis of carbamoylated protein C-14 with trypsin, 30 dansylated peptides were detected on polyamide sheets. This number of peptide spots approximates that expected, i.e., the amino acid composition (Table I) indicated there are 29 arginine residues per molecule of protein C-14.

Protein C-14 not only did not inhibit RNA synthesis but did stimulate the transcription of DNA by 30% in DNA-dependent RNA polymerase systems. As shown in Figure 6, protein C-14 saturated the system in approximately a 1:1 w/w ratio to DNA. Other proteins such as albumin, hemoglobin, and apotransferrin had little or no effect upon transcription compared with protein C-14 (Table II). When apotransferrin was subjected to the same slab gel electrophoretic procedure for its isolation as protein C-14, it had no effect upon transcription, indicating that the electrophoresis step did not contribute unknown stimulatory factors arising from acrylamide.

In analogous transcription experiments with *E. coli* DNA-dependent RNA polymerase I and *E. coli* DNA or Novikoff nucleolar DNA as templates, protein C-14 also stimulated [^{3}H]UMP incorporation.

Most reports on positive and negative effects of nonhistone proteins upon transcription have involved protein fractions rather than individual highly purified proteins. Teng et al. (1971) found that a group of phenol extractable nonhistone proteins caused an increase in transcription when added to DNA. Similarly, Kostraba et al. (1975) found a stimulation of template capacity for proteins extracted by phenol from the 0.35 M NaCl extract of Ehrlich ascites tumor chromatin (Kostraba et al., 1975; Kostraba and Wang, 1975).

This particular group of proteins displayed specificity in that RNA synthesis was only stimulated when homologous DNA was employed; it was enriched in phosphoproteins. Shea and Kleinsmith (1973) found results very similar to those of Kostraba et al. (1975) but with rat liver proteins. However, protein C-14 is not markedly phosphorylated (Olson et al., 1975), and it remains to be established what role its phosphorylation plays in its stimulatory capacity. Recently, Rouviere-Yaniv and Gros (1975) have described a low molecular weight protein (7000) that stimulates the transcription of bacteriophage λ DNA

[3] As shown by two-dimensional polyacrylamide gel electrophoresis, there were large increases in the concentrations of proteins other than C-14 which resulted in a relative decrease in the spot density of protein C-14 in nucleolar extracts.

which may represent a low molecular weight prokaryotic counterpart to protein C-14. Future studies on C-14 will be directed toward the mechanism of its stimulation of RNA synthesis.

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