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Isolation of Nuclear Acidic Proteins from Rat Tissues

CHARACTERIZATION OF ACETYLATED LIVER NUCLEAR ACIDIC PROTEINS

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Nuclear acidic proteins isolated from rat brain, heart, kidney and liver showed similar, complex patterns on electrophoresis in sodium dodecyl sulphate–polyacrylamide gels. The contamination of nuclear acidic proteins by nuclear-membrane acidic proteins was found to the extent of 11%. Incorporation of [^{3}H]acetate into the various nuclear acidic proteins *in vivo*, which were fractionated by polyacrylamide-gel electrophoresis, differed from tissue to tissue. Hydrolysis of these acetylated nuclear acidic proteins with 6M-HCl at 110°C released 70% of the radioactivity, which indicated that labile acetyl groups had been incorporated into these proteins. Analysis of [^{3}H]acetate-labelled nuclear acidic proteins revealed two acetylated amino acid residues, N^2-acetylserine and N^2-acetyl-lysine. The significance of the role played by nuclear acidic proteins in relation to gene regulation is discussed.

It has been reported by several investigators (Wang, 1966, 1967; Benjamin & Gellhorn, 1968; Teng & Hamilton, 1969; Elgin & Bonner, 1970; Shelton & Neelin, 1971; Teng *et al.*, 1971) that nuclear acidic proteins are highly heterogeneous in comparison with histones, and may be involved in the regulation of gene expression. Paul & Gilmour (1968) showed, in their reconstituted chromatin experiments using hybridization of RNA with DNA, that non-histone proteins may be associated with organ-specific masking of the DNA template. It has also been demonstrated that covalent modification of chromosomal protein occurs at times of gene activation (see review, Allfrey, 1971). It has been found that a group of nuclear acidic proteins are selectively modified by acetylation (Jungmann & Schweppe, 1972; Libby, 1972; Liew *et al.*, 1972*a*) and phosphorylation (Johnson & Allfrey, 1972) during the early events of hormone action. However, there was nothing to indicate whether or not the nuclear acidic proteins were contaminated by nuclear-membrane acidic proteins.

In this investigation, we have attempted to compare the sodium dodecyl sulphate–polyacrylamide-gel patterns of nuclear acidic proteins from several rat tissues, taking into account the nuclear-membrane acidic proteins. We have also studied the incorporation of [^{3}H]acetate into the nuclear acidic proteins of liver and kidney *in vivo*.

Materials and Methods

Materials

All chemicals and organic solvents used were of reagent grade. Sodium dodecyl sulphate, ammonium persulphate, *NNN'N'*-tetramethylethylenediamine, acrylamide and *NN'*-methylenebisacrylamide were purchased from Eastman Organic Chemicals, Rochester, N.Y., U.S.A.; sucrose and urea were from Schwarz/Mann, Orangeburg, N.Y., U.S.A.; NCS solubilizer and [^{3}H]acetic acid (sodium salt, specific radioactivity 2.2Ci/mmol) were from Amersham/-Searle, Des Plaines, Ill. U.S.A.; Omnifluor was from New England Nuclear Corp., Boston, Mass., U.S.A.; trypsin was from Sigma Chemical Co., St. Louis, Mo., U.S.A.; Pronase (B grade) was from Calbiochem, Los Angeles, Calif., U.S.A.; N^2-acetylserine, N^2-acetyl-lysine, N^2-acetylmethionine, N^2-acetyl-alanine, N^2-acetylglycine and N^6-acetyl-lysine were from Cyclo Chemical Corp., Los Angeles, Calif., U.S.A.

Animals

Male albino rats (Wistar) weighing 250–300g were used. They were fed on Purina Laboratory Chow and had free access to tap water. Food was removed the night before experiments were carried out.

Isolation of nuclei

The rats were decapitated and brains, hearts, kidneys and livers were each pooled. Organs from six rats were used to isolate heart nuclei and from three rats to prepare nuclei from brain, kidney and liver. Tissues were homogenized in 2 vol. of a medium (Liew & Korner, 1970) consisting of 0.25M-sucrose, 0.02M-Tris–HCl (pH 7.6), 0.1M-KCl, 0.04M-NaCl, 0.005M-$MgCl_2$ and 0.006M-2-mercaptoethanol. The homogenate was centrifuged at 1000*g* for 10min in a Sorvall RC-2B refrigerated centrifuge. The pellet was resuspended in 5vol of a medium consisting of 0.32M-sucrose, 0.01M-Tris–HCl (pH 7.2) and 0.001M-$MgCl_2$. The resuspended pellet was filtered through two layers of nylon cloth and the nuclei were isolated as previously described (Liew *et al.*, 1972*b*) The purified nuclear pellets were suspended in 0.25M-sucrose containing 0.01M-Tris–HCl (pH 7.2) and 0.001M-$MgCl_2$. The resuspended nuclei were centrifuged at 1000*g* for 10min. The pelleted nuclei were then resuspended in 10vol. of the same medium. Samples were taken for determination of DNA, RNA, phosphorus and protein and the rest of the suspension was used for the isolation of nuclear acidic proteins.

Isolation of nuclear acidic proteins

The isolation of nuclear acidic proteins was based on the method of Teng *et al.* (1971). (*a*) The purified nuclear pellets from 2.2M-sucrose were washed with 20 vol. of 0.14M-NaCl in 0.01M-Tris–HCl (pH 7.5) buffer. The pellets were then dispersed twice more in 10 vol. of 0.14M-NaCl with the aid of a Polytron homogenizer (Brinkmann) set at position '4' for 10s and centrifuged at 10000*g* for 10min. The supernatants from the above extraction were pooled and considered as 0.14M-NaCl-soluble proteins. (*b*)The nuclear pellets were extracted twice with 10vol. of 0.25M-HCl for a total of 1h at 4°C. This supernatant was finally precipitated by 10vol. of acetone and considered as basic proteins (i.e. mainly nucleohistones). (*c*) Lipids were extracted from the nuclear pellets with 5vol. of 2:1 (v/v), then 1:1 (v/v) chloroform–methanol containing 0.2M-HCl and finally with ether. This suspension was centrifuged at 17000*g* for 10min. (*d*) The residue was resuspended in 5vol. of 0.1M-Tris–HCl (pH 8.4)–0.01M-EDTA–0.14M-2-mercaptoethanol. An equal volume of phenol which was saturated with Tris–EDTA–mercaptoethanol buffer was added to the resuspension and extracted overnight with continuous stirring at 4°C. The two phases of the resuspension were separated by centrifugation for 10min at 25000*g* and the phenol phase was collected. The nuclear acidic proteins, which are soluble in phenol, were dialysed against 0.1M-acetic acid containing 0.14M-2-mercaptoethanol for 6–8 h at room temperature. This extensive dialysis was implemented to remove possible contamination by basic proteins that were soluble in the solution, as well as to concentrate the nuclear acidic proteins which remain soluble in the phenol phase. The nuclear acidic proteins were then dialysed first in 9.0M-urea, then in 8.6M-urea and finally in 0.01M-sodium phosphate buffer (pH 7.2) containing 0.14M-2-mercaptoethanol and 0.1% sodium dodecyl sulphate at room temperature.

Isolation of nuclear-membrane proteins

The purified nuclei obtained from 5g of liver tissue per centrifuge tube (2.5cm×8.9cm), were used to prepare nuclear-membrane proteins. The nuclear pellet from each tube was resuspended in 20ml of 0.05M-Tris–HCl (pH 7.5)–0.025M-KCl–0.005M-$MgCl_2$, and the nuclei were disrupted by sonication (Biosonik) at a setting of '4' for 10s. This step was repeated once. The nuclear suspension was adjusted and mixed with potassium citrate to a final concn. of 10% (w/v). A similar result can be obtained by 'hypotonic shock', i.e. resuspending the nuclear pellet in 5vol. of water at 4°C for 15min, before the addition of Tris–KCl–$MgCl_2$ buffer and potassium citrate. Centrifugation was carried out at 40000*g* for 45min in a Sorvall refrigerated centrifuge. The resulting pellet was resuspended in sucrose–Tris–KCl–$MgCl_2$ and 10% (w/v) citrate solution of density 1.22g/ml and sheared by a Polytron homogenizer at position '4' for 5s. The nuclear membranes were separated in a discontinuous sucrose density gradient by the method of Kashnig & Kasper (1969), except that the centrifugation was carried out at 75000*g* for 60min in a Beckman SW 27 rotor (Mizuno *et al.*, 1971). The nuclear membrane from each interface was carefully removed. The nuclear-membrane suspension was diluted with 2vol. of potassium citrate in Tris–KCl–$MgCl_2$ buffer and pelleted by further centrifugation at 82000*g* for 1h. The phenol-soluble nuclear-membrane acidic proteins were obtained by a method similar to that described for the isolation of nuclear acidic proteins.

Liver nuclear membrane was also obtained by enzyme digestion, as described by Kay *et al.* (1972). Extraction of nuclear-membrane acidic proteins was then carried out according to the above procedure.

Polyacrylamide-gel electrophoresis

Electrophoresis was performed in a 10% (w/v) polyacrylamide–sodium dodecyl sulphate gel, with the following modification used by Weber & Osborn (1969); *NNN'N'*-tetramethylethylenediamine was decreased from 0.075% to 0.05% in order to delay the polymerization, which was carried out at room

temperature. After 45min of polymerization, the gels were electrophoresed at room temperature for 45min at a constant voltage of 6V/cm before the application of proteins. Protein samples (200μg) containing 10% (w/v) sucrose were layered on the gel (0.6cm×9cm) and electrophoresed for 5h under the same conditions as those for pre-electrophoresis. The gels were stained with 1% Amido Black in 7% (v/v) acetic acid and destained by 7% (v/v) acetic acid. Gels were scanned at 570nm by a densitometer attached to a Unicam SP. 1800 recording spectrophotometer.

Identification of the acetyl group in nuclear acidic proteins

The [^{3}H]acetate-labelled acidic proteins from isolated liver or kidney were hydrolysed for 18h in 6M-HCl at 110°C in sealed evacuated tubes. This procedure was used for releasing the acetyl groups covalently bound to the nuclear acidic proteins. The hydrolysed samples were transferred quantitatively to counting vials and dried at 110°C. The release of the acetyl group was confirmed by hydrolysis with 2M-H_3PO_4 followed by steam distillation and trapping of [^{3}H]acetic acid with 0.3M-NaOH as described by Gershey *et al.* (1968).

To characterize the acetyl group in the amino acid residue, the [^{3}H]acetate-labelled acidic proteins isolated from liver nuclei were incubated with trypsin overnight at 37°C followed by another 24h incubation with Pronase as described by Vidali *et al.* (1968). Substances of low molecular weight were isolated from the hydrolysate by Bio-Gel P-2 column chromatography (1cm×200cm) as previously described (Liew & Gornall, 1973). The fractions that coincided with the standards of N^2-acetylserine, N^2-acetyl-lysine and N^6-acetyl-lysine were pooled and freeze-dried. These samples were further analysed by amino acid analysis to identify the acetylated amino acid residues. For example, N^6-acetyl-lysine, which emerged during 92–94min, and N^2-acetyl-lysine, which came out during 152–154 min from the column (UR-30) of the amino acid analyser, were further identified by paper chromatography as reported by Gershey *et al.* (1968). The radioactive fraction, which emerged from the amino acid analyser in an unretarded position (i.e. 28–30min after the application of the sample to the analyser column) was collected and applied to a Bio-Gel P-2 column for desalting. The radioactive fractions which were eluted with water were pooled, freeze-dried and then subjected to high-voltage electrophoresis in pyridinium acetate buffer (pyridine–acetic acid–water, 1:10:89, by vol., pH 3.5) (Terhorst *et al.*, 1972) and Orange G was used as a marker. The spot that corresponded to authentic standard N^2-acetylserine (R_F 0.66, as compared with acetylglycine 0.61, acetylalanine 0.45 and acetylmethionine 0.44), which could be stained by chlorination (Mazur *et al.*, 1962), was cut out, eluted with 6M-HCl and hydrolysed at 110°C for 18h. The radioactivity had disappeared and a significant amount of serine was recovered from the amino acid analyser. In other double-labelling experiments, where [^{3}H]acetate and [^{14}C]serine were incorporated into nuclear acidic proteins, both radioactivities were detected in one spot by high-voltage paper electrophoresis as described above.

Determination of DNA, RNA, protein and phospholipids

The determination of DNA was based on the method of Burton (1956). RNA concentration was either measured at 260nm or determined by colorimetric methods (Mejbaum, 1939). The method of Lowry *et al.* (1951) was used to determine protein concentration. Total nuclear-membrane phospholipids were determined by the method of Bartlett (1959).

Measurement of radioactivity

Samples used in the determination of radioactivity were solubilized in 0.5ml of NCS (Amersham/Searle) before the addition of 10ml of toluene–Omnifluor (New England Nuclear Corp.).

Nuclear acidic proteins were separated by polyacrylamide-gel electrophoresis and the gels were cut transversely into 1mm slices. Each was placed in a counting vial containing 0.7ml of NCS–water (9:1 v/v) and incubated at 50°C overnight, before the addition of 10ml of toluene–Omnifluor.

Results

Composition of nucleoproteins from purified liver nuclei

The purified nuclei from brain, heart, kidney and liver, which were used in our studies, were shown to be intact by phase-contrast and electron microscopy and had distinct nucleoli and minimal cytoplasmic contamination. The preparations of the various fractions of liver nuclei are summarized in Table 1. The amino acid analysis showed that the ratio of the sum of aspartic acid and glutamic acid to the sum of lysine, histidine and arginine (i.e. acidic to basic amino acids) in all samples was greater than 1.45 and that the ratio was highest in the liver nuclear acidic proteins (not shown).

Polyacrylamide-gel electrophoresis

Equal amounts of the phenol-soluble nuclear acidic proteins from isolated brain, heart, liver and kidney nuclei were separated by 10% (w/v) polyacrylamide–sodium dodecyl sulphate-gel electrophoresis. In

Plate 1, at least 26 separate component bands of nuclear acidic protein can be identified visually. The degree of tissue specificity shown by the electrophoretic patterns of the proteins from these four tissues was consistent from experiment to experiment. The nuclear acidic proteins from brain, heart, kidney and liver were nearly identical in the five major fractions as shown in Plate 1. These results indicated that the nuclear acidic proteins, isolated from the four different tissues, appear to be distributed in different quantitative fashions rather than showing qualitative differences in the proteins that we have separated by one-dimensional sodium dodecyl sulphate–polyacrylamide-gel electrophoresis.

Characterization of nuclear membrane proteins

Isolation of the nuclear membrane was carried out for the purpose of examining the degree of contamination in the nuclear acidic proteins by nuclear-membrane acidic proteins. The nuclear membranes were isolated by the method of Kashnig & Kasper (1969) and also by the enzymic procedure of Kay *et al.* (1972). The chemical compositions of the nuclear membrane are shown in Table 2. The amount of nuclear-membrane acidic protein that was isolated from the nuclear membrane based on the latter method was about 11% of the total nuclear acidic proteins. By using the former method it was found that about 8% of nuclear-membrane acidic proteins were present in nuclear acidic proteins. Amino acid analyses of both preparations indicated that the ratios of acidic to basic amino acids were greater than 1.2 (not shown). We have also examined the effect of 10% (w/v) potassium citrate on the dissociation of nuclear membrane from the isolated nuclei as described by the method of Kashnig & Kasper (1969). It was found that the nuclear acidic proteins were not significantly extracted by 10% (w/v) potassium citrate. Separation of nuclear-membrane acidic proteins by the methods of Kashnig & Kasper (1969) and Kay *et al.* (1972) was carried out by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis as shown in Plates 2(*a*) and 2(*b*). Acidic proteins isolated from nuclear membrane by both methods showed similar electrophoretic patterns. Three major fractions, i.e., fractions a, b and c, of high molecular weight, were similar to the nuclear acidic proteins (e.g. Plate 1 and Fig. 1). The nuclear acidic proteins isolated from liver nuclei before and after removal of the nuclear membrane by the treatments of sonication and potassium citrate are shown in Figs. 1(*a*) and 1(*b*). The electrophoretic gel patterns show at least 26 bands. It was found that most of the fractionated proteins (Fig. 1*b*) are similar to those found without removal of nuclear membrane (Fig. 1*a*), except for some minor changes in fractions a, b and c.

Table 1. *Fractionation of rat liver nucleoproteins*

Nuclear proteins which were soluble in NaCl, HCl and phenol were separated as described in the text. The nucleoprotein/DNA ratio is about 4:1. About 3.8mg of nucleoproteins was obtained from 1g of liver tissue (wet weight). The recovery (%) was calculated from the whole nuclei which were resuspended in 0.25M-sucrose–Tris–$MgCl_2$ buffer. No correction was made in the protein determinations of the fractionated proteins.

	Percentage of total nucleoproteins			
Expt. no.	0.14M-NaCl-soluble	0.25M-HCl-soluble	Phenol-soluble	Recovery (%)
1	30.0	45.5	10.5	86.0
2	24.6	42.0	11.5	78.1
3	27.5	42.4	12.3	82.8
Mean...	27.4	43.3	11.4	82.3

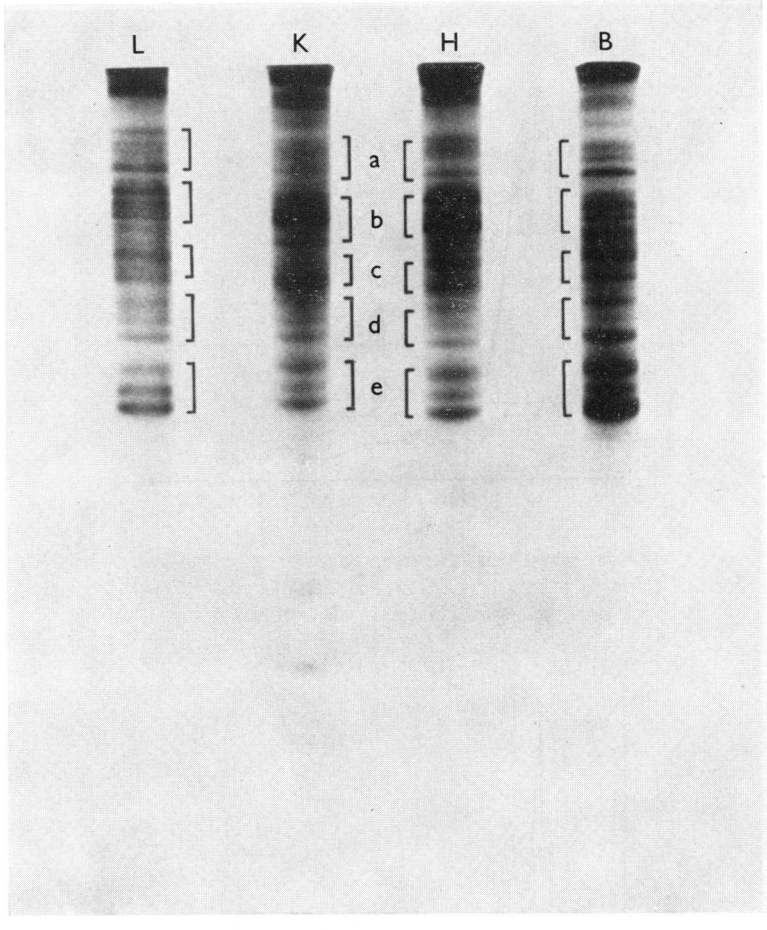
Incorporation of [^3H]acetate into nuclear acidic proteins

Having established that nuclear-membrane acidic proteins were not the main constituents of the nuclear acidic proteins, an attempt was made to examine covalent modification in this class of proteins by acetylation. [^{3}H]Acetate was given to a group of rats 15min before the isolation of nuclei. The isolation of nuclear acidic proteins was performed as

Table 2. *Composition of rat liver nuclei and nuclear membrane*

The results are the mean±S.E.M. of three experiments. The yield of nuclear-membrane protein from the starting nuclei was about 7.4% as obtained by the method of Kay *et al.* (1972) and about 7.2% by Kashnig & Kasper's (1969) method. Acidic proteins were isolated by the method of Teng *et al.* (1971).

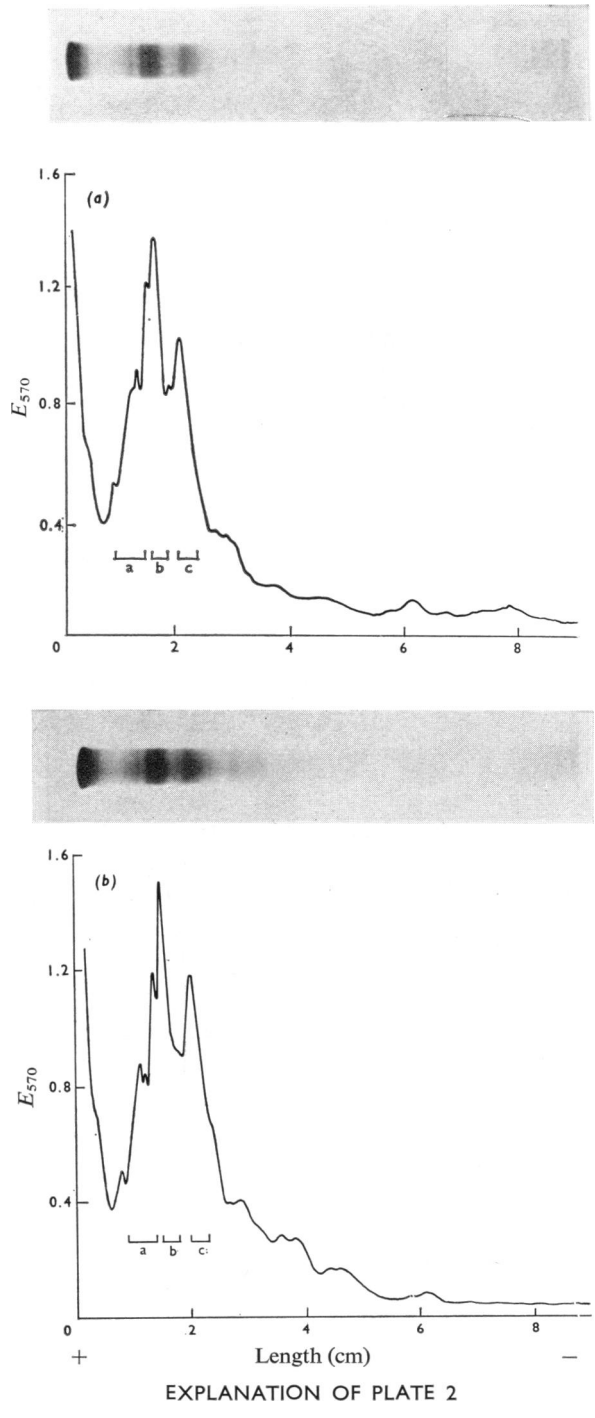
	Content (μg/mg of nucleoproteins in the starting nuclei)			
	DNA	RNA	Phospholipid	Acidic protein
Nuclei	244±11	85.3±2.0	58.7±3.5	114 ±5.2
Nuclear membrane				
(Kay *et al.*, 1969)	4.12±0.6	3.73±0.2	32.3±1.4	13.0±1.9
(Kashnig & Kasper, 1972)	2.62±0.8	5.93±0.9	35.2±2.5	9.3±1.3



EXPLANATION OF PLATE 1

Analysis of nuclear acidic proteins by polyacrylamide-gel electrophoresis

Nuclear acidic proteins which were isolated from liver (L), kidney (K), heart (H) and brain (B) were soluble in 0.01 M-sodium phosphate buffer (pH 7.2) containing 0.1% sodium dodecyl sulphate and 0.14 M-2-mercaptoethanol. Samples containing 200 μg of proteins from each tissue were analysed by 10% (w/v) polyacrylamide-gel electrophoresis at room temperature, at a constant voltage of 6 V/cm. Five major groups of protein fractions are indicated as a, b, c, d and e.



EXPLANATION OF PLATE 2

Analysis of nuclear-membrane acidic proteins by polyacrylamide-gel electrophoresis

The rat liver nuclear membrane was isolated by either (*a*) the method of Kashnig & Kasper (1969) or (*b*) the method of Kay *et al.* (1972) before the isolation of nuclear-membrane acidic proteins as described in the text. Equal amounts (75 μg) of the proteins were fractionated by polyacrylamide-gel electrophoresis. The gels were stained with Amido Black 10B and scanned at 570 nm. Three major fractions (a, b, c) corresponded to those in Plate 1.

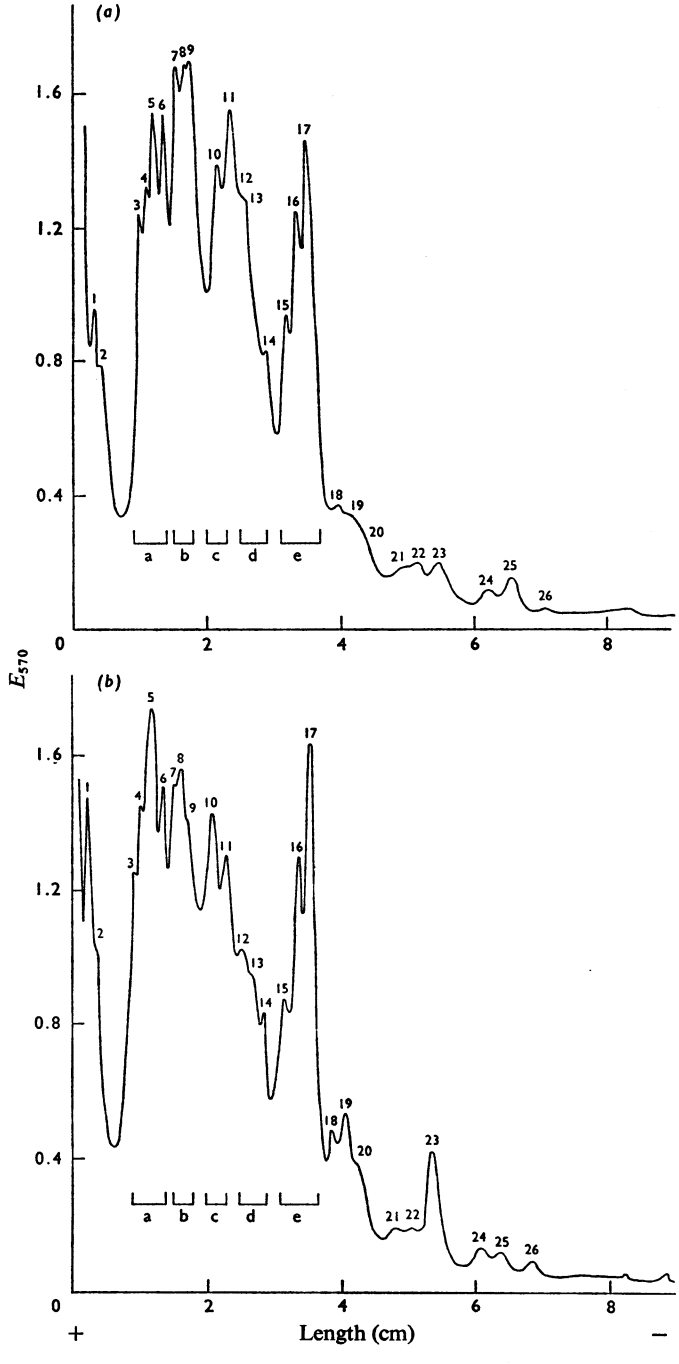


Fig. 1. *Comparison of nuclear acidic proteins associated with and without nuclear membranes*

The nuclear acidic proteins which were isolated (*a*) from total nuclei and (*b*) after removal of the nuclear membrane by the method of Kashnig & Kasper (1969) were obtained from rat liver. Equal amounts of these proteins (200 μg) were fractionated by polyacrylamide-gel electrophoresis. The gels were stained with Amido Black 10B and scanned at 570 nm as described in the Materials and Methods section.

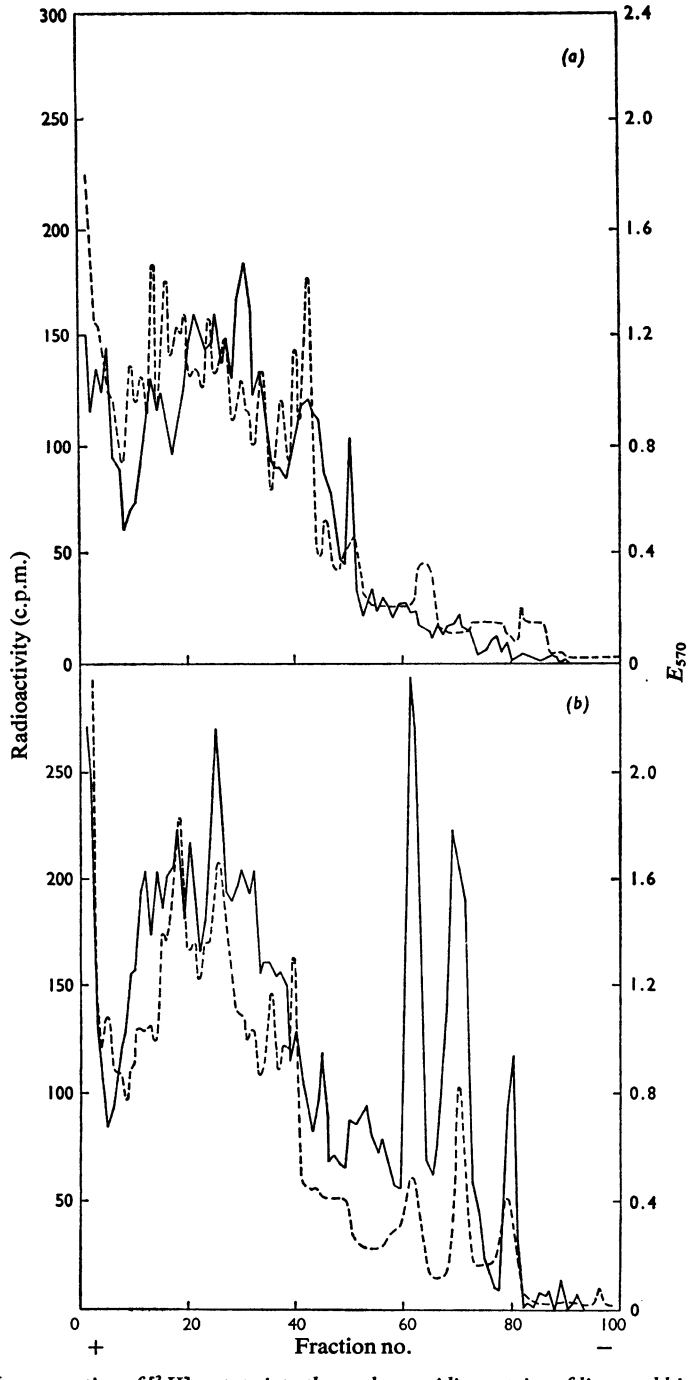


Fig. 2. *Incorporation of [^{3}H]acetate into the nuclear acidic proteins of liver and kidney in vivo*

[^{3}H]Acetate (25mCi/250g) was given to each rat 15min before the isolation of nuclei. The nuclear acidic proteins from liver (*a*) and kidney (*b*) were isolated as described in the Materials and Methods section. Equal amounts (200μg) of nuclear acidic proteins were fractionated by polyacrylamide-gel electrophoresis. The gels were stained with Amido Black 10B and scanned at 570nm (– – – –) and the radioactivity (——) of the fractionated proteins was determined as described previously. The fraction size was 1mm slice of gel.

described in the Materials and Methods section. Equal amounts of nuclear acidic proteins from liver and kidney were analysed by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis. The gels were then sliced transversely into 1mm-thick slices and the amount of radioactivity that was incorporated into the proteins was determined. As shown in Fig. 2, some proteins from kidney (Fig. 2*b*) incorporate [^{3}H]acetate more than those of liver (Fig. 2*a*) and vice versa. We have carried out experiments to show that most of the peaks of the radioactivity coincided with the bands.

Identification of the acetyl group in nuclear acidic proteins

In an attempt to determine whether the [^{3}H]acetate that was incorporated into nuclear acidic protein was covalently bound to these proteins, 6M-HCl was used to hydrolyse these labelled proteins at 110°C for 18h. The results are shown in Table 3. About 70% of the radioactivity was released after such treatment. The release of the labelled acetate group was also confirmed by hydrolysis in 2M-H_3PO_4 followed by steam distillation. The remaining radioactivity after acid hydrolysis was further characterized by the amino acid analyser. This radioactive residue was mainly glutamic acid.

In a separate experiment, liver nuclear acidic proteins were treated both with trypsin and Pronase as described previously. The acetylated amino acid residues were partially purified by exclusion chromatography and were then identified by the amino acid analyser, paper chromatography and high-voltage paper electrophoresis. The radioactivity was detected mainly as N^2-acetylserine and N^2-acetyl-lysine. The radioactivity that coincided with the N^6-acetyllysine was minimal. We have also found that some other amino acid residues can be modified by acetylation.

Table 3. *Acid hydrolysis of* [^{3}*H*]*acetate-labelled nuclear acidic proteins*

The animals were given 25mCi of [^{3}H]acetate 15min before the isolation of the nuclear acidic proteins. Proteins (100μg) were hydrolysed in 6M-HCl in an evacuated tube at 100°C for 18h. The results are the mean of three separate experiments that were in good agreement. In a separate experiment, the labelled proteins were hydrolysed with 2M-H_3PO_4 after steam distillation as described in the text.

	Radioactivity (c.p.m./100μg of protein)		
	Before treatment	6M-HCl-treated	Percentage remaining
Liver	1760	520	29.6
Kidney	2430	680	28.1

Discussion

Speculative inquiry into nuclear acidic proteins has been aimed at determining their significance in the regulation of gene expression (Kleinsmith *et al.*, 1966; Paul & Gilmour, 1968; Shelton & Allfrey, 1970; Kamiyama & Wang, 1971; Teng *et al.*, 1971). We have isolated highly purified nuclei from rat tissues and examined the properties of these phenol-soluble proteins. We found that nuclear acidic proteins are composed of at least 26 fractions and that the variation in heterogeneity of these proteins from tissue to tissue is rather limited. These findings were in agreement with those of MacGillivray *et al.* (1972), who compared the non-histone proteins of mouse and bovine chromatins and showed that the differences in gel patterns were quantitative rather than qualitative. Other investigators also (Benjamin & Gellhorn, 1968; Elgin & Bonner, 1970) demonstrated a 'limited' heterogeneity among the tissues. One should not neglect the fact that other proteins in amounts either too small to be detected, or overlapped by larger fractions, could determine 'specificity' in a qualitative manner. Besides, it must be stressed that the separation by gel electrophoresis of non-histone proteins solubilized in detergents was on the basis of differences in molecular weights (Weber & Osborn, 1969). Hence the differences in primary structure between peptides of the same molecular weight would not be detected.

The degree of contamination by nuclear-membrane acidic proteins of the nuclear acidic proteins was also examined. As reported by Tata *et al.* (1972), most of the methods for the preparation of chromatin produced material that contained fragments of nuclear membrane. Certainly, whether or not the isolated nuclear acidic proteins are contaminated by nuclear-membrane acidic proteins should be determined, especially in studies of the covalent modification of these proteins at times of gene activation. We estimated that nuclear acidic proteins contained nuclear-membrane acidic proteins to the extent of 11%. It was found that three major fractions of the nuclear-membrane acidic proteins are similar to the nuclear acidic proteins. The nuclear-membrane acidic proteins obtained by either of the two methods outlined previously exhibited similar electrophoretic patterns. Further, the nuclear acidic proteins isolated from the pellets from which the nuclear membranes had been removed showed similar electrophoretic patterns to the proteins from the intact nuclei, except that the amounts of nuclear acidic proteins in fractions a, b and c were quantitatively different. These observations indicated that most of the nuclear acidic proteins being isolated were indeed nuclear in origin. Teng *et al.* (1971) have also shown that nuclear acidic proteins isolated either from

chromatin or directly from purified nuclei exhibit similar electrophoretic patterns.

We have investigated the nature of nuclear acidic proteins by labelling with [^{3}H]acetate *in vivo* and found that about 70% of [^{3}H]acetate was released from these proteins by acid hydrolysis. When the labelled acidic proteins were treated with trypsin and Pronase, at least two amino acid residues in the hydrolysate were identified as N^2-acetylserine and N^2-acetyl-lysine. The presence of N^6-acetyl-lysine was minimal in the liver nuclear acidic proteins. This suggests that acetylation may be involved in the process of protein biosynthesis. In our preliminary experiments, we found that administration of puromycin *in vivo* before injection of [^{3}H]acetate into the partially hepatectomized rats produced a 60% decrease in the incorporation of [^{3}H]acetate into the liver nuclear acidic proteins as compared with the sham-operated animals. Dixon and his co-workers (Dixon, 1972; Louie & Dixon, 1972) found that acetylation of histones occurred shortly after synthesis. They suggested that acetylation and phosphorylation of histones in trout testis might be obligatory processes for correct binding with DNA. In addition, Shelton & Allfrey (1970) and Teng & Hamilton (1969) have shown that a selective stimulation of the synthesis of some of the acidic-protein fractions is evident after acute hormone administration. We have also reported, as have others, that the patterns of acetylation vary from one tissue to another and that they are subject to hormonal regulation (Liew *et al.*, 1973; Libby 1972; Jungmann & Schweppe, 1972), an analogy with phosphorylation reported by other investigators (Johnson & Allfrey, 1972).

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