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EFFECT OF ALDOSTERONE ON RENAL RIBOSOMAL PROTEIN PHOSPHORYLATION

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SUMMARY

The phosphorylation of ribosomes in kidney cortex slices was measured during 60 min of incubation 2 h after intravenous injection of aldosterone into adrenalectomized rats. No significant differences from controls were observed in the incorporation of [^{32}P] into total ribosomes, ribosomal RNA or ribosomal protein. The phosphorylation of ribosomal proteins separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis was also compared in renal cortical slices from aldosterone-treated and control animals by a double-labeling technique using [^{32}P] and [^{33}P]. After aldosterone administration the phosphorylation of at least two proteins with low mobilities (high molecular weights) increased, while that of at least two others with higher mobilities (lower molecular weights) decreased.

INTRODUCTION

The phosphorylation of serine and threonine residues of proteins associated with eukaryotic ribosomes by protein kinases bound to the ribosomes or free in the cytoplasm has been demonstrated in a number of systems *in vivo* and *in vitro* [1–15]. In all systems so far examined, with the exception of reticulocyte ribosomes, ribosomal protein phosphorylation is stimulated by 3',5'-cyclic AMP (cAMP). The functional role of ribosomal protein phosphorylation is as yet unknown. It is conceivable that certain hormones could act directly or indirectly on the activity of the translation machinery through a series of reactions involving protein phosphorylation. Glucagon [2] is reported to increase the phosphorylation of rat liver ribosomal proteins. Thyroidectomy was found to result in a 35 per cent decrease in the phosphate content of liver ribosomal proteins; 3,5,3'-triiodothyronine administration restored the phosphate content to control levels [8]. Garren *et al.* [16] suggested that ACTH, acting through cAMP, regulates adrenal function by modulating protein synthesis at the level of the translation of mRNA. They demonstrated a cAMP-dependent protein kinase that phosphorylates protein tightly associated with the ribosomes and postulated that this event has a regulatory role in the hormone-mediated translational control of adrenal cortical protein synthesis [6, 15].

In our laboratory Majumdar and Trachewsky [17] demonstrated that aldosterone administration increased the ability of kidney cortex ribosomes isolated from adrenalectomized rats to synthesize polypeptides and polyphenylalanine in the presence of endogenous mRNA and polyuridylic acid (poly U), respectively. The poly U-directed binding of phenylalanyl-tRNA to ribosomes from aldosterone-treated rats was also increased. These alterations in binding capacity and in the ability to synthesize polypeptides were shared by other mineralocorticoids but not by 17β-estradiol [18]. Our conclusion was that the treatment with aldosterone (and other mineralocorticoids) so altered the renal cortical ribosomes that their functional capacity increased. Covalent modification of ribosomal protein(s) would be one way to produce alteration in structure and function of the ribosomal apparatus. We have now examined the effect of administering aldosterone to adrenalectomized rats on the phosphorylation of ribosomal proteins in renal cortical slices.

EXPERIMENTAL

Materials

Carrier-free [^{32}P]-H_3PO_4 and [^{33}P]-H_3PO_4 in 0·02 N HCl were obtained from New England Nuclear Corp. N,N,N',N'-tetramethylethylenediamine (TEMED), acrylamide, and N,N'-methylenebisacrylamide were purchased from Eastman Organic Chemicals. Pronase was from K & K Laboratories. PCS solubilizer for scintillation counting was from Amersham–Searle.

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Methods

Treatment of rats. Male hooded rats weighing 175–200 g were bilaterally adrenalectomized. Their drinking water contained 1% NaCl and 5% glucose; this and Purina laboratory chow (240 μ-equiv K^+/g dry weight) were supplied *ad lib.* 72 h after adrenalectomy the rats received a single intravenous injection of either aldosterone (4 μg/rat) in 0·5 ml of saline or 0·5 ml of saline alone. One hour after the injections the rats were decapitated, the kidneys were excised and decapsulated, and cortical slices (0·5 mm) were rapidly prepared with a Stadie–Riggs tissue slicer. The tissues were kept on ice and moistened with Krebs–Ringer bicarbonate buffer [19] until used for incubations.

Incubation of kidney cortex slices

Two hours after hormone or saline injections the slices were incubated with constant shaking at 37 C in Krebs–Ringer bicarbonate buffer containing 100 mg glucose/100 ml (KRBG buffer) and [^{32}P]-H_3PO_4 or [^{33}P]-H_3PO_4, as indicated, in stoppered flasks with an atmosphere of 95% O_2–5% CO_2.

Preparation of ribosomes

At the end of the incubation the slices were chilled on ice and rinsed several times with large volumes of cold KRBG buffer. Ribosomes were prepared as already described [18]. The tissue was homogenized in 2 vol. of medium consisting of 0·25 M sucrose, 0·01 M $MgCl_2$, 0·08 M KCl, and 0·05 M Tris–HCl, pH 7·8. The sucrose used here and in subsequent centrifugations was RNAase-free. The homogenate was centrifuged for 15 min at 15,000 *g* to remove mitochondria, nuclei and debris.

The 15,000 *g* supernatant was filtered through a double layer of cheesecloth and then centrifuged 2 h at 100,000 *g* to sediment the microsome fraction. The microsomes were re-suspended by 10 up-and-down strokes in a Potter Elvehjem vessel with a motor driven Teflon pestle in a mixture of 3·25 ml of homogenizing medium and 1·0 ml of 2·5 M KCl in 0·01 M $MgCl_2$. To the milky suspension in the homogenizer vessel was added 0·25 ml of 10% (w/v) Lubrol WX (General Biochemicals) in 0·01 M $MgCl_2$; mixing was achieved by four additional up-and-down strokes with the pestle turning slowly. Finally 0·5 ml of 10% (w/v) sodium deoxycholate in water was added, and an additional 10 up-and-down strokes were done. Appreciable clarification of the milky suspension occurred upon addition of the detergents. These concentrations of the detergents are known to completely dissolve the microsomal lipoprotein membranes [21] without apparent damage to the ribosomes which retain their physical integrity and ability to incorporate amino acids [21]. The detergent-treated microsomes were then layered in 5 ml aliquots over 7 ml of 1·0 M sucrose (ion composition of microsomal homogenizing mixture, i.e. 0·6 M KCl, 0·01 M $MgCl_2$, and 0·05 M Tris–HCl, pH 7·8) in centrifuge tubes; centrifugation was at 200,000 *g* for 5 h in the SB-283 rotor of the International B-60 ultracentrifuge. This treatment was designed to ensure the removal of other proteins adhering nonspecifically to the ribosomes [13, 20, 21]. The ribosomal pellets were suspended in an all-glass homogenizer in 0·01 M sodium phosphate buffer, pH 7·2 containing 0·5 M urea.

Assay for purity of the ribonucleoprotein particles

The absorbance of the ribosomal suspension was determined at 235, 260, and 280 nm and the absorbance ratios 260:235 and 260:280 nm were calculated and used to assess the purity of the particles. In our ribosomal preparations the ratio 260:280 nm exceeded 1·75 and the ratio 260:235 nm exceeded 1·45. Ribosomes with a 260:280 nm absorbance ratio > 1·75 were found to correspond to an RNA/protein ratio > 1·25 [18].

Determination of specific activities of phosphorylated ribosomes and ribosomal constituents

The ribosomal solutions were precipitated with one volume of ice-cold 20% trichloroacetic acid (TCA) and after 20 min on ice were centrifuged at 3000 *g* for 10 min. The resulting precipitate was washed successively with ice-cold 7·5, 5 and 1% TCA. The combined supernatants gave the "cold-acid extract" of the ribosomes. The ribosomes were next extracted with 10% TCA at 90 C for 15 min and, after the solution had been cooled to 0 C for 20 min, they were sedimented again. The extraction was repeated with 5% TCA at 90 C for 5 min. The residue was washed with 5% and again with 1% ice-cold TCA. The hot-acid extracts and the washes were combined to give the ribosomal RNA fraction. RNA concentrations were determined by the method of Mejbaum [22], with yeast soluble RNA as standard.

After an ether wash of the residue, phospholipids were extracted with chloroform–methanol (1:1, v/v) at 60 C for 15 min and with ethanol–ether (3:1, v/v) at 60 C for 5 min. The residue was washed with ether. The combined extracts and ether wash gave the organic-solvent-extractable fraction. The protein residue was dissolved in 3N NaOH at 37 C overnight. Protein quantities were determined by the method of Lowry *et al.* [23].

To determine the amount of radioactivity associated with inorganic phosphate and other phosphorus-containing small molecules in the homogenate and in the

100,000 *g* supernatant (cytosol), protein was determined in 10-fold diluted aliquots subjected to 10% ice-cold TCA precipitation and cold acid washes of the protein as described above for the ribosomes; radioactivity was measured in an aliquot of the combined supernatants.

Separation of phosphorylated ribosomal proteins by gel electrophoresis

Ribosomes were cold-washed as described above and were dissolved by homogenization in 0·01 M sodium phosphate buffer, pH 7·2 containing 1% sodium dodecyl sulfate (SDS), 0·5 M urea, and 1% 2-mercaptoethanol and were placed in a boiling water bath for 90 s. Before electrophoresis the samples were dialyzed for 4 h against two changes of 250 vol. of 0·01 M Tris–HCl buffer, pH 7·2 containing 1% SDS, 0·5 M urea, and 0·1% 2-mercaptoethanol and then dialyzed against 2 changes of 0·01 M sodium phosphate buffer, pH 7·2 containing 1% SDS, 0·5 M urea, and 0·1% 2-mercaptoethanol.

Disc gel electrophoresis was performed with stacking gels on 0·6 × 12 cm separating gels according to the method of Warner [24]. Gels contained 10% acrylamide, 0·27% N,N′-methylenebisacrylamide, 0·5% SDS, 0·5 M urea, 0·075% ammonium persulfate, 0·05% TEMED, and 0·1 M sodium phosphate buffer, pH 7·2. Stacking gels were prepared by adding to the sample, in 0·01 M sodium phosphate buffer, pH 7·2 containing 1% SDS, 0·5 M urea, and 0·1% 2-mercaptoethanol, one-fifth the volume of a solution of 15% acrylamide, 4% N,N′-methylenebisacrylamide, 0·35% TEMED, and 0·03% riboflavin. The electrophoresis buffer was 0·1 M sodium phosphate, pH 7·2 containing 0·5% SDS.

Electrophoresis took place at room temperature at 7 mA/gel for 10 h. Migration was toward the anode. After electrophoresis, gels were fixed with isopropyl alcohol, stained with Coomassie blue according to the method of Fairbanks *et al.* [25], and fractionated into 2 mm crushed slices by a Gilson Aliquogel Fractionator. Radioactivity was measured in 10 ml of PCS solubilizer solution. Stained gels were stored in 10% acetic acid. Densities of the stained protein bands were recorded by a Vitatron UR Linear Recorder.

Preparation of ribosomal RNA for gel electrophoresis

Cold acid-washed ribosomal preparations were dissolved in 0·005 M Tris–HCl, pH 7·4 containing 0·4% SDS and were digested with pronase (2 mg/ml) at 37°C for 30 min. After the incubation an equal volume of 0·1 M sodium acetate, pH 5·1 containing 0·02 M EDTA and 1·6% SDS was added and rRNA was extracted according to the method of Oda and Joklik [26]. The RNA precipitate was dissolved in 0·01 M sodium phosphate buffer, pH 7·2 containing 1% SDS, 0·5 M urea, and 0·1% 2-mercaptoethanol, and was dialyzed before electrophoresis as described above for the ribosomal preparations.

Determination of the [^{32}P]/[^{33}P] ratios in polyacrylamide gel slices

The c.p.m. or d.p.m. of the two isotopes in each others presence were calculated by means of simultaneous equations [27], the efficiencies of the isotopes in each of two channels at appropriate settings being taken into consideration. Ratios were computed from the d.p.m.'s obtained. One channel was set to detect the higher energy isotope, [^{32}P], at a setting that registered only negligible counts from the lower energy isotope, [^{33}P]; the other was set to detect [^{33}P] maximally with the lowest possible overlap (11–14%) of [^{32}P] counts. [^{32}P]/[^{33}P] ratios were determined only on gels in which the lowest net c.p.m. for either isotope exceeded 45.

RESULTS

Effect of aldosterone on the phosphorylation of ribosomes, ribosomal proteins and ribosomal RNA

Figure 1 shows the time course of [^{32}P]-H_3PO_4 incorporation into total ribosomes and also into the

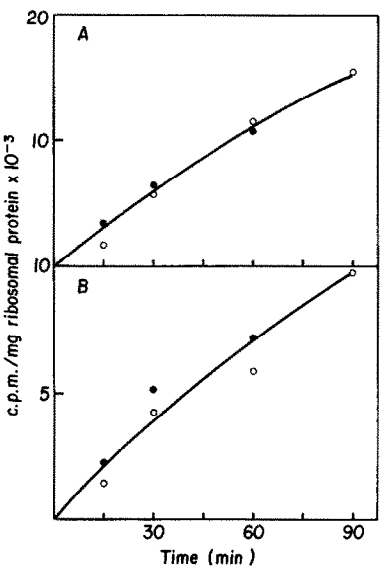

Fig. 1. Time course of [^{32}P] phosphate incorporation into ribosomes (A) and ribosomal proteins (B) in kidney cortex slices of aldosterone-treated (○) and control (●) adrenalectomized rats 2 h after intravenous hormone injection (4 μg/rat). 1·5 g of tissue was incubated in 7·5 ml of medium containing 67 μCi of [^{32}P]-H_3PO_4 per ml. In each experimental group, tissues from each of six rats contributed equally to each time period of incubation. These experiments were repeated three times. The average values given are representative of each individual experiment.

Table 1. Solubility characteristics of phosphorylated ribosomal constituents

Solubilization procedure	Experimental group	Radioactivity solubilized as percentage of washed ribosome radioactivity
Hot trichloroacetic acid	Aldo*	39·2
	Control	40·9
Organic solvents	Aldo	10·8
	Control	10·9
Protein residue	Aldo	38·4
	Control	44·4
Recovery	Aldo	88·4
	Control	96·2

For each group values are the averages obtained on six different preparations. These averages are representative of each individual preparation. Ribosomes were prepared from [^{32}P] labeled kidney cortex slices of aldosterone-treated and control adrenalectomized rats. 2 h after hormone treatment 1·8 g of tissue was incubated at 37°C for 60 min in 7·5 ml of medium containing 100 μCi of [^{32}P]-H_3PO_4 per ml. Ribosomes were prepared, precipitated with 10% cold TCA, and washed several times. 30–40% of the total ribosomal radioactivity was cold acid-soluble.

* Aldo, aldosterone-treated.

ribosomal protein residues which remained after TCA extraction of the ribosomal preparations at 90 C (to remove RNA) and organic solvent extraction (to remove phospholipids). The kinetics of radiophosphorus incorporation were similar in tissues from aldosterone-treated and control animals and incorporation was linear up to at least 60 min. Of the radioactivity in the total ribosomal preparations, 30–40% could be washed off with ice-cold TCA and was therefore, probably due to nucleotides, inorganic phosphate, or other phosphorus-containing small molecules. Of the remaining radioactivity 40 per cent was extractable with TCA at 90 C, and an equal portion remained in the protein residue (Table 1). Ribosomal RNA and proteins, therefore, were labeled to about the same extent. Organic solvents extracted about 11 per cent of the ribosomal radioactivity; this portion was probably associated with phospholipids.

Since the amount of radioactivity associated with inorganic phosphate and nucleotides can influence the labeling, the extents of phosphorylation of total ribosomes, of ribosomal RNA, and of ribosomal protein were determined not only in terms of their specific activities ([^{32}P] incorporated per unit of ribosomal protein or RNA), but also in terms of these specific activities relative to the amount of radioactivity in the acid-soluble homogenates and post-microsomal (100,000 *g*) supernatants per mg of homogenate or cytosol protein (Table 2). In none of these measures were there significant differences from controls.

Comparison of the phosphorylated ribosomal proteins from aldosterone-treated and control animals on SDS-polyacrylamide gels

Chemical extraction methods for the separation of ribosomal proteins from rRNA have variable yields and are, in general, not quantitative. For this reason we electrophoresed total ribosomal preparations on 10 per cent acrylamide gels containing SDS. On such gels rRNA and proteins are segregated; the proteins, complexed to SDS, enter the gel and separate according to their molecular weights [3,24]. The electrophoretic

Table 2. Phosphorylation of ribosomes, ribosomal proteins and ribosomal RNA in kidney cortex slices of aldosterone-treated and control adrenalectomized rats

Preparation	Experimental group	S.A. (c.p.m./mg ribosomal protein or RNA × 10^{-3})	S.A. × 10^{-3} c.p.m. acid-sol. homog./mgHomog. protein	S.A. × 10^{-3} c.p.m. acid-sol. cytosol/mgCytosol protein
Total ribosomes	Aldo	22·1 ± 5·75	17·0 ± 4·36	7·7 ± 1·27
	Control	20·0 ± 7·77	15·5 ± 2·45	7·1 ± 1·18
Ribosomal protein	Aldo	15·7 ± 7·67	10·9 ± 1·32	5·1 ± 1·34
	Control	13·7 ± 7·67	9·8 ± 1·83	4·7 ± 1·77
Ribosomal RNA	Aldo	8·1 ± 1·36	6·5 ± 2·35	2·9 ± 0·59
	Control	7·5 ± 2·57	5·9 ± 1·11	2·7 ± 0·37

Values are means ± S.D. for six separate incubations.

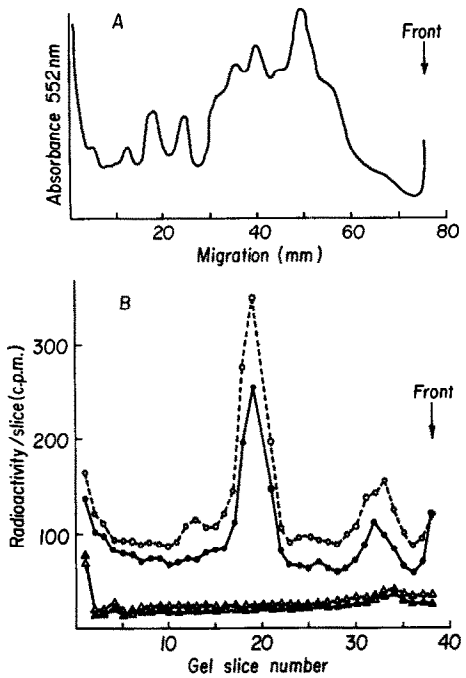

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of ribosomes and extracted ribosomal RNA. Kidney cortex slices from aldosterone-treated animals were incubated with [^{33}P]-H_3PO_4, and those from the controls with [^{32}P]-H_3PO_4 (1·8 g of tissue in 7·5 ml medium containing 200 μCi of [^{32}P] or [^{33}P] per ml) for 60 min, 2 h after hormone injection. (A) Densitometric tracing of Coomassie blue-stained ribosomal proteins, (B) radioactivity profiles. (○) ^{33}P-Labeled ribosomes; (●) ^{32}P-labeled ribosomes; (△) ^{33}P-labeled extracted rRNA; (▲) ^{32}P-labeled extracted rRNA. The same amount of RNA (730 μg) was analyzed on each gel.

mobilities of protein–SDS complexes are inversely proportional to the logarithms of the polypeptide chain molecular weights. 28S and 18S rRNA (but not 5S or 4S RNA) are excluded from such gels [28].

To confirm that under our experimental conditions rRNA was indeed excluded from entering the gels, RNA was extracted from a mixture of labeled ribosomes obtained from aldosterone-treated and control rats and was electrophoresed under the conditions used for the separation of total ribosomal preparations. In Fig. 2 the radioactivity profile of total ribosomes separated on one gel and that of the extracted RNA, applied on a separate gel, are plotted together. [^{32}P] Radioactivity indicates the profiles of total ribosomes or extracted rRNA from control rats; [^{33}P] radioactivity denotes the same from aldosterone-treated animals. Extracted rRNA gave no appreciable radioactivity in the gel.

Total ribosomes were resolved into at least 14 Coomassie blue-stained protein bands associated with one major (R_f 0·47–0·59) and several minor radioactive peaks. The protein banding pattern of ribosomes from control and aldosterone-treated animals was the same. Each of these protein bands probably represents several protein species, since eukaryotic ribosomes are known to contain 70 or more distinct proteins [29, 31, 32]. Ribonuclease digestion [30] of the ribosomal preparations with pancreatic ribonuclease and T_2 ribonuclease prior to gel electrophoresis did not alter the radioactivity profile of the Coomassie blue-stained protein banding pattern. Pronase treatment of the ribosomal preparations followed by dialysis prior to electrophoresis removed all the radioactive peaks (Fig. 3), and Coomassie blue-stained proteins could not be seen on the gel. When pronase-digested ribosomes were electrophoresed without prior dialysis, the radioactive peaks of the total ribosomes moved faster, which indicates conversion to smaller molecules. We concluded that the phosphorylated ribosomal constituents resolved on our gels were phosphoproteins.

The phosphorylation of individual proteins of ribosomes was compared in aldosterone-treated and control rats by a double-labeling technique. Kidney cortex slices from aldosterone-treated animals were labeled with [^{33}P], those from the controls with [^{32}P]. The tissues were pooled and the ribosomes were isolated, purified, and electrophoresed together. The effect of hormone treatment on ribosomal protein phosphorylation could then be assessed by the [^{32}P]/[^{33}P] ratios in different areas of the gel. This ratio per gel slice is an indication of the relative rates of phosphorylation of the proteins in that fraction. If all ribosomal proteins from the aldosterone-treated rats were phosphorylated at the same rate as the corresponding proteins from the controls, all the [^{32}P]/[^{33}P] ratios would be the same.

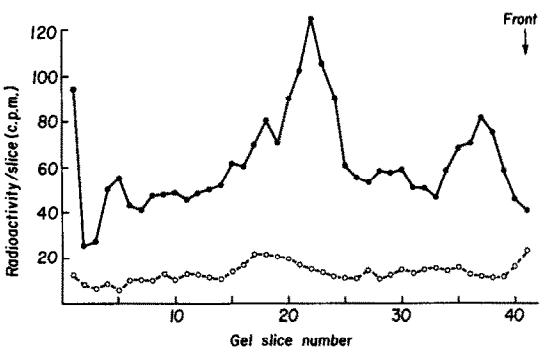

Fig. 3. Effect of pronase digestion on the polyacrylamide gel electrophoretic pattern of ribosomes labeled with [^{32}P]. Ribosomal preparations containing 840 μg protein were either electrophoresed directly or after pronase digestion (2 mg/ml, 37°C, 30 min) followed by dialysis. (●) Untreated, (○) pronasedigested ribosomes.

The variability in $[^{32}P]/[^{33}P]$ ratios inherent in the experimental design was determined by labeling ribosomes in kidney cortex from control animals with $[^{32}P]$ and $[^{33}P]$ separately, pooling the tissue, and isolating and electrophoresing the ribosomes as for the experimental group. In order to present succinctly the consistent changes in these ratios due to aldosterone treatment we have computed the averages of standardized ratios determined on several control and experimental gels, (Fig. 4) by taking the $[^{32}P]/[^{33}P]$ ratio in each gel slice and dividing by the ratio of total $[^{32}P]$ radioactivity to total $[^{33}P]$ radioactivity recovered from the gel. In this way $[^{32}P]/[^{33}P]$ ratios from different gels could be compared. Figure 4 presents the averages of standardized ratios from 6 gels in which 4 separate ribosomal control preparations were electrophoresed and those from 7 gels in which five separate experimental preparations (mixture of $[^{33}P]$-labeled ribosomes from hormone-treated and of $[^{32}P]$-labeled ribosomes from control animals) were separated. Thus, nine separate experiments were performed in which a minimum of 1 gel per experiment was run. The coefficient of variation (S.D. $\pm$ 0·035) of the averaged standardized $[^{32}P]/[^{33}P]$ ratios from the mean gel ratio (i.e. 1·0) on control gels was $\pm$3·5 per cent.

Lower $[^{32}P]/[^{33}P]$ ratios in the experimental gels indicate an increase in phosphorylation after hormone treatment; higher ratios, a decrease in phosphorylation. On all 7 gels in which mixtures of ribosomes from hormone-treated and control rats were separated, there was, in the region of R_f 0·11–0·14 (slices 4–5), a mean 14–16% decrease in the $[^{32}P]/[^{33}P]$ ratio. This decrease was significantly different ($P < 0·01$) from the averaged ratios at the same R_f on control gels. At R_f 0·19–0·24 (slices 7–9) the ratios were lower than the control by 12–15% ($P < 0·02$ to $< 0·05$ in the different gels). In the region of R_f 0·11–0·24 there were at least two Coomassie blue-stained protein bands. At R_f 0·43–0·49 (slices 16–18) the increase in averaged ratios in this region over the control was 9% ($P < 0·1$), while at R_f 0·57–0·62 (slices 21–23) the mean ratios were 8% higher than the control ratios ($P < 0·1$). Again, at least two Coomassie blue-stained protein bands were found in the region of R_f 0·43–0·62.

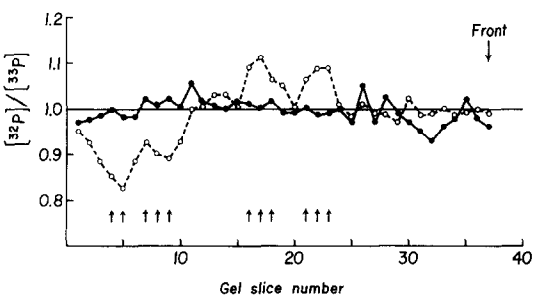

Fig. 4. Comparison of the phosphorylation of ribosomal proteins from aldosterone-treated and control rats on SDS-polyacrylamide gels by means of a double labeling technique. Kidney cortex slices from aldosterone-treated rats were incubated with $[^{33}P]$-H_3PO_4 (230 μCi/ml) and those from the controls with $[^{32}P]$-H_3PO_4 (460 μCi/ml) as described for Fig. 2. Ribosomes isolated from a mixture of these tissues were separated on gels and the standardized ratios of $[^{32}P]/[^{33}P]$, (○), were determined in each gel slice after fractionation (five separate experiments). Control standardized ratios (●) were determined on gels in which ribosomes prepared from a mixture of tissues from control animals, labeled with $[^{32}P]$ and $[^{33}P]$ separately in the same way, were electrophoresed (4 separate experiments). Computation of the mean standardized ratio is described in the text. Arrows indicate areas of gels where the extent of phosphorylation changed after aldosterone administration.

The fact that we do see significant changes relative to the controls indicates that changes do occur in the phosphorylation of ribosomal proteins migrating to certain regions of these gels after aldosterone administration. There appears to be an increase in the phosphorylation of a couple of ribosomal proteins of high molecular weight (low electrophoretic mobility) and a decrease in the phosphorylation of other ribosomal proteins of lower molecular weight (higher electrophoretic mobility). The ribosomal proteins which are decreased in phosphorylation migrate in the region of the main radioactive peak (R_f 0·47–0·59) shown in Figs. 2 and 3.

DISCUSSION

Distinguishing between ribosomal and non-ribosomal proteins is a matter of operational definition. The ribosomes in this study were isolated, after solubilization of the microsomal fraction by treatment with the detergents Lubrol WX and deoxycholate, by high-speed centrifugation over sucrose in a high-KCl medium. Such a procedure is designed to remove extraneous protein [13, 20, 21]. In previous studies [17, 18] ribosomes isolated from rat kidney cortex by the same method supported protein synthesis *in vitro* and, when isolated from the animal after aldosterone administration, had an increased ability to synthesize synthetic polypeptides and to bind aminoacyl-tRNA. The ribosomal preparations in the present study, therefore, contain the factor(s) necessary for ribosome function and also those necessary to bring about the hormone-induced changes in function.

The double-labeling technique used to compare the phosphorylation of the ribosomal protein species of hormone-treated and control animals is a more sensitive and accurate method than the comparison of specific activities of proteins in different regions of separate gels that is necessary when a single label is used.

Specific activity determinations on gels are not only laborious, but may also be unreliable since protein–dye complexes may deviate from Beer's law [33].

To ensure that they were free of contaminating phosphorus-containing materials of small molecular weight, the ribosomal preparations were washed several times with cold TCA and dialyzed against several changes of Tris–HCl and then a phosphate buffer before electrophoresis. That the proteins separated on the gels were phosphoproteins was shown by the disappearance of radioactive peaks after pronase digestion (Fig. 3). The increases observed, after hormone treatment, in the phosphorylation of ribosomal proteins migrating to the R_f 0·11–0·24 region were significant ($P < 0·01$ to $< 0·05$); the decrease in the phosphorylation of proteins migrating with a greater mobility (R_f 0·43–0·62) was also consistent in all experiments but the differences were significant only at the $P < 0·1$ level. It was the proteins in the major radioactive peak (R_f 0·47–0·59) that were decreased in their phosphorylation.

In a preliminary report Liew *et al.* have stated [34] that the *in vivo* labeling of whole kidney ribosomal proteins with [^{32}P]-H_3PO_4 decreased by 23% after aldosterone treatment of adrenalectomized rats. Our report demonstrates a specific effect of aldosterone on increases and decreases in the phosphorylation of separated renal ribosomal proteins.

Although the function(s) of ribosomal protein phosphorylation remains unknown it is presumably important since this event has been retained during mammalian evolution. Phosphorylation of ribosomal proteins has been shown to occur in a similar pattern in non-nucleated, non-dividing reticulocytes, as well as in rapidly proliferating mouse tumor cells [5, 11]. It is conceivable that cyclic phosphorylation and dephosphorylation play a part in altered ribosome function. Both a positive [2, 6, 8] and a negative correlation [35] between ribosomal phosphorylation and protein synthesis has been reported. Eil and Wool [36], however, recently found no appreciable and consistent difference in the *in vitro* functional capacity of phosphorylated and nonphosphorylated rat liver ribosomes. They may not, however, have tested the proper function, or it may be possible that liver ribosomes are already maximally phosphorylated before they are isolated, so that the *in vitro* phosphorylation is only a small fraction of what can occur.

The demonstration that renal cortical ribosomes isolated from adrenalectomized rats support more protein synthesis and more binding of aminoacyl-tRNA after aldosterone administration led to the conclusion that treatment by the hormone altered the ribosomes so that their functional capacity increased [17]. Perhaps the aldosterone-induced increases and decreases in renal ribosomal protein phosphorylation reported here may play a part in such alterations. The recent study of Walton and Gill [15] suggests that the phosphorylation of ribosomal proteins decreases the affinity of these proteins for the ribosomal structure, possibly by altering the protein-nucleic acid and/or protein–protein interactions within the ribosome. Such alterations in turn could conceivably alter ribosome function.

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