Phosphorylation of Chromatin-associated Proteins in *Lemna* and *Hordeum*[1]

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ABSTRACT

Sterile embryos of barley (*Hordeum vulgare*) and cultures of *Lemna perpusilla* have been labeled with ^{32}Pi and the chromatin proteins prepared and separated by acid-urea and sodium dodecyl sulfate gel electrophoresis. Under these conditions chromatin proteins became labeled and the gel radioactivity profiles which were complex indicated a probable minimum of 15 to 20 proteins phosphorylated with molecular weights ranging from 10^4 to 10^5. The majority of the radioactivity, 80 to 90% of the total, is found in the acidic protein fraction and this can be recovered as serine phosphate after partial acid hydrolysis.

Nuclei have been isolated from *Lemna* and barley and found to possess endogenous kinase activity. *In vitro* labeling of these nuclei with ^{32}P-adenosine triphosphate indicated that similar proteins appear to become labeled as *in vivo* labeling with ^{32}Pi but the proportions of label in each protein were different.

Protein phosphorylation is an important element in the molecular basis of animal hormone action (6). We have already reported the presence of ribosomal protein phosphorylation in *Lemna* and pea and its catalysis by a kinase attached to the ribosomes (9, 15). As part of a continuing program to examine the presence and possible function of this regulatory system in plants this paper describes the presence of phosphorylated chromatin proteins and indicates that the phosphorylation may be carried out by endogenous kinases in the nucleus.

MATERIALS AND METHODS

Plants. *Lemna perpusilla* was grown under sterile conditions on E medium (3) at 28C with a photoperiod of 16 hr at 300 ft-c. Under these conditions, cultures doubled their fresh weight every 1.25 days. Sterility was checked upon each transfer as described previously (14).

For *in vivo* labeling of *Lemna* with ^{32}Pi, KH$_2$PO$_4$ in the medium was reduced: (*a*) for 3-hr labeling, to 5 μM with ^{32}Pi at

50 μCi/ml; (*b*) for 18-hr labeling, to 50 μM with ^{32}Pi at 25 μCi/ml; and (*c*) for 8-days labeling, to 1 μM with ^{32}Pi at 4 μCi/ml. Growth, measured as frond number, continued normally on these media for the duration of the different labeling periods.

Hordeum vulgare L. cv. COHO seeds were dehusked by soaking for 3 hr in 50% H$_2$SO$_4$, and subsequently sterilized for 5 min in 1% sodium hypochlorite. They were then washed five times and imbibed for 3 hr in sterile distilled H$_2$O. Embryos were removed with a sharp spatula, sterilized 10 min in 0.2% sodium hypochlorite, and stored in sterile distilled H$_2$O at 2 C. Sterility was checked in the same way as for *Lemna*.

Barley embryos were labeled *in vivo* with 200 μCi/ml ^{32}Pi in the basal medium of Joy and Folkes (7), in which the phosphate concentration was reduced to 10^{-8} M. Amino acids were supplied in the form of 600 μg/ml of an enzymic hydrolysate of casein. Sterilized embryos were placed in 2 ml of the above medium in a 50-ml conical flask, left at 2 C overnight, and further incubated at 25 C for 3 hr.

Isolation of Chromatin. Chromatin was isolated by a modification of the procedure of Huang and Bonner (5). *Lemna* was ground in 10 volumes 0.05 M tris-HCl, pH 8, 0.25 M sucrose, 10 mM MgCl$_2$, 0.12 M mercaptoethanol, 1 mM NaHSO$_3$ at 0 C. The homogenate was filtered through two and four layers of gauze and two layers of Miracloth on a Büchner funnel under suction. The combined filtrates were immediately made 2% in Triton X-100 and centrifuged at 2,000g for 30 min. The precipitate was resuspended in 1 volume of grinding medium, treated once more with Triton X-100, centrifuged at 10,000g for 10 min, and washed 4 to 5 times, with scrapings, in washing medium (5). Finally, the chromatin, after resuspension in 5 ml of washing medium, was layered over 25 ml of 10 mM tris-HCl pH 8, 1.7 M sucrose, 10 mM mercaptoethanol, 1 mM NaHSO$_3$; the upper third of the tube was gently mixed to form a crude sucrose gradient and the contents were spun at 50,000g for 3 hr. The gelatinous precipitate, still containing some starch, was used as the purified chromatin. Yields varied between 17.5 and 18.8 μg DNA/g fresh weight of tissue. This constitutes about 30% of the DNA extracted directly from the tissues.

In vivo labeled *Lemna* plants were homogenized with a mortar and pestle. After the last Triton X-100 treatment, the resuspended precipitate was combined with that from non-labeled *Lemna*, prepared at the same time from plants that were grown under the same conditions.

Barley embryo chromatin was isolated by essentially the same method. The tissue was homogenized with a mortar and pestle in 2 volumes of grinding medium, then made up to 10 volumes. Treatments with Triton X-100 were omitted. Yields varied between 70 and 72 μg DNA/g fresh weight.

Isolation of Nuclei. All steps were carried out at 0 to 4 C. *Lemna* plants were homogenized in a Potter-Elvehjem homog-

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enizer with a motor-driven Teflon pestle at 200 rpm in 6 volumes of ice-cold 50mM tris-HCl, pH 7.6, 0.3 M sucrose, 5 mM MgCl₂, 0.5% mercaptoethanol. The homogenate was filtered twice, with shaking, through Miracloth. The residue was re-extracted with 1 volume of isolation medium and filtered in the same way. The combined filtrates were made 2% in Triton X-100 and centrifuged at 270g for 10 min, using a swing-out rotor. The precipitate was resuspended in 2 volumes of 0.05 M tris-HCl, pH 7.6, 0.3 M sucrose, 5 mM MgCl₂, 5 mM mercaptoethanol and the centrifugation step repeated. The resulting precipitate was resuspended in 10 ml of buffer and centrifuged at 4000g for 30 min through a cushion of 15 ml 0.01 M tris-HCl, pH 7.6, 1.78 M sucrose, 5 mM MgCl₂, 5 mM mercaptoethanol. The final white precipitate consisted of nuclei, as evidenced by phase-contrast and light microscopy after staining with methyl green, starch grains, and calcium oxalate crystals. Yields of about 12.5 μg DNA/g fresh weight of tissue were routinely obtained.

Barley embryos were homogenized and the homogenate was filtered as described for *Lemna*. The combined filtrates were made 2% in Triton X-100, layered over 1 ml of 10 mM tris-HCl, pH 7.6, 1.78 M sucrose, 5 mM MgCl₂ in a conical tube and centrifuged at 270g for 6 min. The upper layer was discarded and the ring of material at the boundary of the two layers was gently resuspended in the 1.78 M sucrose layer with a Teflon rod. This nuclei-containing layer was then carefully removed without disturbing the starch precipitate, and made 0.3 M sucrose by dilution with 10 mM tris-HCl, pH 7.6, 5 mM MgCl₂. This step was repeated twice. The final nuclei-containing fraction was made up to 5 ml with buffered 1.78 M sucrose and centrifuged at 7000g for 30 min through a cushion of 15 ml 10 mM tris-HCl, pH 7.6, 1.93 M sucrose, 5 mM MgCl₂ to yield the final nuclei preparation. Yields varied between 96 and 124 μg DNA/g fresh weight of tissue.

In Vitro **Labeling and Preparation of Chromatin from Nuclei.** Nuclei were resuspended in 0.5 ml of 0.5 M tris-HCl, pH 7.6, 0.3 M sucrose, 5 mM MgCl₂, 5 mM mercaptoethanol and incubated for 20 min at 25 C in the presence of 10 μM ATP, containing 50 to 80 μCi γ-³²P-ATP. The reaction was stopped by the addition of 5 ml of 10 mM tris, 1 mM EDTA, 1 mM NaHSO₃, pH 8.0, and chromatin was prepared by the washing and sucrose gradient procedure as outlined above. In the case of *Lemna*, chromatin could not be separated from the starch, as it sedimented as an amorphous mass together with the starch.

Isolation of Chromatin Proteins. For the separation of total chromatin proteins and DNA, the purified chromatin was resuspended in 0.5 ml of 10 mM tris-HCl, pH 8, 3 M NaCl, 6 M urea, 1% mercaptoethanol, 1 mM NaHSO₃ (13). One μg of RNase, which had been previously heated to 60 C for 10 min to remove any residual DNase activity, was added to digest any remaining contaminating RNA and the mixture was centrifuged at 74,000g for 16 hr. The protein containing supernatant was used for protein determination and gel analysis.

Acrylamide Gel Electrophoresis of Proteins. Acidic urea gel electrophoresis at pH 3.2 was performed as described by Panyim and Chalkley (12). In the earlier experiments, electrophoresis was performed at room temperature for 4 hr at 2 mamps/gel. Increased resolution was obtained in the later experiments by running the gels for 16 to 18 hr at 1 mamp/gel at 2 C.

SDS[4] gel electrophoresis at pH 9.05 was carried out as described by Neville (11). Electrophoresis was conducted at room temperature at 30 v for 1 hr, then at 1 mamp/gel until the tracking dye band reached the bottom of the gels. After electrophoresis the gels were removed from the tubes and stained in 0.25% of Coomassie blue in 45% methanol, 9% acetic acid for 4 hr. They were then destained by washing in 5% methanol, 7.5% acetic acid at 55 C, and scanned at 575 nm with a Gilford spectrophotometer, equipped with a gel scan attachment. Subsequently, the gels were frozen quickly on Dry Ice and cut into 1-mm slices with a Mickle gel slicer. After drying on filter paper strips, the slices were individually immersed in a scintillation mixture containing 4 g of PPO and 100 mg of POPOP/liter of toluene, and counted for ³²P.

Radioactive Chemicals. ³²Pi, γ-³²P-ATP (25–50 Ci/mmole), α-³²P-ATP (3 Ci/mmole) were obtained from Amersham/ Searle and tetraethylammonium ³²P-ATP from New England Nuclear.

RESULTS

Chromatin preparations were characterized by chemical and spectral analysis. After suspension of the final chromatin preparation from the sucrose gradient spectral ratios (A_{246}: A_{280}:A_{260}) of 0.84:1:0.59 were obtained from *Lemna* and 0.78:1:0.57 for barley. The compositional characteristics for the two preparations (DNA:acid-soluble protein:acid-insoluble protein) were for *Lemna* 1:0.87:0.46 and barley 1:0.75:0.91. To obtain these measurements it was found necessary to prepare barley chromatin via a preliminary isolation of nuclei; otherwise much higher protein to DNA ratios were obtained. This seems to be the result of contamination of chromatin preparations by storage protein bodies which are centrifuged down at 2000g but not at 275g.

The nucleic acids of the chromatin preparations were also isolated and separated by gel electrophoresis. Scanning such gels at 265 nm revealed the presence of a single peak which was resistant to the action of ribonuclease and could be stained with methyl green, both characteristics of DNA. Traces of ribosomal RNA were only occasionally seen and the results indicated an upper limit of 2% ribosomal RNA of the total nucleic acid.

Chromatin Protein Phosphorylation in Barley Embryos. Phosphorylation of barley chromatin proteins was initially detected by incubating 3-hr-old embryos for a further 3 hr in ³²Pi, isolating the chromatin and separating the labeled proteins by acid-urea and SDS gel systems (Fig. 1). Both separations show that radioactivity is spread over a considerable length of the gel interspersed with a number of discrete peaks. The majority of the radioactivity appears to be associated with the slowly migrating acidic proteins. Initial attempts to detect the presence of serine phosphate in these proteins were unsuccessful mainly because of the low total radioactivity incorporated with embryos of this age. This problem of low labeling was circumvented by preparing isolated nuclei from this tissue and incubating them in γ-³²P-ATP of high specific radioactivity. In this case phosphorylation was catalyzed by a protein kinase found in the nuclear preparations. Figure 2 shows such an experiment, in which nuclei were isolated from 5.5-hr-old embryos, and were incubated either in γ-³²P-ATP or α³²P-ATP. The chromatin proteins prepared from these labeled nuclei were separated by urea/acetic acid and SDS gel electrophoresis. When ATP of high specific radioactivity is used, much more label is incorporated into the separated proteins than when labeling *in vivo*. Although the gel profiles of radioactivity in Figures 1 and 2 look distinctly different, careful examination indicated that similar protein bands become labeled. For example, in the urea/acetic acid system radioactive bands at 1.6, 2.4, and 4.7 cm are labeled both *in vivo*

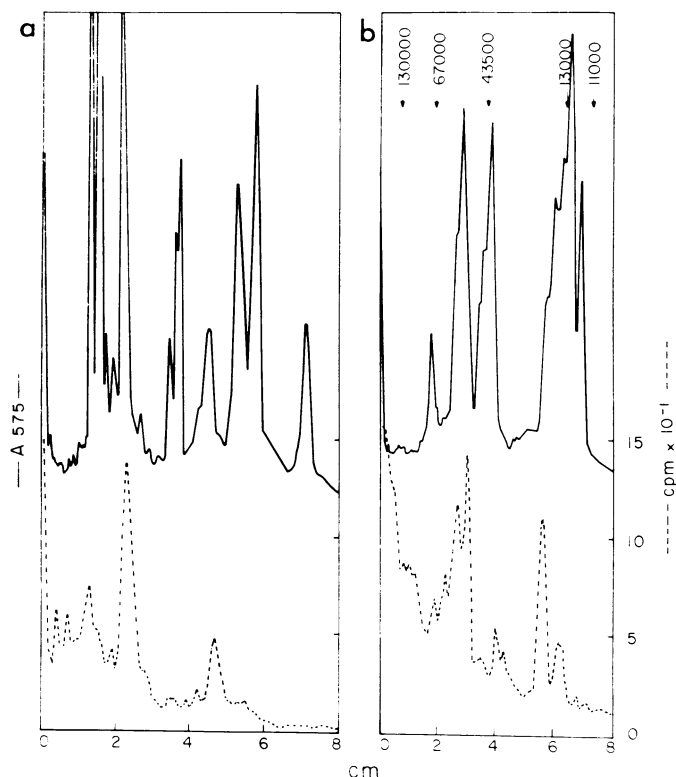[4] Abbreviation: SDS: sodium dodecyl sulfate.

FIG. 1. Protein profiles and phosphorylation patterns of *in vivo* labeled barley embryo chromatin proteins. Three-hr-old barley embryos were incubated for a further 3 hr in ^{32}Pi, and the chromatin proteins were prepared. After gel electrophoresis proteins were stained with Coomassie blue, scanned at 575 nm, cut into 1-mm slices, and counted. a: Acid-urea gel electrophoresis of 74 μg of protein separated at 2 C for 18 hr at 70 v/gel. b: SDS gel electrophoresis on 181 μg of chromatin protein. Mol wt markers used were β-galactosidase (130,000), BSA (67,000), ovalbumin (43,500), carboxypeptidase A (34,600), trypsin (23,300), and chymotrypsin (13,000 and 11,000). The mobilities of these markers are indicated by arrows at the top of the figure. A_{575} (———); radioactivity (– – –).

and *in vitro* but the proportions of label incorporated into these bands under these two experimental conditions are substantially different. When the nuclei were labeled with α^{32}P-ATP, incorporation was hardly detectable above the background. Even when allowing for the 10-fold lower specific activity of the α^{32}P-ATP compared to the γ^{32}P-ATP, the total incorporation is less than 5% of that obtained with γ^{32}P-ATP. Differences in the A_{575} profile which can be seen by comparing Figures 1 and 2 appear to arise by contamination of ordinary chromatin preparations by storage protein bodies. Preparations made through a prior nuclear isolation, (Fig. 2) are free from this problem as described earlier.

Figure 3 shows an experiment in which nuclei from 5-hr-old embryos were labeled with γ^{32}P-ATP. The chromatin was prepared and divided into two and one part was extracted with dilute sulphuric acid to solubilize histone material; the two samples were then separated by urea-acetic acid electrophoresis. Only a minor fraction of the radioactivity was acid-soluble, most being associated with a single peak migrating at 4.9 cm. When total chromatin proteins were separated, a broad band of radioactivity could be seen between 4.4 and 5.0 cm and occasionally this was observed to split into two and sometimes three bands. Only the most rapidly migrating zone was solubilized by acid extraction. Chromatin proteins labeled as described under Figures 2 and 3 were also hydrolyzed with 6 N

HCl for 6 hr at 105 C and the products separated by paper electrophoresis (Fig. 4). Peaks of serine phosphate, inorganic phosphate, an unknown peptide, and traces of threonine phosphate can be seen in the separation. Under these hydrolysis conditions serine phosphate has a half-life of about 6 hr (8). The inorganic phosphate may thus arise solely from breakdown of serine phosphate.

Chromatin Protein Phosphorylation in *Lemna perpusilla*. Phosphorylation of chromatin-associated proteins in *Lemna perpusilla* was first detected by incubating cultures in ^{32}Pi for 3 or 18 hr, preparing the labeled chromatin and separating the associated proteins by gel electrophoresis. Figure 5, a and c, shows urea-acetic acid separations of cultures labeled for 3 hr and 18 hr respectively. Figure 5b shows the acid-soluble components of the proteins shown in 5a, and Figure 5d shows SDS gel separations of the proteins in Figure 5c. As noticed in barley, there is an extensive spread of radioactivity over the gel separations with the majority of labeled material in the more slowly migrating acidic proteins. Figure 5d shows the mol wt of this material to range from 10,000 to 100,000. However, unlike barley, considerably more of the radioactivity is acid-soluble (about 25–30%) with two distinct peaks running under strongly staining bands. These may therefore represent histone material. The nature of the diffuse labeled material covering the first 3 cm of Figure 5b is unknown but this may represent contaminating acid-soluble proteins which have been reported by some workers to be present in histone preparations made in this way (2, 4).

Phosphorylation of chromatin proteins of *Lemna* was also carried out *in vitro* using the endogenous kinase in nuclei together with γ^{32}P-ATP. Typical urea-acetic acid and SDS separations are shown in Figure 6. Comparison with Figure 5
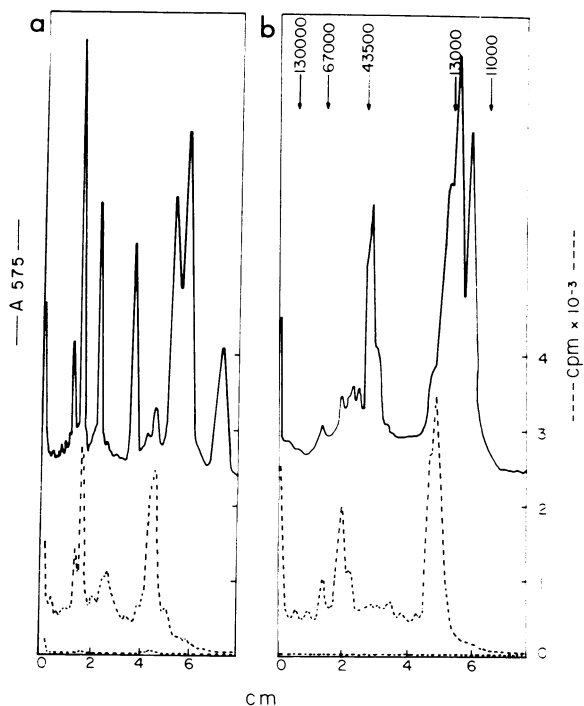

FIG. 2. Protein profiles and phosphorylation patterns of *in vitro* labeled barley embryo chromatin proteins. Nuclei were isolated from 6-hr-old embryos and incubated for 20 min in the presence of 80 μCi of γ^{32}P-ATP (upper broken curve) or 80 μCi of α^{32}P-ATP (lower broken curve). Thirty μg of total chromatin proteins were subjected to acid-urea gel electrophoresis at 2 C for 18 hr at 70 v (Fig. 3a) and 50 μg for SDS electrophoresis (Fig. 3b). Mol wt markers as under Figure 2. A_{575} (———); radioactivity (– – –).
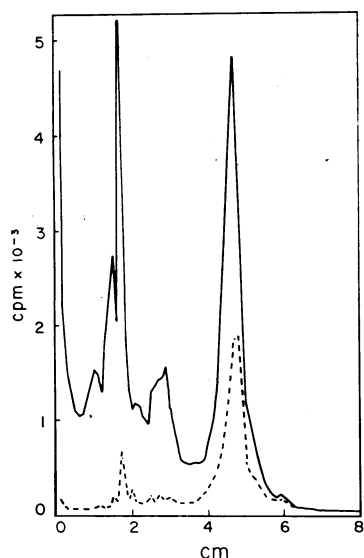
FIG. 3. Phosphorylation patterns of total chromatin and acid-soluble chromatin protein labeled *in vitro*. Nuclei were isolated from 6-hr-old barley embryos and incubated for 20 min in 70 μCi of γ^{32}P-ATP. Chromatin was prepared from the labeled nuclei, and the acid-soluble chromatin proteins were extracted as previously described (1). Gel electrophoresis using the acid-urea system was conducted as described in Figure 2. Radioactivity, 22 μg of total chromatin proteins (———); radioactivity, 10 μg of acid-soluble protein (– – –).
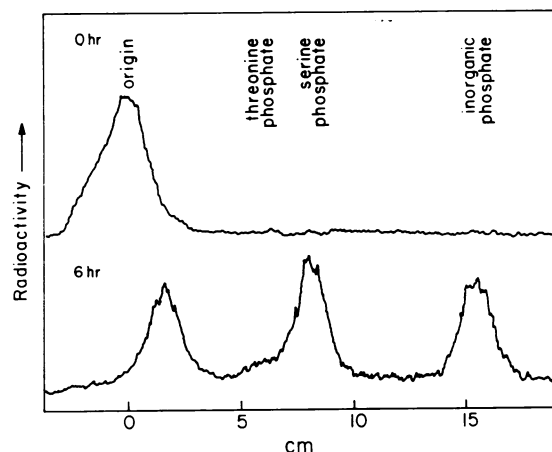

FIG. 4. Paper electrophoresis separation of a partial acid hydrolysate of barley chromatin proteins labeled *in vitro*. Nuclei were isolated from 6-hr-old embryos and incubated in 100 μCi of γ^{32}P-ATP for 20 min. The chromatin proteins were prepared and subjected to hydrolysis in 6 N HCl for 0 hr (upper control curve) and 6 hr (lower curve) at 105 C. Markers of Pi, serine phosphate, and threonine phosphate were subjected to electrophoresis at the same time and their position was revealed with phosphomolybdate reagent. The sample strips were counted using a chromatogram scanner.
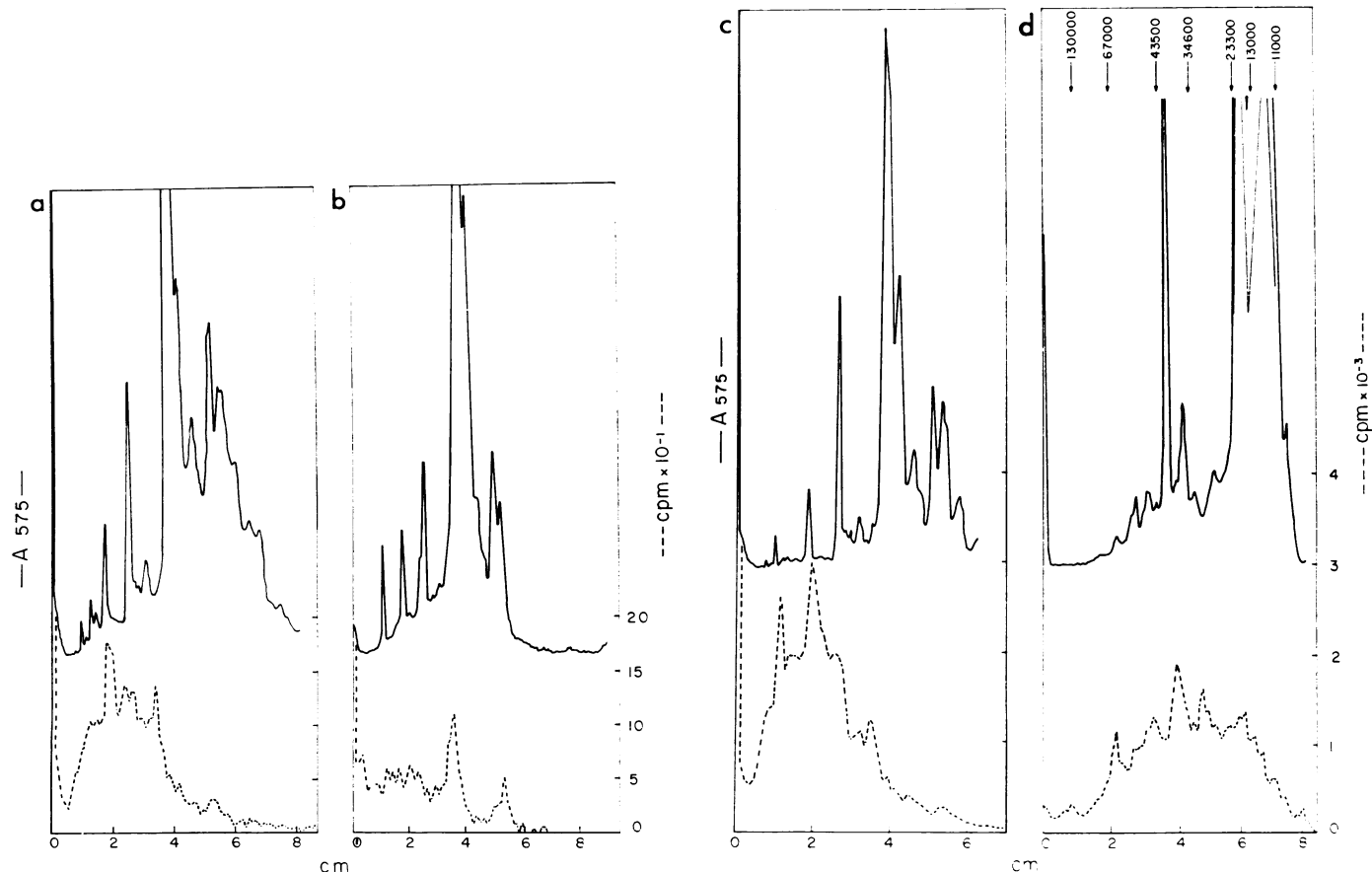

FIG. 5. Protein profiles and phosphorylation patterns of *in vivo* labeled *Lemna* chromatin proteins. Plants were labeled for 3 hr on medium containing 50 μCi/ml ^{32}Pi (a,b) and for 18 hr on medium containing 25 μCi/ml ^{32}Pi (c,d). Chromatin was prepared, and the labeled proteins were separated by the acid-urea system (a–c) with electrophoresis at room temperature for 3.5 to 4 hr at 110 to 120 v or the SDS system (d). a: 126 μg total chromatin proteins; b: 82 μg acid-soluble chromatin protein; c and d: 200 μg total chromatin protein. Other details as described in Figure 1. A_{575} (———); radioactivity (– – –).
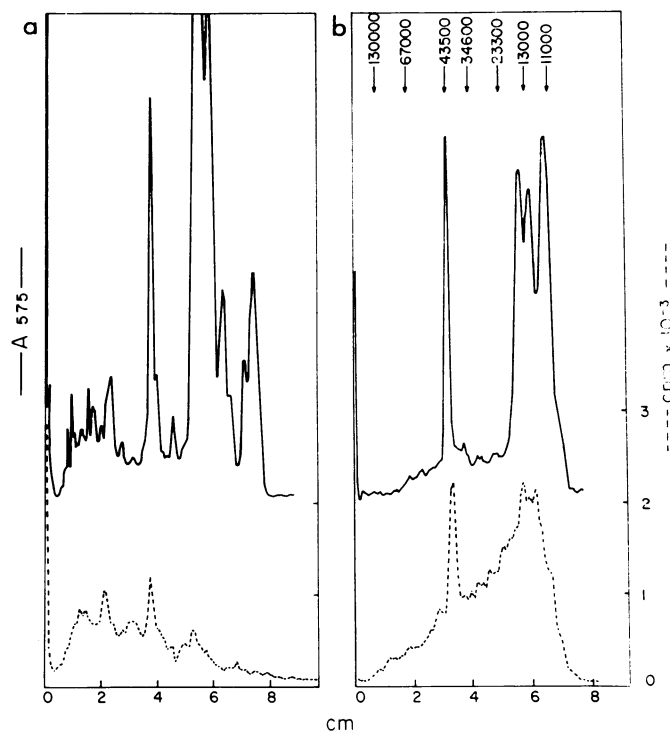
FIG. 6. Protein profiles and phosphorylation patterns of *in vitro* labeled *Lemna* chromatin proteins. Isolated nuclei were incubated for 20 min in the presence of 100 μCi of γ^{32}P-ATP. The labeled proteins were prepared and 30 μg were subjected to acid-urea gel electrophoresis at 2 C for 17 hr at 70 v (a) and 100 μg to SDS gel electrophoresis (b). A_{575} (———); radioactivity (– – –).

shows that there are similarities between the distribution of radioactivity in the urea/acetic acid gels with a number of peaks common to both *in vivo* and *in vitro* labeling but the proportion of label incorporated into the various gel regions has been modified with a higher amount of label in the faster migrating proteins. This is essentially confirmed by comparing the SDS separations in Figures 5d and 6b which show a greater emphasis on labeling of proteins in the region of 10,000 to 20,000 mol wt.

When chromatin proteins of *Lemna* were labeled as described under Figure 6 and subjected to partial acid hydrolysis, separation of the products by paper electrophoresis revealed the presence of inorganic phosphate, serine phosphate, an unknown peptide, and traces of threonine phosphate precisely as described for barley (Fig. 4).

DISCUSSION

This paper provides evidence for the presence of phosphorylated proteins in the chromatin of barley and *Lemna*. That the ^{32}Pi was not loosely associated with the proteins but covalently bound follows from the observations that: (a) serine phosphate could be isolated from an acid hydrolysate of the chromatin-associated proteins, (b) the majority of such label was acid-insoluble (c) migrated with proteins during gel electrophoresis, and (d) was transferred from γ^{32}P-ATP but not from α^{32}P-ATP. That the ^{32}Pi was attached to proteins themselves follows from the observations that the macromolecules moved as cations in urea-acetic acid and as anions in SDS.

The major portion (90% in barley and 70–75% in *Lemna*)

of the labeled proteins was insoluble in dilute acid and thus acidic in nature, a situation similar to that found in animal systems (10). Gel electrophoresis reveals that the pattern of phosphorylation of these acidic proteins is complex with minimal estimates of 15 phosphorylated proteins from the urea-acetic acid separations and clearly a considerably greater number from SDS separations.

Labeling *in vivo* with ^{32}Pi or of isolated nuclei with γ^{32}P-ATP resulted in phosphorylation patterns in which the same proteins appear to be phosphorylated, although in different proportions. The reasons for the differences in the relative amounts of ^{32}P incorporated into individual proteins are not clear but may be sought in: differential losses during nuclei and chromatin preparations; the involvement of multiple protein kinases which are differently active under *in vivo* and *in vitro* conditions; differences in phosphatase activity; selective labeling of some parts of the tissues during ^{32}Pi incubation; or differences in the regulation of protein phosphorylation under the two conditions. Our evidence does not allow discrimination between these hypotheses at the present time. The situation is not comparable, however, to that shown by us (9) for ribosomal protein phosphorylation in which the substrate specificity of the kinase appears to be lost during *in vitro* incubation and many proteins become labeled which are not labeled *in vivo*. The maintenance of discrimination by the kinase shown here in Figure 2 and Figure 6 suggests that this *in vitro* labeling may be an acceptable method of detecting alterations in the pattern of chromatin protein phosphorylation.

LITERATURE CITED

1. BONNER, J., G. R. CHALKLEY, M. DAHMUS, D. FAMBROUGH. F. FUJIMURA. R.-C. C. HUANG, J. HUBERMAN, R. JENSEN, K. MARUSHIGE. H. OHLENBUSCH, B. OLIVERA, AND J. WIDHOLM. 1968. Isolation and characterization of chromosomal nucleoproteins. Methods Enzymol. 12B : 3 65.
2. CHAE, C.-B., M. C. SMITH, AND J. L. IRVIN. 1972. Effect of *in vitro* histone phosphorylation on template activity of rat liver chromatin. Biochim. Biophys. Acta 287 : 134–153.
3. CLELAND, C. F. AND W. R. BRIGGS. 1967. Flowering responses of the long-day plant *Lemna gibba* G 3. Plant Physiol. 42 : 1553–1561.
4 FAMBROUGH, D. M., F. FUJIMURA, AND J. BONNER. 1968. Quantitative distribution of histone components in pea plants. Biochemistry 7 : 575–585.
5. HUANG, R.-C. C. AND J. BONNER. 1962. Histone. a suppressor of chromosomal RNA synthesis. Proc. Nat. Acad. Sci. U.S.A. 48 : 1216–1222
6. JOST. J.-P. AND H. V. RICKENBERG. 1971. Cyclic AMP. Annu. Rev. Biochem. 40 : 741–774.
7. JOY, K. W. AND B. F. FOLKES. 1965. The uptake of amino-acids and their incorporation into the proteins of excised barley embryos. J. Exp. Bot. 16 : 646–666.
8. KABAT, D. 1970. Phosphorylation of ribosomal proteins in rabbit reticulocytes. Characterization and regulatory aspects. Biochemistry 9 : 4160–4175.
9. KEATES, R. A. B. AND A. J. TREWAVAS. 1974. Protein kinase associated with ribosomes of peas and *Lemna*. Plant Physiol. 54 : 95–99.
10. LANGAN. T. A. 1968. Phosphorylation of proteins of the cell nucleus. In: A. SanPietro, M. R. Lamborg, and F. T. Kenney, eds., Regulatory Mechanisms for Protein Synthesis in Mammalian Cells. Academic Press, New York. pp. 101–118.
11. NEVILLE, D. M. 1971. Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoresis in a discontinuous buffer system. J. Biol. Chem. 246 : 6328–6334.
12. PANYIM, S. AND R. CHALKLEY. 1969. High resolution acrylamide gel electrophoresis of histones. Arch. Biochem. Biophys. 130 : 337–346.
13. SHAW, L. M. J. AND R.-C. C. HUANG. 1970. A description of two procedures which avoid the use of extreme pH conditions for the resolution of components isolated from chromatins prepared from pig cerebellar and pituitary nuclei. Biochemistry 9 : 4530–4542.
14. TREWAVAS, A. 1970. The turnover of nucleic acids in *Lemna minor*. Plant Physiol. 45 : 742–751.
15. TREWAVAS. A. 1973. The phosphorylation of ribosomal protein in *Lemna minor*. Plant Physiol. 51 : 760–767.