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High Mobility Group Like Chromosomal Proteins from Amoebas of the Acellular Slime Mold *Physarum polycephalum*†

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ABSTRACT: Amoebas of the slime mold *Physarum polycephalum* contain four major non-histone chromosomal proteins called AS-1, AS-2, AS-3, and AS-4. These proteins were extracted from chromatin with 0.35 M NaCl and were soluble in 2% trichloroacetic acid. Fractions AS-1, AS-2, and AS-3 were also quantitatively extracted with 5% perchloric acid, while a low yield of AS-4 was obtained with this method. Two minor non-histone proteins, AS-5 and AS-6, were extracted from nuclei, but not from chromatin, with either 5% perchloric acid or 0.35 M NaCl. These proteins had electrophoretic mobilities similar to HMG-18 and HMG-19 of calf. On acetic acid–urea–polyacrylamide gel electrophoresis, none of the four major *Physarum* AS proteins comigrated with the standard calf high mobility group (HMG) proteins. On sodium dodecyl sulfate gel electrophoresis, AS-1 migrated slower than calf HMG-1 and HMG-2 while AS-2 migrated near these two calf proteins. The AS-3 and AS-4 proteins, with apparent molecular weights of 16 500 and 16 000, respectively, migrated in the same region as calf HMG-14. The four major *Physarum* AS proteins were fractionated by exclusion chromatography on Bio-Gel P-100, and the amino acid compositions of the isolated proteins were determined. The four AS proteins contained high levels of both acidic and basic residues, a distinctive feature shared by most HMG proteins. Although some similarities in amino acid composition were seen between the *Physarum* and the calf HMG proteins, the results suggested that considerable divergence had occurred. A greater similarity was seen when the *Physarum*, *Tetrahymena*, and yeast HMG-like proteins were compared. The presence of HMG-like proteins in the slime mold *Physarum polycephalum* is further proof of the ubiquitous occurrence of the HMG proteins in eucaryotes.

It is only recently that histones from the acellular slime mold *Physarum polycephalum* were isolated and characterized. Using either plasmodia (Chahal et al., 1980; Mende et al., 1983; Champagne et al., 1982) or amoebas (Côté et al., 1982), two different developmental stages of this lower eucaryote, it was shown that *P. polycephalum* contained recognizable H1, H2A, H2B, H3, and H4 histones.

In addition to the histones, considerable attention has been focused on another group of chromosomal proteins, the high mobility group (HMG) proteins originally isolated from calf thymus nuclei (Goodwin et al., 1973). Although some of the HMG proteins appear clustered in the transcriptionally active regions of chromatin (Levy-Wilson et al., 1979; Weisbord et al., 1979), the precise function of these non-histone chromo-

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somal proteins still remains to be determined. Nevertheless, since the early studies on calf, proteins resembling HMGs have been isolated and characterized from a wide variety of eucaryotic cell types [for a review, see Mayes (1982)], including two lower eucaryotes: *Tetrahymena* (Hamana et al., 1979) and yeast (Petersen & Sheridan, 1978; Spiker et al., 1978; Weber & Isenberg, 1980).

Matthews et al. (1979) reported that *Physarum* plasmodia contained a small group of proteins that, like mammalian HMG proteins, can be extracted from nuclei by 0.35 M NaCl and are soluble in 2% trichloroacetic acid. During the course of our work on the characterization of histones from *Physarum polycephalum*, we isolated an acid-soluble protein, "AS", which had an amino acid composition resembling that of HMG proteins from higher eucaryotes (Côté et al., 1982). In the present work, we used 0.35 M NaCl and 5% perchloric acid (PCA) to extract four major and two minor HMG-like proteins from *Physarum* amoebas. The major proteins were separated by exclusion chromatography and characterized by gel electrophoresis and amino acid composition.

Materials and Methods

Strain. The haploid amoebal strain Cld-Axe (McCullough et al., 1978) was grown in 500-mL baffled flasks containing 200 mL of Daniel and Baldwin's semidefined medium supplemented with hematin (Daniel et al., 1964).

Nuclear Isolation. Nuclei were isolated by using a slightly modified method previously described (Côté et al., 1982). All steps were carried out at 4 °C. Briefly, amoebas in late exponential growth [(3–6) × 10^7 cells/mL] were collected from 800 mL of culture medium by a 5-min centrifugation at 500*g*. The cells were suspended and gently stirred for 5 min in 250 mL of washing buffer [0.25 M sucrose and mM ethylenediaminetetraacetic acid (EDTA), pH 8.0] and collected by centrifugation. The washed cells were then suspended in 100 mL of homogenization buffer [0.25 M sucrose, 3 mM $MgCl_2$, 0.5% (w/v) Triton X-100, 1 mM phenylmethanesulfonyl fluoride (PMSF), and 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.5] and broken by 10 strokes of a Potter-Elvehjem tissue grinder. The homogenate was centrifuged at 1000*g* for 5 min. The resulting pellet was suspended in 75 mL of homogenization buffer and centrifuged under the same conditions. This step was repeated once more before the nuclear pellet was washed twice in 50 mL of TMK buffer (10 mM Tris-HCl, 1 mM $MgCl_2$, 25 mM KCl, and 1 mM PMSF, pH 7.5) and centrifuged each time at 1000*g* for 5 min.

Histone Isolation. Total basic nuclear proteins were extracted at 4 °C by stirring nuclei in 0.3 N HCl and 1.0 M $CaCl_2$ for 30 min (Mohberg & Rusch, 1969). Nuclei were pelleted at 12000*g* for 10 min, and solid trichloroacetic acid (Cl_3CCOOH) was added to the supernatant to a final concentration of 25% (w/v). The precipitated histones were collected by centrifugation at 12000*g* for 10 min, solubilized in 0.02 M H_2SO_4, and reprecipitated with 5 volumes of ethanol.

HMG Protein Isolation. The method of Goodwin & Johns (1973), as modified by Weisbord et al. (1981), was used. Isolated nuclei were washed twice in 50 mL of TEN buffer (10 mM Tris-HCl, 0.2 mM EDTA, 75 mM NaCl, and 1 mM PMSF, pH 7.5) and centrifuged for 10 min at 9800*g*. The resulting chromatin pellet was resuspended in 20 mL of 0.35 M NaCl, 5 mM Tris-HCl, and 1 mM PMSF, pH 7.0, and sonicated in ice. A Heat-Systems Ultrasonics sonicator with a microtip was used at an amplitude of 3.5 for two 10-s bursts. The solution was then adjusted to 2% Cl_3CCOOH by the addition of 100% Cl_3CCOOH with rapid stirring, left on ice for 5 min, and centrifuged at 12000*g* for 10 min. The resulting supernatant was adjusted to 25% (w/v) Cl_3CCOOH and left again on ice for at least 1 h. The precipitated proteins were recovered by a 10-min centrifugation at 12000*g*, suspended in 1.5 mL of 0.3 M HCl, and precipitated with 6 volumes of acetone.

In some experiments, *Physarum* HMG proteins were isolated by the PCA extraction method (Goodwin et al., 1977). Briefly, isolated nuclei were directly suspended in 20 mL of 5% (w/v) PCA,, sonicated, and stirred for 20 min at 4 °C. Solubilized proteins in the supernatant were precipitated as described above.

Bio-Gel Chromatography. Protein sieving was carried out at room temperature as described by Nadeau et al. (1977). Dried proteins (20 mg) were solubilized in 0.01 N HCl and 0.02% (w/v) NaN and loaded onto a Bio-Gel P-100 column (120 × 2.5 cm). Proteins were eluted with 0.01 N HCl and 0.02% (w/v) NaN. One-milliliter fractions were collected, and their absorbance was measured at 230 nm. The appropriate fractions were pooled and then concentrated with an Amicon ultra filtration cell. The resulting reduced volumes were adjusted to 0.3 M HCl and precipitated with acetone.

Amino Acid Analyses. Amino acid compositions of isolated proteins were performed on a Beckman 119 BL amino acid analyzer with a Model 126 peak integrator. Samples were hydrolyzed in 6 N HCl at 100–110 °C for 24 h in sealed tubes. No corrections were made for degradative losses. All analyses were done at least in duplicate.

Relative differences in amino acid profiles were compared by the difference index of Metzger et al. (1968), calculated by the expression DI = $\sum|\Delta(\text{mol }\%)|^1/_2$.

Gel Electrophoresis. Proteins were analyzed by acetic acid–urea–polyacrylamide slab gel electrophoresis, carried out according to the method of Panyim & Chalkley (1969). Sodium dodecyl sulfate (SDS) gel electrophoresis was performed essentially as described by Thomas & Kornberg (1975). Apparent molecular weights were determined with calf thymus histones as standards.

Results

Gel Electrophoresis Analysis. The proteins extracted from *Physarum* chromatin with 0.35 M NaCl and soluble in 2% Cl_3CCOOH were compared by two-dimensional acetic acid–urea–SDS gel electrophoresis to total HCl–$CaCl_2$-extracted proteins. As shown in Figure 1A, the five histones are clearly visible in 0.3 N HCl–1.0 M $CaCl_2$ extracts. Among the numerous non-histone proteins also extracted from *Physarum* nuclei, four predominant polypeptides were identified and labeled AS-1, AS-2, AS-3, and AS-4 in order of increasing mobility in first-dimension electrophoresis. Figure 1B shows that these four proteins were selectively extracted from chromatin by using 0.35 M NaCl. Histones H1 and H2B were relatively less extractable in 0.35 M NaCl, while the other histones were not extracted.

When analyzed on a one-dimensional acid–urea–polyacrylamide gel system, the 0.35 M NaCl soluble proteins gave only four bands (Figure 2B). AS-1 and histone H1 migrated together to form the slowest running band on the gel. Protein AS-2 migrated so close to histone H2B that both proteins were found in the second band of the gel, while the third and fourth bands contained proteins AS-3 and AS-4, respectively. In a total basic protein extract (Figure 2C), AS-3 migrated between arginine-rich histones H3 and H4 and corresponded to the "AS" protein described in a previous paper (Côté et al., 1982), AS-4 was faintly visible, but AS-1 and AS-2 were concealed

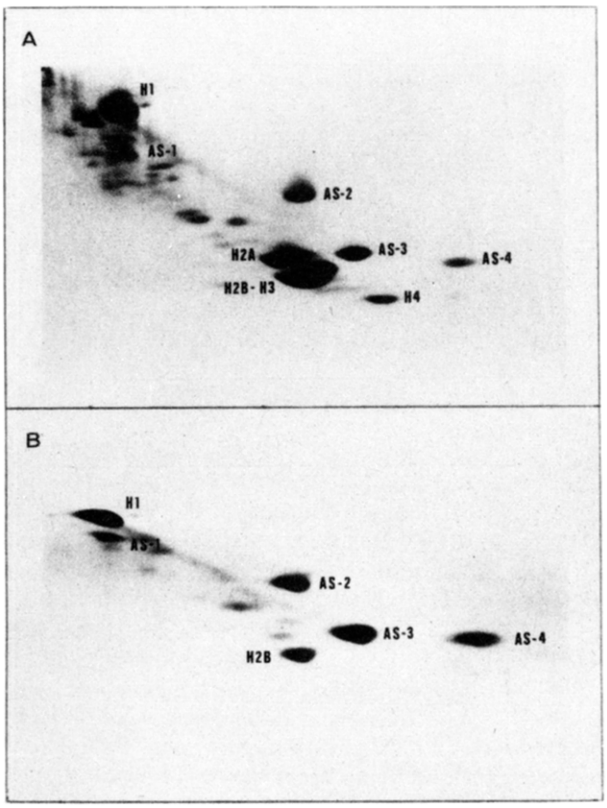

FIGURE 1: Two-dimensional acetic acid–urea–SDS gel electrophoresis of *Physarum polycephalum* chromosomal proteins. (A) Proteins solubilized by 0.3 N HCl–1.0 M $CaCl_2$; (B) proteins solubilized by 0.35 M NaCl.

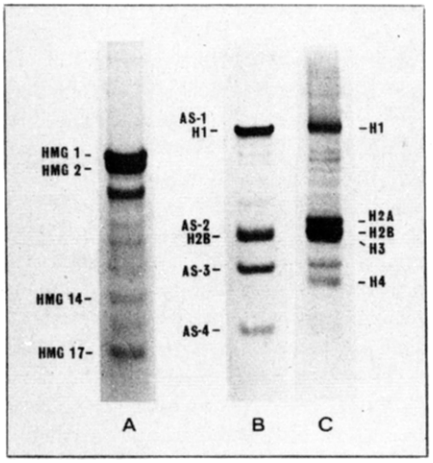

FIGURE 2: One-dimensional electrophoresis of *Physarum* HMG-like proteins, *Physarum* histones, and calf HMG proteins on polyacrylamide–acid–urea gels. (A) Calf thymus HMG proteins. (B) *Physarum* chromosomal proteins extracted with 0.35 M NaCl. (C) *Physarum* chromosomal proteins extracted with 0.3 N HCl–1.0 M $CaCl_2$.

by histone bands. None of the *Physarum* AS proteins comigrated with the standard calf HMG proteins (Figure 2A).

Fractionation of HMG-like Proteins. Proteins from the 0.35 M NaCl extract of *Physarum* chromatin were fractionated by exclusion chromatography on Bio-Gel P-100. A typical elution profile is shown in Figure 3. The fractions, A–F, were pooled and analyzed by polyacrylamide gel electrophoresis (Figure 4). No stainable proteins remained at the electrophoretic origins (not shown).

It can be seen from these results that the Bio-Gel column gave satisfactory separation of most salt-extracted chromosomal proteins. The large peak A of Figure 3 contained the *Physarum* histone H1. In all preparations [see also Côté et al., (1982)], this fraction always contains a minor band reminiscent of an $H1^o$-like histone (Brown et al., 1981). This band did not coincide electrophoretically with any other component in both systems (Figures 4 and 5). Peaks B, C, D, and E contained proteins having electrophoretic mobilities on acid–urea gels identical with those of proteins AS-1, AS-2, AS-3, and AS-4, respectively. *Physarum* histone H2B was found in peak F.

On SDS gel electrophoresis (Figure 5), protein AS-1 migrated slower than calf HMG-1 and HMG-2 with an apparent molecular weight of 26 100 (Figure 5B). AS-2 migrated in the same general region as calf HMG-1 and HMG-2 with an apparent molecular weight of 20 500 (Figure 5C). The AS-3 and AS-4 proteins, with relative masses of 16 500 and 16 000, respectively, migrated near HMG-14 (Figure 5D,E). These protein fractions were considered to be at least 90% free from polypeptide contaminants.

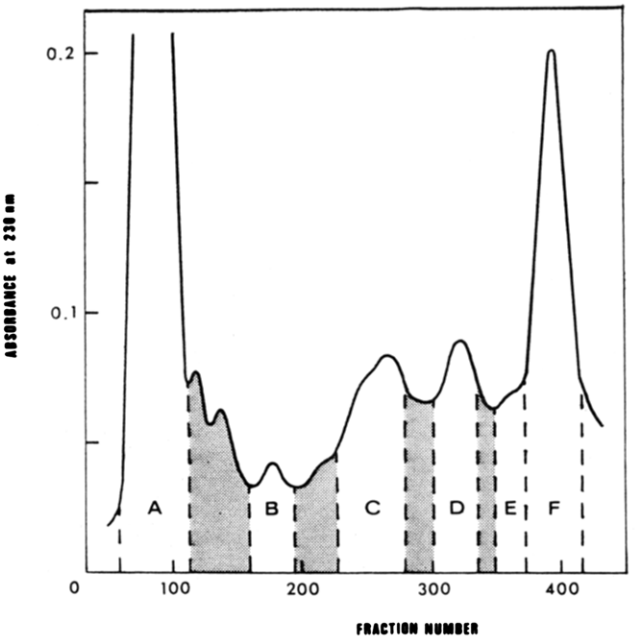

FIGURE 3: Chromatogram of *Physarum* chromosomal proteins extracted with 0.35 M NaCl and eluted from a column of Bio-Gel P-100 with 0.01 N HCl–0.02% NaN_3. Fractions A through F were collected.

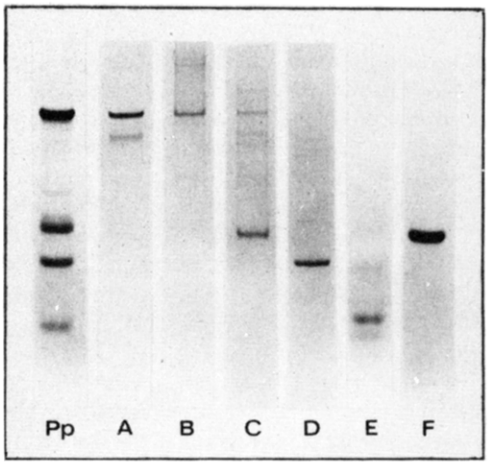

FIGURE 4: Acetic acid–urea gel electrophoresis of Bio-Gel P-100 purified proteins. The letters on each lane refer to the peaks eluted from the chromatogram shown in Figure 3. Total *Physarum* chromosomal proteins extracted with 0.35 M NaCl (Pp) are shown for comparison.

Table I: Amino Acid Composition (mol %) of *Physarum* 0.35 M NaCl Extractable Proteins[a]

	AS-1	AS-2	AS-3	AS-4	histones H1	histones H2B
Asx	8.8	7.8	7.1	8.0	3.4	6.6
Thr	7.2	6.8	5.5	4.4	7.7	6.5
Ser	7.0	6.1	6.9	2.9	9.0	9.4
Glx	14.3	15.3	12.8	11.1	7.4	9.6
Pro	5.4	5.2	4.7	3.5	10.7	3.7
Gly	7.0	6.2	7.2	12.8	3.9	8.2
Ala	9.8	10.9	12.9	14.1	20.8	13.7
Val	5.6	5.2	4.7	8.2	3.3	7.2
Cys	ND[b]	ND	ND	ND	ND	ND
Met	0.9	0.8	0.6	0.4	0.4	0.8
Ile	3.6	3.2	2.5	3.7	2.7	2.9
Leu	5.6	5.0	6.1	5.4	2.8	4.1
Tyr	1.2	1.3	1.8	1.0	0.8	3.0
Phe	2.6	2.3	2.9	1.7	1.2	1.4
His	2.8	2.3	1.2	3.9	3.4	3.1
Lys	15.2	18.2	18.0	16.1	19.2	16.4
Arg	3.1	3.5	5.1	3.5	3.0	3.4
Asx + Glx	23.1	23.1	19.9	19.1	10.8	16.2
basic amino acids	21.1	24.0	24.3	23.5	25.6	22.9
Lys/Arg ratio	4.9	5.2	3.5	4.6	6.4	4.8

[a] Compositions are all uncorrected for degradative losses. [b] ND, not determined.

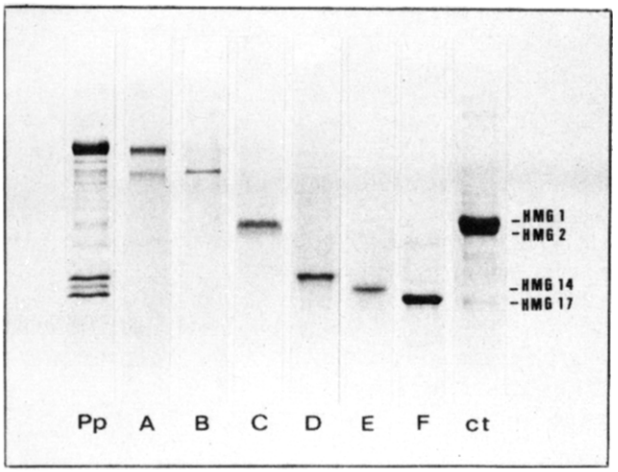

FIGURE 5: Polyacrylamide–SDS gel electrophoresis of fractions from Bio-Gel P-100 chromatography. The letters on the lanes refer to the peaks eluted from the chromatogram shown in Figure 3. Total *Physarum* chromosomal proteins extracted with 0.35 M NaCl (Pp) and calf thymus HMG (ct) are shown for comparison.

Amino Acid Analysis. The amino acid compositions of the proteins in peaks A through F were determined, and the results are summarized in Table I. As indicated by their mobilities in both acid–urea and SDS–polyacrylamide gels, fractions A and F appeared to contain histones H1 and H2B, respectively, and this was confirmed by amino acid compositions. Like histone H1 (Côté et al., 1982), protein in peak A was relatively rich in lysine, alanine, and proline with low levels of arginine and acidic residues, but this fraction tended to be slightly poorer in the former, and slightly richer in the latter as well as in methionine, histidine, and hydrophobic residues. This may be attributable to variable amounts of the H1°-like component. The H2B fraction in peak F was rich in serine, alanine, glutamic acid, and especially, in lysine, but low in proline. These amino acid compositions are typical for *Physarum* H1 and H2B histones (Côté et al., 1982; Mende et al., 1983).

The proteins found in peaks B, C, D, and E contained high levels of both acidic (19–23%) and basic residues (21–24%), a distinctive feature of most HMG proteins. When compared to calf high mobility group proteins (Mayes & Johns, 1982), the AS proteins, however, were richer in most of the hydrophobic residues (i.e., valine, isoleucine, leucine, tyrosine, and phenylalanine), and none of the AS proteins had levels of proline reported for calf HMG proteins. The minor contaminants could not account for the quantitative differences in composition.

Despite a difference in their apparent molecular weights (Figure 3), the amino acid composition of AS-1 and AS-2 suggested that these two proteins are similar, perhaps even sharing a relatively lysine-rich, homologous sequence. Moreover, they resembled calf HMG-1 and HMG-2 proteins in having a high level of acidic residues (mainly glutamic acid) and a moderate alanine content.

AS-3 was the most abundant of all AS proteins isolated from *Physarum* chromatin and comprised the AS fraction previously reported (Côté et al., 1982). Although similar in many ways to fractions AS-1 and AS-2, the composition of this protein differed principally in glutamic acid and in alanine. Consistent differences were also observed in histidine and arginine contents. Except for the presence of more hydrophobic residues, AS-3 resembled the mammalian HMG-14 protein in that the glutamic acid/alanine and lysine/alanine ratios of both proteins were similar.

The amino acid composition of AS-4 was different in many respects from that of the three other AS proteins. It was particularly rich in glycine, valine, alanine, and histidine, while it contained less serine, proline, and phenylalanine. Like AS-3, protein AS-4 contained less glutamic acid and was thus less acidic than AS-1 and AS-2. The high proportions of glycine and alanine found in the AS-4 protein were also seen in calf HMG-17, but the proportion of hydrophobic residues was much higher in the former.

The relatedness of *Physarum* salt-solubilized chromosomal proteins to standard calf thymus HMG proteins was estimated by the difference index (DI) of Metzger et al. (1968). If a DI of 13 is taken as the threshold between close similarity and dissimilarity between two proteins, the values of Table II suggest that *Physarum* AS and calf HMG proteins have diverged considerably. The DI of 14.1 between AS–2 and HMG-2 in Table II was the lowest value obtained and indicated a "likely" relationship for proteins of this size, at least more likely than between any other pair of *Physarum* AS and calf HMG proteins.

Table II: Difference Indexes Based on Amino Acid Compositions[a]

	AS-1	AS-2	AS-3	AS-4
		Calf Thymus		
HMG-1	18.4	15.5	18.5	27.0
HMG-2	15.9	14.1	15.4	26.0
HMG-14	19.8	16.1	15.3	24.0
HMG-17	33.7	31.2	29.0	25.4
		Yeast		
HMG a	13.5	13.4	13.2	23.8
S1	9.9	10.3	10.3	15.0
S3	14.7	16.1	16.9	21.6
S4	10.9	14.7	12.4	14.2
		Tetrahymena pyriformis		
LG-1	15.7	12.9	16.7	24.7
LG-2	12.2	8.9	10.2	17.9

[a] Amino acid compositions for *Physarum* are in Table I, calf thymus in Mayes & Johns (1982), yeast in Weber & Isenberg (1980), and *Tetrahymena* in Hamana & Iwai (1979).

The same comparative method was applied to HMG-like proteins isolated from yeast (Weber & Isenberg, 1980) and from the ciliated protozoan *Tetrahymena pyriformis* (Hamana & Iwai, 1979). Considering the much smaller phylogenetic distance between these two organisms and *Physarum polycephalum*, it was reasonable to find lower difference index values than between calf and *Physarum* (Table II). Moreover, the DI values indicated a "near certainty" relationship (Cornish-Rowden, 1980), especially between AS-1 and yeast S1 protein (9.9 value) and between AS-2 and *Tetrahymena* LG-2 protein (8.9 value). Amino acid compositions of S1 and LG-2 are found in Weber & Isenberg (1980) and Hamana & Iwai (1979), respectively.

Perchloric Acid Extractions. Isolation of HMG proteins from higher eucaryotic cells using 5% PCA directly on nuclei or even whole tissue was developed as an alternative procedure to the 0.35 M NaCl extraction of chromatin (Sanders & Johns, 1974). Since HMG proteins appear very susceptible to proteolytic degradation and since extraction with PCA prevents such a degradation (Goodwin et al., 1978), both extraction methods were tried on either purified nuclei or chromatin from *Physarum polycephalum*.

Proteins AS-1, AS-2, AS-3, and AS-4 were extracted with 0.35 M NaCl from either nuclei or chromatin (Figure 6B,D). In addition, other proteins were also extracted. For example, two minor bands, AS-6 and AS-6, were seen in NaCl extracts of nuclei. These proteins had electrophoretic mobilities similar to those of HMG-18 and HMG-19 of calf (Figure 6). The AS-5 and AS-6 proteins were not seen in a 0.35 M NaCl extract of chromatin. When nuclei or chromatin was extracted with 5% PCA, proteins AS-1, AS-2, and AS-3 were evident; the amount of AS-4, however, was greatly reduced relative to the quantity obtained with NaCl extractions. The minor proteins AS-5 and AS-6 were again found in 5% PCA extracts of nuclei, but not chromatin. The presence of proteins AS-1, AS-2, and AS-3 in both NaCl and PCA Extractions suggests that they are not products of degradation during extraction after nuclear isolation. The absence of AS-4 in PCA extracts may indicate that this polypeptide is a degradation product or that it has a low solubility in PCA.

Discussion

The present experiments suggest that at least four major non-histone proteins with high contents of both acidic and basic amino acids (over 40%) are extractable from the chromatin of the acellular slime mold *Physarum polycephalum*. Like the high mobility group proteins in higher eucaryotic cells,

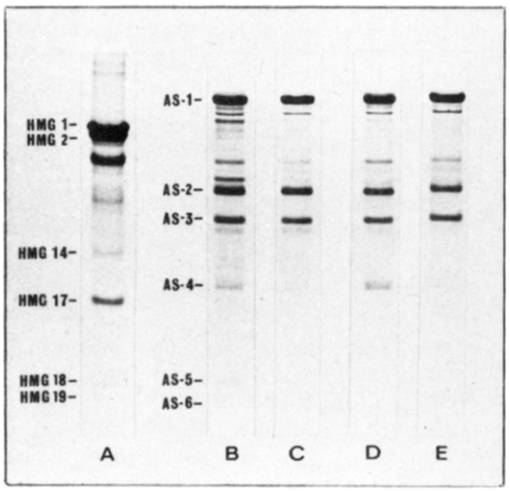

FIGURE 6: Polyacrylamide–acid–urea gel electrophoresis of calf and *Physarum* chromosomal proteins. (A) Calf HMG proteins. (B and C) *Physarum* HMG-like proteins extracted from nuclei with 0.35 M NaCl (B) and 5% perchloric acid (C). (D and E) *Physarum* HMG-like proteins extracted from chromatin with 0.35 M NaCl (D) and 5% perchloric acid (E).

these proteins can be selectively extracted with 0.35 M NaCl and are not precipitated with 2% Cl_3CCOOH (Johns, 1982).

The presence of *Physarum* lysine-rich histone H1 among the salt-solubilized material is not surprising since this protein was extracted in most studies on HMG proteins. It has been known for many years that partial dissociation of a typical H1 histone from chromatin occurs with 0.4 M NaCl (Johns & Forrester, 1969). The partial solubilization of *Physarum* histone H2B with 0.35 M NaCl was unexpected since H2B histones from other sources are extracted by much higher salt concentrations (Larue & Pallotta, 1976). The solubility of *Physarum* H2B was not caused by a too violent chromatin isolation procedure since this histone was extracted even when whole nuclei were extracted with 0.35 M NaCl. It has been pointed out that chromatin with substantial amounts of light "euchromatin" tends to be partially solubilized by 0.35 M NaCl, which results in partial extraction of histones (Goodwin & Johns, 1977b). The existence of a unique chromatin structure in *Physarum polycephalum* (Scheer et al., 1981) might be related to the salt solubilization behavior of H2B. Alternatively, *Physarum* H2B may be less tightly bound to DNA than are H2B histones from other sources since it is known that phosphorylated histones are more easily extracted from transcriptionally active cells (Urban et al., 1981) than from mature cells (Brasch et al., 1974).

Most H2B histones are insoluble in 5% PCA, but a PCA-soluble H2B was reported for wheat germ (Spiker et al., 1976; Fazal & Cole, 1977) and more recently in sea urchin embyro (Katula, 1983). *Physarum* H2B is also soluble in 5% PCA as shown by two-dimensional electrophoresis of PCA-extracted proteins (not shown).

The identification of *Physarum* amoebal HMG-like proteins was based on extraction properties, electrophoretic mobility, and amino acid composition. Until the sequence of a protein is determined, caution must be exercised in claiming homologous relationships between proteins of phylogenetically distant organisms. The difference index of Metzger et al. (1968) offers an objective estimate of similarity in amino acid compositons. The DI between *Physarum* HMG-like and calf HMG proteins indicates a weak relationship at best, but not necessarily that these proteins are unrelated. This may be illustrated by comparing calf thymus HMG-17 and trout H6 protein amino acid compositions (Watson et al., 1979). De-

spite a DI of 15.4 between these proteins, which suggests only a "likely" relationship (Cornish-Bowden, 1980), sequence analysis has nevertheless shown 69% homology (Watson et al., 1979).

The DI values of 9.9 between *Physarum* AS-1 and yeast S1 protein and of 8.9 between AS-2 and *Tetrahymena* LG-2 protein indicate relatedness with "near certainty". These values are even more impressive if we consider that *Physarum* and yeast H4 histones have a difference index of 10.4 even though this protein is known as one of the most conserved proteins. *Physarum* and calf H4, for example, have a difference index of 3.9 (Wilhelm & Wilhelm, 1984; Waterborg et al., 1983).

As is true for mammalian HMG proteins, *Physarum* HMG-like proteins can be extracted with either 5% PCA or 0.35 M NaCl (Figure 6). With both procedures, two fast-migrating proteins, AS-5 and AS-6, were extracted from nuclei but were almost completely absent from chromatin. These proteins have respective mobilities similar to 5% PCA-soluble HMG-18 and HMG-19 on acid–urea–polyacrylamide gels. Since the characterization of AS-5 and AS-6 was not undertaken, it is not known whether they are homologous with calf HMG-18 and HMG-19. It is interesting to note, however, that like *Physarum* AS-5 and AS-6, a better yield of these two calf thymus HMG proteins was obtained from nuclei than from chromatin (Nicolas & Goodwin, 1982).

Although fresh material was used and PMSF was added to all solutions during the preparation, one or more of the *Physarum* proteins isolated in the present work might correspond to breakdown products of other proteins, as was the case for some of the original, putative HMG proteins (Goodwin et al., 1978). *Physarum* AS-4 protein is particularly suspect, since it is almost completely absent from nuclear material extracted with perchloric acid, a solvent which limits proteolysis during extraction. However, an estimate of amino acid residue differences based on relative molecular weights (Figure 5) and compositions (Table I) indicates that AS-4 has more glycine than any other AS and more alanine, valine, and histidine than AS-2 or AS-3. Similarly, AS-3 contains more arginine than AS-1 or AS-2; thus, the smaller polypeptides could not be derived from the larger.

Chambers et al. (1983) have recently published studies on *Physarum* H1 histone. When this histone is digested with chymotrypsin, two large fragments are generated. As judged by SDS gel electrophoresis, one of these fragments, the N-terminal region of *Physarum* H1 histone, has a mobility similar to that of the AS-3 protein described in the present paper. The amino acid composition of the amino-terminal chymotryptic fragment of H1, however, is different from the composition of the AS-3 protein (Mende et al., 1983). This and other results indicate that AS-3 is not a breakdown product of H1. All AS proteins are of course too large to be degradation products of the other histones.

Mende et al. (1983) reported that when the H1 histone component from acid–urea–Triton gel electrophoresis of total *Physarum* plasmodial histones was rerun into a 17.5% SDS gel, the lysine-rich histone showed a substantial band running at an apparent molecular weight of 23 000. Since protein AS-1 and *Physarum* histone H1 were found to migrate together, forming one band in an acid–urea gel (Figure 2), AS-1 and the protein reported above might be related. However, the possibility that the 23 000 molecular weight protein described by Mende et al. (1983) corresponds to a degradation product of H1 must be considered since we have found that this histone is particularly acid labile, even in very mild conditions, and that the "satellite band" near H1 in Figures 4 and 5 is derived from it (S. Côté and Pallotta, unpublished results). Moreover, because of its amino acid composition (Table I), AS-1 could not be a degradation product of H1.

We have isolated and characterized four HMG-like proteins from the true slime mold *Physarum polycephalum*. Amino acid composition indicated some homologies between the *Physarum* and calf HMG proteins. A greater homology was seen between the *Physarum*, *Tetrahymena*, and yeast HMG-like proteins. Amino acid sequencing of the *Physarum* proteins is necessary to assess their actual relationship to other nuclear proteins. The presence of HMG-like proteins in the lower eucaryote *Physarum polycephalum* is further proof of the ubiquitous occurrence of this class of proteins. This result supports the idea that HMG proteins play an essential role in chromatin structure or function.

While our manuscript was in preparation, Czupryn & Tockzko (1984) reported the isolation of three HMG-like proteins from *Physarum* plasmodia. The identification of two of their proteins as HMG-like is preliminary. Neither of these proteins comigrated on polyacrylamide gels with the calf HMG proteins or with the amoebal HMG proteins studied in the present work. In addition, amino acid analyses of these presumptive plasmodial HMG-like proteins were not carried out. Their third plasmodial HMG-like protein (HMG-14/17P) had the same electrophoretic migration as did the amoebal AS-3 protein. We also noticed the AS-3 protein in preparations of chromosomal basic proteins of plasmodia (Côté et al., 1982). It is noteworthy that Czupryn & Toczko (1984) found the plasmodial HMG-like proteins associated with transcriptionally active chromatin.

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