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HMG-Like Chromosomal Proteins in *Trypanosoma cruzi*

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Abstract HMG-like chromosomal proteins from *Trypanosoma cruzi* were studied. Four HMG-like proteins, designated HMG A, HMG-B, HMG-C, and HMG-E, were isolated and found to have molecular weights of 35.5 kd, 27.5 kd, 21.8 kd and 10.4 kd, respectively. Immunological relatedness was demonstrated between the mammalian HMG 1,2 and the HMG-A and HMG-B from *T. cruzi*. The relative amounts of HMG-C and HMG-E proteins vary in *T. cruzi* depending to the proliferative stage of the cells. HMG-E protein is increased in proliferating cells when compared to its level in non-proliferating cells. HMG-C is increased in the non-proliferating cells. Probably, the shifts observed in the relative amounts of HMG-like proteins are related to the proliferating cells of this flagellate. The results are consistent with those described for other lower eukaryotes where the HMG-like proteins isolated are similar but not identical to HMG proteins from vertebrates.

Key words: flagellate, HMG proteins, immunological relatedness, proliferating cells, non-proliferating cells

Chromatin is a dynamic structure, constantly changing during transcription, mitosis, meiosis, DNA replication, and repair. These processes require the participation of histones as well as nonhistone chromosomal proteins. Among the nonhistone chromosomal proteins, the high mobility group (HMG) have been associated with active nucleosomes [Weisbrod, 1982]. Furthermore, a correlation between the amount of some HMG chromosomal proteins and the proliferative rates of different tissues has been suggested [Seyedin and Kistler, 1980; Kennedy and Davies, 1980; Seyedin et al., 1981; Bhorjee, 1981; Gordon et al., 1981; Bucci et al., 1984].

Vertebrate cells contain four well-characterized HMG proteins, namely, HMG 1, HMG 2, HMG 14, and HMG 17. These proteins have low molecular weights (<30,000) [Shooter et al., 1974; Goodwin et al., 1975; Walker et al., 1977; Bustin et al., 1978; Pipkin et al., 1981] and an unusual amino-acid composition containing approximately 25% basic and 30% acid residues [Goodwin et al., 1973; Bustin and Neihart, 1979; Weisbrod, 1982]. In contrast to the mammalian HMG chromosomal proteins, those of lower eukaryotes are poorly characterized.

We report here the presence of HMG-like chromosomal proteins in *Trypanosoma cruzi*, the protozoan which causes American tripanosomiasis or Chagas disease. These proteins were characterized by their solubility characteristics, electrophoretic mobilities, and immunological properties. Moreover, it was found that their relative amounts vary in relation to the proliferative activity of the parasite in culture.

METHODS

Cell Culture

T. cruzi epimastigotes strain Tulahuen were grown in Diamond medium [Diamond, 1968] supplemented with 2.5% fetal calf serum and 7.5 μM hemin at 28°C. The cells were collected by centrifugation, washed three times in phosphate-buffered saline (PBS), and used immediately.

Extraction of HMG Proteins

The HMGs were extracted as described C. Sanders [Sanders, 1977]. *T. cruzi* epimastigotes or calf thymus were homogenized twice in 0.75 M perchloric acid (PCA). Histone H1 was precipitated from the supernatant adding 3.5 volumes of acetone (−20°C) and 0.07 M HCl. HMG proteins were precipitated by addition of 2.5 volumes of acetone (−20°C) to the supernatant.

Histones Isolation

Histones from *T. cruzi* epimastigotes or calf thymus were isolated from chromatin with 0.4

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N sulfuric acid by following the procedure described previously [Toro and Galanti, 1988].

SDS-Polyacrylamide Gel Electrophoresis (PAGE)

Histones and HMG-like proteins were analyzed by electrophoresis in 15% polyacrylamide—0.1% SDS gel slabs (16 × 14 × 0.2 cm) performed essentially according by U. Laemmli [Laemmli, 1970] and modified as described J. Bhorjee [Bhorjee, 1981], adding 0.5 M urea and 0.005 M EDTA to the separating and stacking gel solutions prior to polymerization. After electrophoresis the proteins were fixed in the gel with 15% trichloracetic acid (TCA) and 45% methanol for 12 h, stained with 0.6% amido black in 7.0% acetic acid, and destained in 7.0% acetic acid. Protein concentrations were determined by the Bradford method [Bradford, 1976]. Densitometric measurements of the relative amount of each protein band were performed in a Schimatsu UV-190 double beam spectrophotometer at 594 nm.

Estimation of *T. cruzi* HMG Molecular Weights

The apparent molecular weights of HMG proteins were determined by measuring their relative migration on SDS-PAGE gels as described by Weber and Osborn [1969]. Carbonic anhydrase (29 Kd), trypsinogen (24 Kd), β-lactoglobulin (18.4 Kd), lyzozyme (14.3 Kd), cytochrome c (12.4 Kd), and aprotinin (6.5 Kd), were used as molecular weight standards.

Preparation of Antiserum Against Calf Thymus HMG 1-2

Total HMG was isolated from calf thymus and the four major HMGs, HMG 1,2,14, and 17, were purified by chromatography in CM-Sephadex G-25 as described by Sanders [Sanders, 1977]. The fractions containing HMG 1-2 were pooled and used to immunize rabbits by following the procedure described by Bustin [Bustin et al., 1978]. The specificity of the serum obtained was assayed by dot blots using calf thymus HMG 1,2,14,17, and histones as antigens. A 1/50 dilution of serum was assayed against different amounts of proteins (5–30 μg of HMG and 100 μg of histones). To perform the dot blots, the nitrocellulose membranes containing the different proteins were incubated with the rabbit serum diluted 1/50 in TBS and then the procedure described previously was followed for western immunoblots [Imschenetzky, 1991].

Immunoblotting

Immunoblotting was carried out essentially following Towbin [1979]. Proteins were separated on SDS-polyacrylamide mini gels (7 × 6 × 0.1 cm) and transferred electrophoretically to nitrocellulose during 1.5 h. This condition is optimal for HMG chromosomal proteins though it was previously determined that 15 min are sufficient for transference of *T. cruzi* histones [Toro and Galanti, 1988]. The gels were overloaded with proteins. The nitrocellulose membranes were then blotted and afterwards incubated for 1.5 h at 37°C with anti-HMG 1-2 calf thymus rabbit serum. Detection of the antigen-antibody complexes was achieved by using the biotin-streptoavidin-peroxidase system as described previously [Imschenetzky et al., 1991].

DNA Labeling

T. cruzi epimastigotes (1.5×10^6) were preincubated for 20 min at 28°C in PBS, pH 7.2, and then were incubated for 1.0 h at 28°C with 10 μl of [3H]-methylthymidine [60 Ci/mM, New England Nuclear (NEN, Boston, MA). The cells were collected by centrifugation and washed with cold PBS. Afterwards, 1.0 ml of 5.0% (w/v) cold TCA was added and the precipitated material was washed twice in 5.0% TCA, once in 80% ethanol and once in ethanol/ether (3:1 v/v). Finally, the pellet was suspended in 1.0 N sodium hydroxide (NaOH) and the radioactivity associated to the TCA insoluble material was determined in a liquid scintillation counter Beckman model LSC.

RESULTS

Electrophoretic Comparison of Mammalian HMGs With HMG-Like Proteins Isolated From *T. cruzi*

The electrophoretic patterns in SDS polyacrylamide gels of the HMGs from calf thymus and those from *T. cruzi* epimastigotes (7 days of culture) that were isolated by following an identical experimental protocol are compared in Figure 1A. As shown, the calf thymus HMGs exhibit the typical pattern reported elsewhere [Godwin et al., 1973; Godwin et al., 1975; Sanders, 1977; Bustin et al., 1978], confirming that the procedure utilized to isolate the HMGs is adequate. Since histone H1 is often coextracted with HMG proteins in other cells, *T. cruzi* and calf thymus histones were separated in the same electrophoretic system (Fig. 1B). Five HMG-like

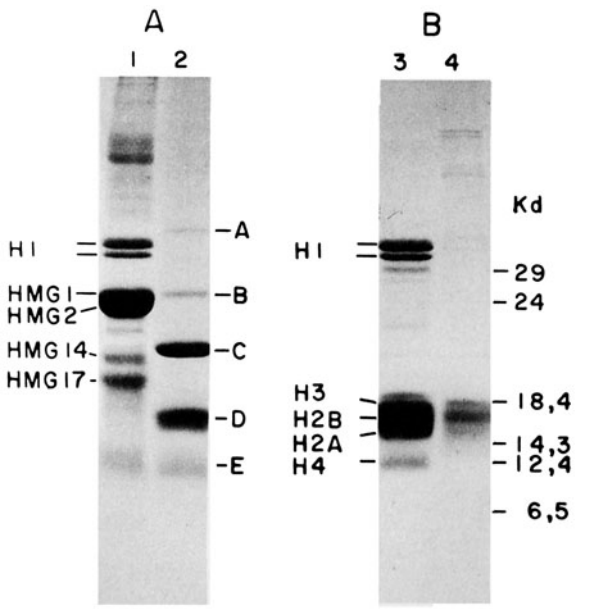

Fig. 1. HMG proteins analyzed by 15% polyacrylamide—0.1% SDS electrophoresis with 0.5 M urea and 0.005 M EDTA following Bhorjee [Bhorjee, 1981]. **A:** Lanes (1) Calf thymus HMG, (2) *T. cruzi* HMG-like proteins. **B:** Lanes (3) calf thymus histones, (4) *T. cruzi* histones. Molecular weights markers: carbonic anhydrase (29.000), trypsinogen (24.000), β-lactoglobulin (18.400), lyzozyme (14.300), cytochrome c (12.400), and aprotinin (6.500).

proteins are evident in *T. cruzi* (HMG-A, HMG-B, HMG-C, HMG-D, and HMG-E) (Fig. 1, lane 2). HMG-B protein presents a similar mobility to HMG 1, while HMG-C has approximately the same mobility as HMG 14. HMG-A protein migrates much slower than HMG 1 while HMG-D and -E migrate below HMG 17 from calf thymus.

From the measurement of the relative electrophoretic mobilities of *T. cruzi* HMG-like proteins, the apparent molecular weights estimated were A, 35.5 Kd; B, 27.5 Kd; C, 21.8 Kd; and E, 10.4 Kd. Such estimations were also performed for the isolated calf thymus HMG, and the results obtained were in close agreement with data previously published [Bustin et al., 1978].

Immunological Relatedness of *T. cruzi* HMG-Like Proteins and Mammalian HMG 1 and 2

The immune cross-reactivity between *T. cruzi* HMG-like proteins and calf thymus HMG 1 and 2 was determined by anti-calf HMG 1,2 sera that was elicited in rabbits for this purpose. The specificity of the sera obtained was determined by measuring its reactivity against HMGs purified from calf thymus. The profiles of elution from the chromatography on CM Sephadex of the four mammalian HMG and the electrophoretic analysis of these purified proteins are shown in Figure 2. The individual HMGs were then used to determine the specificity of the rabbit anti-HMG1,2 serum by dot blots. A positive reaction was observed for HMG 1 and 2 (Fig. 3A) and no cross reactivity was detected with HMG 14 and 17 or with histones (Fig. 3B).

This anti-calf HMG 1,2 serum was used to determine the immunological relatedness between mammalian HMG 1,2 and the HMG-like proteins isolated from *T. cruzi* by western immunoblots. As shown in Figure 4, the HMG-like proteins A and B cross-reacted with the serum anti-HMG 1,2, indicating that these two proteins are immunologically related to mammalian HMG 1 and 2. No cross reactivity was observed for HMG-like proteins C,D, and E, as well as for *T. cruzi* and calf thymus histones, confirming the results previously obtained by dot blots.

HMG-Like Chromosomal Protein Levels in Proliferating and Non-Proliferating Cells of *Trypanosoma Cruzi*

To investigate the relative amounts of HMG-like proteins from *T. cruzi* in a proliferating cells as compared to the non-proliferating cells, these proteins were isolated from *T. cruzi* epimastigotes harvested in the fifth day of culture (proliferating cells) and in the 14th day (non-proliferating cells). The proliferative activity was determined by counting the number of cells, and by [3H]-methylthymidine incorporation into DNA. The [3H]-methylthymidine incorporation into DNA in proliferating and non-proliferating cells was 42.229 cpm and 4.345 cpm in 1.5×10^6 cells, respectively. Clearly, HMG E is prominent in chromatin from cells in active proliferation while the same protein is hardly visible in non-proliferating cells (Fig. 5). Thus, the relative amounts of HMG C and E change dramatically, HMG-C being prominent in stationary cells and HMG-E showing prominence in proliferating cells, while HMG-D remains stable (Fig. 5).

DISCUSSION

The presence of four HMG-like chromosomal proteins named A,B,C, and E is described in the parasitic flagellate *Trypanosoma cruzi*. Their identification is based in their solubility properties, electrophoretic migration, molecular weights, and immunological relatedness with

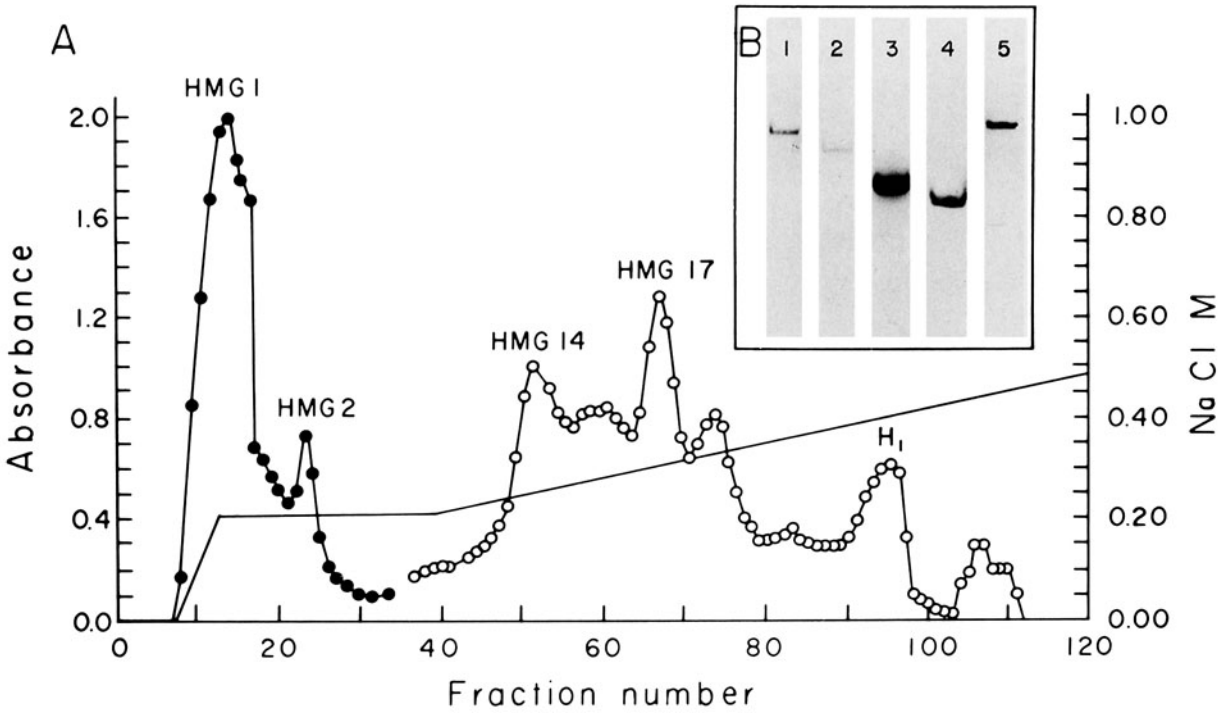

Fig. 2. A: CM-Sephadex chromatography of calf thymus HMG proteins. (●—●), absorbance at 280 nm; (○—○), absorbance at 230 nm; (—), NaCl (M). B: Electrophoretic analysis of the purified calf thymus HMG proteins (SDS-PAGE). Lanes: calf thymus proteins: (1) HMG-1, (2) HMG-2, (3) HMG-14, (4) HMG-17, and (5) histone H1.

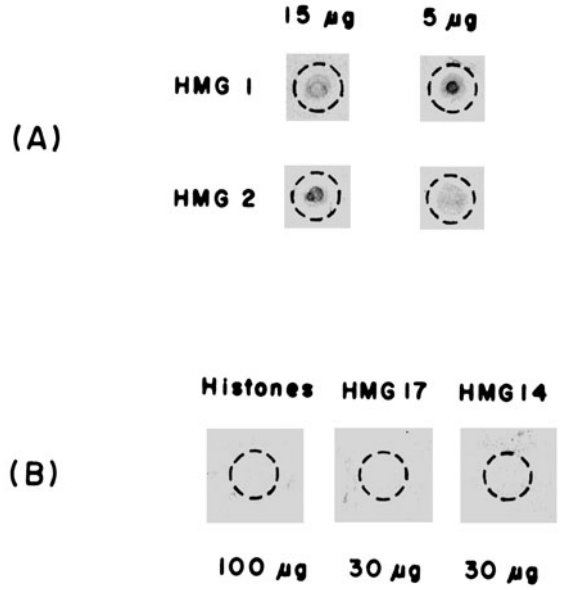

Fig. 3. Specific determinations by dot blots of the anti-HMG 1,2 rabbit serum. A: HMG 1 (15 µg, 5 µg); HMG 2 (15 µg, 5 µg). B: calf thymus histones (100 µg); HMG 17 (30 µg); HMG 14 (30 µg).

mammalian HMG 1 and 2. These results are consistent with those described previously for other protozoa such as *S. cereviciae* [Weber and Isenberg, 1980; Estruch et al., 1986], *T. termophyla* [Levy-Wilson et al., 1983], and *P. polycephalum* [Côte et al., 1982; Czupryn and Toczko, 1984; Côte et al., 1985]. In all these species, as well as in *T. cruzi*, the HMG-like proteins isolated are similar but not identical to HMG from vertebrates.

Taking together the electrophoretic similarities and the immune cross-reactivity observed between the HMG-like proteins A and B and HMG 1 and 2, it seems clear that these two pairs of proteins are very closely related, in fact, HMG-B comigrates with HMG 1. Obviously, a more precise assignment could only be achieved by the comparison of the sequences of these proteins, which are unknown thus far.

The immunological similarity between *T. cruzi* HMG-like C,D, and E and calf thymus HMG 14 and 17 should be clarified by the use of an antiserum directed against mammalian HMG 14 and 17 which was not available at present.

The relative amounts of two HMG-like proteins vary in *T. cruzi* in proliferating cells as compared to non-proliferating cells. HMG-like protein E is increased in proliferating cells and almost undetectable in resting cells, while HMG-like protein C is scarce in proliferating cells and significantly increases in the non-proliferating

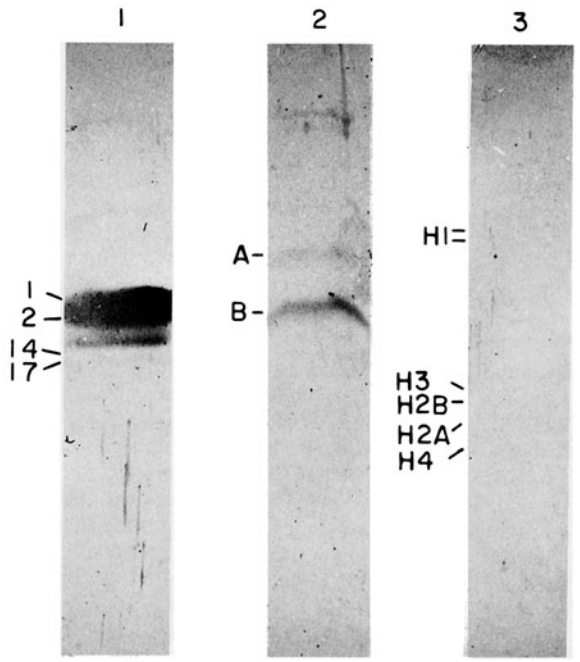

Fig. 4. Western blots of HMG-like chromosomal proteins from *T. cruzi*. Proteins were transferred electrophoretically to nitrocellulose paper and probed with a rabbit antiserum raised against calf thymus HMG-1 and -2. Electrophoretic separation of HMG and histones was as in Figure 1. Western blots immunoreactivity in nitrocellulose paper: lanes (1) calf thymus HMG, (2) *T. cruzi* HMG-like proteins, and (3) calf thymus histones.

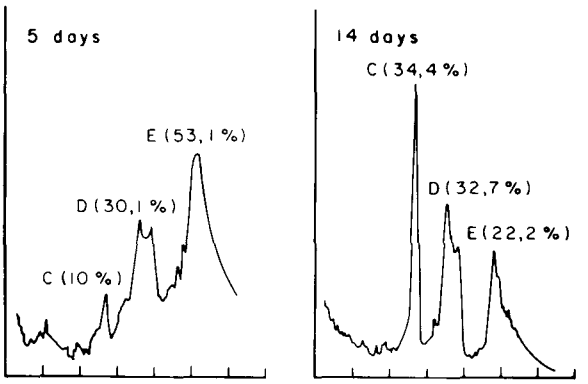

Fig. 5. Densitometric determinations of *T. cruzi* HMG-like proteins separated by SDS-PAGE. The cell cultures correspond to 5 and 14 days, respectively. The contribution of each peak (C,D, and E) to the total spectrum is given in percentage.

epimastigotes. These results are consistent with previous reports indicating a decrease of individual HMG proteins at the end of the proliferative phase of synchronized HeLa cells [Bhorjee, 1981], mouse neuroblastoma cells [Seyedin et al. 1981], and in processes leading to cell differentiation as spermatogenesis [Seyedin and Kistler, 1980; Kennedy and Davies, 1980; Bucci et al., 1984] and myogenesis of chicken skeletal muscle [Gordon et al., 1981].

Protein HMG-D was not considered as a genuine HMG protein because applying the Sanders method [Sanders, 1977] for H1 isolation from *T. cruzi* epimastigotes, we obtained a protein that co-migrates in SDS-polyacrylamide and urea-acetic acid gels with HMG-D (data not shown). Also, this protein does not cross-react with the serum against HMG proteins from calf thymus and does not vary in amount in relation to cell proliferation. This protein named HMG-D might correspond to an atypical H1 histone as was previously demonstrated in our laboratory [Toro and Galanti, 1988; Toro and Galanti, 1990].

The general molecular features of the HMG-proteins isolated from *T. cruzi* and the shifts observed in their relative amounts related with cell proliferation strongly suggest a possible role of these proteins in the proliferation of this parasitic flagellate as has been suggested previously for higher eukaryotes.

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