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The Biodiversity of the Basic Chromatin Proteins of Archaebacteria in China

WEN JIANFAN LI JINGYAN

Kunming Institute of Zoology, Academia Sinica, Kunming, Yunnan 650223, *China*.

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ABSTRACT

Using a novel acid extraction procedure, the acid-soluble proteins from a thermo-and acido-philic archaebacterium *Suifosphaerellus thermoacidophilum* were isolated, and analysised by SDS-PAGE and AUT-PAGE with the calf thymus histones, which were isolated by the same procedure, as a control. The neutral Azocarmin G staining technique, which specifically stains basic chromatin protein, was used. The results suggested that the archaebacterium owns three major basic chromatin proteins, the molecular weight of all the basic proteins was smaller than calf thymus histone H4 (MW 11 282). Different artchaebacterium possesses very different basic chromatin (histone-like) proteins. The biodiversity of these proteins was very rich. The significance of their biodiversity to the origin and evolution of histone was discussed.

Key wards archaebacteria, basic chromatin protein, biodiversity histone evlution

Archaebacteria are a group of prokaryotes, which are very different from another group of prokaryotes—eubacteria such as *E. coli*. Since the discovery of archaebacteria in the late 1970s, the growing evidences, especially of molecular biology, strongly suggests that they have much closer evolutionary relationship with eukaryotic cell than eubacteria do. The discovery of archaebacteria sheds new light on the mystery of the origin and evolutin of eukaryotic cell.

Almost every typic eukaryotic cell owns the basic chromatin protein —— histone. It is also clear that histone (H1, H2A, H2B, H3, H4) is very conserved among different eukaryotes, and the four core histones have a high homology to each other, they must come from a common ancestral protein (Reeck et al. 1978). But what is the ancestral protein and where to find it? Although a lot of work about this have been done on lower eukaryotes such as dinoflagellates and on prokaryotes eubacteria, no significant clue was found. While on archaebacteria this is not the case at all. Up to now, no common basic chromatin protein was discovered in them, they are different in different species. It is the difference (or biodiversity) that provides the possibility for us to find some evolutionary clue of histone in archaebacteria. In fact, some interesting results have been obtained, the HMf, HMt, HMv proteins, for instance, are three basic chromatin proteins discovered in three methanogenic archaebacteria, they have a very high homology to the four core histones (Sandman et al. 1990; Tabassum et al. 1992; Agha—Amiri and Klein 1993). In the present work, we have investigated the

basic chromatin proteins from an archae-bacterium strain, which is one of the two species reported in China.

1 MATERIALS AND METHODS

1.1 Materials

Sulfosphaerellus thermoacidophilum was isolated from an acid thermal spring in Yunnan province, and was characterized and cultured successfully by Zhong et al. (1982) and Li et al. (1988). The culture conditons were determined according to Zhong et al. (1982).

Fresh calf thymus was taken from a new-born calf.

1.2 Extraction of basic chromatin proteins from archaebacteria

The bacteria were harvested at the beginning of the stationary phase of growth by centrifugation (8, 000×g, 10 min). Basic proteins were extracted from about 0. 2g of the cells by the following procedure, which was a simple and effective method established in our laboratory. (1) The bacteria cells were prefixed by cold methanol at 4℃ for 40 min, then pelleted by centrifugation (8, 000×g, 10 min). (2) 0. 2ml of 0. 3N HCl solution was added, and the bacteria cells were ruptured by pestling in a mortar, then 4. 8ml 0. 3N HCl solution was added. The acid extraction was carried out at room temperature (20℃) for 30 min under gentle agitation. The extract was collected by centrifugation (10, 000×g, 5 min). (3) The acid extract was diluted by 8 times volume of cold acetone and kept at -10℃ overnight. (4) The precipitated protein in acetone was pelleted by centrifugation (8, 000×g, 5 min) and 5 ml of 0. 3N HCl solution were added to solve the the pellet again. The acid-insoluble substance was discarded after high speed centrifugation (18, 000×g, 10 min), the supernatant was collected and the step (3) was repeated. Finally the precipited protein was washed 3 times with cold acetone and then lyophilized.

1.3 Extraction of histone from calf thymus

0. 2g of fresh calf thymus was taken and washed with 0. 9% N_aCl solution. Histone was extracted by the same procedure described above, except that the tissue was sectioned into small pieces before prefixatin and sheared into meat paste before acid extraction.

1.4 Electrophoretic analysis

SDS-PAGE was performed as described by Laemmli (1970) in gels of linear gradient concentration of 15～27% acrylamide. Loading buffer contained 4% SDS. After electrophoresis, the gel were cut into two parts (both had been loaded the same samples symmetrically). One was stained with Coomassie brilliant blue R250 and destained with 7% acetic acid; the other one, after fixation with 10% TCA, was put into an acetic acid-methanol-water (3: 3: 4) solution containing 0. 5% Triton X-100 to release SDS overnight, washed with distilled water until the gel was neutral, stained in 0. 1% Azocarmin G staining solution (pH 7) for 2 hours and destained with 15% methanol.

AUT-PAGE was carried out according to Vernet et al. (1990). Slab gels containing 6. 25M urea, 8mM Triton X－100 and 18% Acr/0. 2% Bis were made. Loading buffer contained 8mM urea, 4% sucroes, 0. 1% 2-mercaptoethanol. The tray buffer was 0. 9N acetic acid solution (pH 3) containing 2. 5M urea and 0. 4M glycine. Gels were stained with Coomassie brilliant blue R250.

Densitometry scans were performed on a LKB 2202 Ultrascan Laser Densitometer in conjunction with a LKB 2220 Recording Integrator.

2 RESULTS

2.1 SDS-PAGD analysis

Calf thymus histone has five fractions (H1, H2A, H2B, H3, H4). The electrophoric patterns stained with Coomassie brilliant blue was shown in Fig. la. Lane A shows six bands which correspond to the five fractins (H1 has two sub-bands), no fraction was lost and no nonhistone contaminant appeared. This suggested that the acid extraction procedure used was effective for isolating basic chromatin proteins. The basic proteins extracted from *S. thermoacidophilum* by this procedure gave seven bands in Fig la (lane B). But only three bands (No. 5, 6, 7), which migrated faster than calf thyums histone H4, were the major bands. The protein molecular weight of them was samller than H4 (MW 11 282). According to the staining shade, band 5 has the highest protein content followed by band 7 and band 6.

Fig 1b. was the densitometry scans patterns of the gel of Fig la. It showed the same results more directly and clearly.

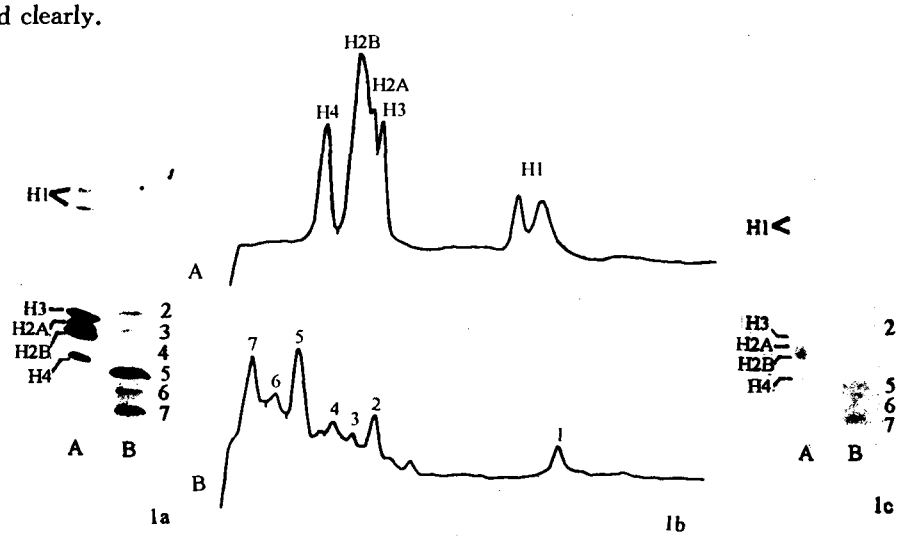

Fig la. SDS-PAGE patterns of basic proteins from *S. thermoacidophilum* (lane B) with calf thymus histone (lane A) as a control. The gel was stained with Coomassie brilliant blue R250.

Fig 1b. Laser densitometry scans patters of the gel of Fig la.

Fig 1c. The same to Fig la. except the gel was stained with Azocarmin G (pH 7).

At pH 7, Azocarmin G staining solution stained specially proteins of strong basicity such as basic chromatin proteins (Li et al. 1978). Fig 1c. showed the results of the staining. All the five histones were stained (lane A), while only four bands of the basic proteins from the bacterium were stained, they corresponded to the band 2, 5, 6, 7 in Fig la. But the staining shade grade was different, band 7 was stained deepest followed by band 5 and band 6, and band 2 was stained very slightly. This meant that there were mainly three proteins of strong basicity (band 5, 6, 7) and band 7 was the protein

possessing the strongest basicity.

2.2 AUT-PAGE analysis

The AUT-PAGE patterns was shown on Fig. 2a. According to the patterns of calf thymus his tones in the same electrophoric system (Vernet et al. 1990), The sites of the five histone fractions were determined (Fig 2a. lane A). Lane A also showed the complete five histone fractions without remarkable contaminants or loss of any histone fraction. The basic proteins of the bacterium gave six bands (No. 1', 2', 3', 4', 5', 6') in the gel.

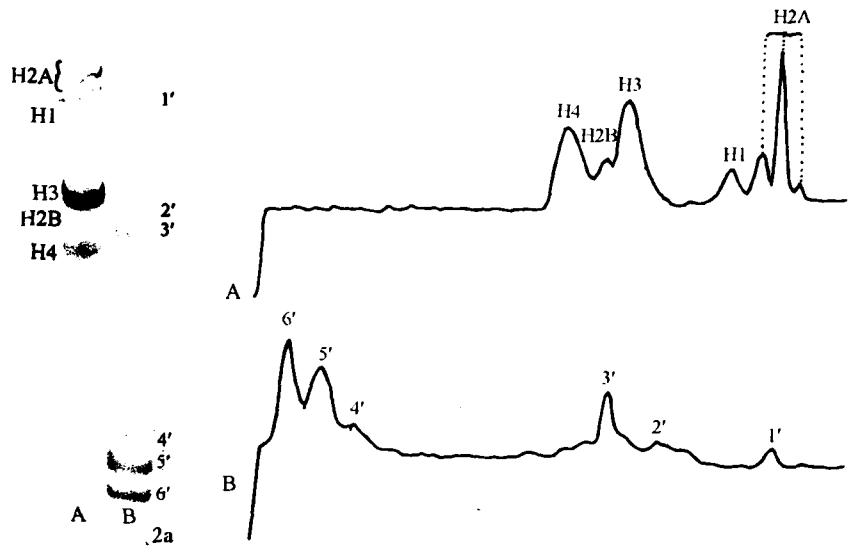

Fig 2a. AUT-PAGE patterns of basic proteins from *S. thermoacidophilum* (lane B) with calf thymus histone (lane A) as a control. The gel was stained with Coomassie brilliant blue R250.

Fig 2b. Laser densitometry patterns of the gel of Fig 2a.

As in Fig la., only three bands (No. 3', 5', 6') were the major bands, their protein contents were much higher than those of other bands, but the mobility changed. Two bands (5', 6') migrated faster than calf thymus histone H4, the mobility of band 2' was between H2B and H4. The densitometry scans patterns of the gel of Fig la. was shsown on Fig. 2b., it showed the same results more clearly.

3 DISCUSSION

Owing to the previous methanol fixation, the acid extraction procedure used in the present work had the following advantages: (1) the methanol fixation deactivated all endogenous proteolytic enzymes, hydrolysis of protein was avoided. This is especially important to work on lower organisms because protesase inhibitors which were used in the conventional extraction procedure for isolating histones from higher organism were uncertainly effective for lowder organism. (2) neutral and even slightly basic proteins, which were originally acid-soluble, became acid-insolube after fixation, only proteins of strong basicity could be extracted by eluted acid after the fixation. This led to the improvement of purity of the isolated protein. The above results showed that calf thymus histone was

extracted succesfully without remarkable contamination of nonhistone or loss of any histone fraction. This suggested that the procedure was effective for isolating basic chromatin ptotein.

When this procedure was applied to *S. thermoacidophilum*, both SDS-PAGE and AUT-PAGE analyses showed that three major fractions were obtained. According to the following aspects: (1) the reliable extraction procedure; (2) the much higher contents of the three proteins than other basic proteins extracted (generally, in a cell basic chromatin protein has the most content among proteins of strong basicity.); (3) the strong basicity of the three proteins (they are still acid-soluble after fixation and can be stained by neutral Azocarmin G solution); (4) the small molecular weight of the three proteins (reportedly, the basic chromatin proteins of other archaebacteria all own a small molecular weight which are generally samller than histone H4 (MW 11 282)). We suggested that the three major proteins were the basic chromatin proteins of *S. thermoacidophilum*.

All typic eukaryotic cells owned the famous conserved basic chromatin protein —— histone. A common chromatin basic protein —— HUprotein also could be found in all eubacteria. However, in archaebacteria the basic chromatin proteins showed a rich biodiversity. They were very different in different species. For example, in *Thermoplasma* the HTa protein was discovered; while in *Sulfolobus* another cluster of proteins was reported. All these proteins were very different from each other (Li 1990). Interestingly, recent researches reported that three basic chromatin proteins HMf, HMt, HMv were discovered respectively in three species of methanogenic archaebacteria, both HMf and HMt had differentiated into two polypeptieds HMf-1, HMf-2 and HMt-1, HMt-2, all these proteins had very remarkable homology to each other and to the four core histones of eukaryote (Sandman et al. 1990; Tabassum et al. 1992; Agha-Amiri and Klein 1993). We suggested that the rich biodiversity of basic chromatin protein of ancient archaebacteria might provide materials for the evolution, one of these proteins had evolved into the histone of eukaryote, another one had evolved into HUprotein of eubacteria. While in the archaebacteria existing today, the biodiversity of these proteins is still retained.

In this papar, we reported three major basic chromatin proteins in *S. thermoacidophilum*. The homological relationship between them and the previous reported basic chromatin proteins of other archaebacteria and histone is under studying. The preliminary results implied that one of them had very close relation with HTa —— a basic chromatin protein from archaebacterium *Thermoplasma acidophilum*.

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