A Histone-like Protein (HTa) from *Thermoplasma acidophilum*

I. PURIFICATION AND PROPERTIES*

Robert J. DeLange
From the Department of Biological Chemistry, School of Medicine, and the Molecular Biology Institute, University of California, Los Angeles, California 90024

George R. Green and Dennis G. Searcy
From the Zoology Department, University of Massachusetts, Amherst, Massachusetts 01003

A histone-like protein (HTa) has been isolated from cell extracts of *Thermoplasma acidophilum* by column chromatography on DNA-cellulose, hydroxylapatite, and Sephadex G-75. HTa elutes from DNA-cellulose in two fractions, one of which contains an 89-residue form of the protein with an NH₂-terminal sequence of Val-Gly. The other fraction apparently contains the 89-residue species, in addition to a 90-residue form of the protein with the NH₂-terminal sequence Met-Val. The sequence of 47 residues from the NH₂ terminus of the 89-residue protein was established by automated Edman degradation.

HTa is characterized by the following properties: 22% of its residues are lysine and arginine; the lysine:arginine ratio is 2.33; the absorption spectrum of the protein is distinctive due to the lack of tryptophan and the presence of 1 tyrosine and 5 phenylalanine residues; and the protein stabilizes DNA against thermal denaturation (Stein, D. B., and Searcy, D. G. (1978) Science 202, 219-221) and condenses DNA into spherical particles. All of these characteristics indicate that HTa resembles eukaryotic histones, but there are distinctive differences.

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*Thermoplasma acidophilum* is a prokaryotic organism that grows best at 59°C and pH 1 to 2 (1). It has been isolated only from spontaneously burning refuse piles associated with coal mines (2). Since it is without a cell wall, it has been classified with the mycoplasmas. However, on the basis of both its 16 S ribosomal RNA (3) and its unusual membrane lipids (4), it is not closely related to other typical mycoplasmas and should be classified with the archaebacteria (3).

One of the adaptations helping *T. acidophilum* to survive in its extreme environment is a small histone-like protein (HTa) that is tightly bound to its DNA (5). Like eukaryotic histones, HTa can stabilize DNA against thermal denaturation by up to 38°C. This stabilization has been shown to be important under conditions that can occur physiologically, and is probably the true function of the protein (6).

The HTa protein of *T. acidophilum* resembles eukaryotic histones in also condensing DNA into spherical particles, although these particles are smaller and simpler than eukaryotic nucleosomes (7). In *T. acidophilum*, each nucleoprotein particle is composed of a loop of 40 base pairs of DNA around 4 molecules of the protein. This compares to about 146 base pairs around 8 histone molecules (four different histones) in each eukaryotic nucleosome (8).

There are also several other features of *T. acidophilum* that are suggestive of eukaryotic cells (9, 10). For example, the organism has no cell wall, but like animal cells, its shape appears to be influenced by an internal cytoskeleton. Cooling causes the cells to lose their irregular shapes and become spherical; in addition, growth of *T. acidophilum* is inhibited by cytochalasin B. It has also been possible to prepare extracts from *T. acidophilum* that undergo gelation and contraction (10). Filaments 6 nm in diameter (the same size as actin) can be seen in these gels. However, the filaments did not bind heavy meromyosin from rabbit muscle,¹ and so the extent of their homology to eukaryotic actin remains uncertain.

Another similarity to the cytoplasm of eukaryotic cells is the respiratory metabolism of *T. acidophilum*. Most of the cellular respiration can be attributed to soluble cytoplasmic enzymes that resemble those of the microbodies in eukaryotic cells (9). Oxidative phosphorylation is apparently absent from *T. acidophilum*.²

In addition, certain other eukaryotic features have been associated with other archaebacteria (11), but have not yet been specifically examined in *T. acidophilum*. For example, in archaebacteria, the 5' terminal nucleotide of the initiator tRNA is base-paired and carries unformylated methionine, in contrast to the initiator tRNA of typical euabacteria. Also, protein synthesis in archaebacteria is sensitive to certain inhibitors of protein synthesis that were characteristically thought to be eukaryote-specific.

Thus, there is reason to suspect an evolutionary connection between *T. acidophilum* and the ancestor of the nucleus and cytoplasm of eukaryotic cells. One way to test this hypothesis is to look for sequence homology in certain highly conserved proteins. In this regard, histones are among the most slowly evolving of all known proteins (12, 13). Therefore, for its evolutionary interest, as well as to better understand its interaction with DNA, we have isolated and characterized the HTa protein of *T. acidophilum* (this paper) and determined its amino acid sequence (the accompanying article, Ref. 14).

MATERIALS AND METHODS

*Culture—* *T. acidophilum* (strain 122-182, ATCC 25906) was cultured and stored frozen as described previously (5).

Isolation of the Histone-like Protein—Frozen cells (10 g) were

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¹ J. Pustell and D. G. Searcy, unpublished observation.
² D. G. Searcy and F. R. Whatley, manuscript in preparation.
suspended in 50 ml of 2 M NaCl, 0.05 M EDTA, pH 7, to lyse the cells. After centrifugation at 20,000 × g for 15 min, the supernatant solution was collected. PEG 6000 (polyethylene glycol) in 2 M NaCl was added to a final concentration of 10% (w/v) polyethylene glycol. The mixture was stirred at 4°C for 30 min and then centrifuged at 10,000 × g for 15 min. The supernatant solution, now free of DNA (15), was collected, diluted with an equal volume of water, and mixed with 10 g of DNA-cellulose that had been prepared according to Litman (16), and contained about 10 mg of native calf thymus DNA per g of cellulose. The combined slurry of extract plus DNA-cellulose was diluted slowly to a final concentration of 0.5 M NaCl, stirred at 25°C for 10 min, allowed to settle, and then washed by decantation twice with 0.5 M NaCl in 10 mM sodium phosphate, 1 M EDTA buffer, pH 6.8. The DNA-cellulose was packed into a chromatography column, washed again with the 0.5 M NaCl-phosphate/EDTA buffer, and then eluted at a flow rate of 200 ml/h with a gradient of 0.5 to 2.0 M NaCl in the same buffer. The elution was monitored continuously at 220 nm (1-cm light path), the HTa protein being located in the absorbance peaks eluting at 0.7 to 0.8 M NaCl (Fig. 1). The HTa protein was precipitated by dialysis against several changes of 0.05 M NaCl in 0.05 M NaCl in 1.0× phosphate buffer, pH 6.8, and applied to chromatography columns, each containing 5 ml of DNA-cellulose that had been prepared according to Litman (16), and contained about 10 mg of native calf thymus DNA per g of cellulose. The combined slurry of extract plus DNA-cellulose was collected. After centrifugation at 20,000 × g for 15 min, the supernatant solution, now free of DNA (15), was collected, diluted with an equal volume of water, and mixed with 10 g of DNA-cellulose that had been prepared according to Litman (16), and contained about 10 mg of native calf thymus DNA per g of cellulose. The combined slurry of extract plus DNA-cellulose was diluted slowly to a final concentration of 0.5 M NaCl, stirred at 25°C for 10 min, allowed to settle, and then washed by decantation twice with 0.5 M NaCl in 10 mM sodium phosphate, 1 M EDTA buffer, pH 6.8. The DNA-cellulose was packed into a chromatography column, washed again with the 0.5 M NaCl-phosphate/EDTA buffer, and then eluted at a flow rate of 200 ml/h with a gradient of 0.5 to 2.0 M NaCl in the same buffer. The elution was monitored continuously at 220 nm (1-cm light path), the HTa protein being located in the absorbance peaks eluting at 0.7 to 0.8 M NaCl (Fig. 1). The HTa protein was precipitated by dialysis against several changes of 0.05 M H2SO4, 70% ethanol.

For further purification, the proteins in Fractions A and B (Fig. 1) were dissolved separately in 5 mM urea, 10 mM sodium phosphate, pH 6.8, and applied to chromatography columns, each containing 5 ml of hydroxyapatite (Bio-Rad) that was eluted with 100 ml of a salt gradient of 0 to 1.0 M NaCl in 10 mM phosphate buffer, pH 6.8 (no urea). The HTa protein eluted at about midway in the gradient and was precipitated by dialysis against ethanol/sulfuric acid as before. The final step of purification was chromatography in 10 mM HCl on Sephadex G-75 as described previously (5).

Amino Acid Analysis—Samples of protein (about 1 mg each) were hydrolyzed for 24 h at 110°C in sealed, evacuated tubes using 1 ml of glass-distilled, constant-boiling HCl (5.7 N) and 1 drop of 5% phenol. The hydrolysates were taken to dryness over solid NaOH in a heated vacuum disiccator at 40-50°C and were analyzed using a Beckman program 030176 with a Beckman 890C Sequencer. The thiazolinone derivatives of residues N-terminal to Val bonds in the protein (see Table I) are only partially hydrolyzed and the other half are apparently the same as Fraction B (no methionine).

Manual Edman Degradation—Samples of the proteins (35 nmol each) were subjected to Edman degradation according to the method of Peterson et al. (18). The thiazolinone derivatives of residues removed were hydrolyzed for 16 h at 150°C in 0.5 ml of 5.7 N HCl containing 1 drop of 5% phenol. An aliquot (10%) of each hydrolysate was examined by high voltage electrophoresis at pH 1.9, and the remainder was analyzed as indicated above.

Automated Edman Degradation—Samples of the protein (270 nmol) were subjected to automatic Edman degradation using Beckman program 06901 with a Beckman 890C Sequencer. The thiazolinone derivatives were converted as described by Edman and Begg (19) to the phenylthiohydantoin derivatives that were then identified by gas-liquid chromatography with a Beckman G-65 instrument equipped with columns containing 10% DC-560 (Alltech). Residues that could not be found by other specific methods.

Amino Acid Composition of HTa—Samples of protein (1 mg each) were hydrolyzed for 24 h. No tryptophan or cysteine could be found by other specific methods.

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Amino Acid Composition of HTa—Samples of protein (1 mg each) were hydrolyzed for 24 h. No tryptophan or cysteine could be found by other specific methods.
not be identified by this method were hydrolyzed for 4 h in 0.5 ml of 5.7 N HCl containing SnCl₄ according to the method of Mender and Lai (20) and analyzed to determine whether serine or threonine had been removed. Some of the other phenylthiodyantoinas were hydrolyzed for 16 h at 150°C and analyzed to confirm the results obtained by gas-liquid chromatography. Aliquots of the aqueous layer (1 N HCl) of each sample were also checked by the phenanthroquinone method (21) to determine whether arginine had been removed.

Hydrolysis of HTa with Carboxypeptidases—HTa (36 nmol) was hydrolyzed first with carboxypeptidase A (20 μg, Worthington) in 0.2 ml of 0.2 μM NH₄HCO₃, pH 8.0 at 40°C for 30 min, after which 20 μl were removed and added to 1 drop of 30% acetic acid. To the remainder of the mixture were added 20 μg of carboxypeptidase B (Worthington), and the mixture was incubated for 2.5 h, after which the digestion was stopped by the addition of 0.2 ml of 30% acetic acid. After both samples had been taken to dryness over P₂O₅ in a vacuum desiccator, all of the carboxypeptidase A hydrolysate, 10% of the carboxypeptidase A + B hydrolysate, and amino acid standards were subjected to high voltage paper electrophoresis at pH 1.9 (22). The paper was oven-dried at 60°C, dipped in ninhydrin stain (0.1% ninhydrin in 95% ethanol/collidine 95:5, v/v), and colors were developed in an oven at 60°C. The remainder of the carboxypeptidase A + B hydrolysate was analyzed with a Beckman 121 analyzer.

RESULTS

Purification of HTa—HTa protein eluted from the DNA-cellulose column in two peaks (Peaks A and B) at about 0.7 to 0.8 M NaCl in the gradient as shown in Fig. 1. When this material was analyzed by gel-exclusion chromatography in the same buffer in which it eluted from DNA-cellulose, it had an apparent molecular weight of 35,000 to 36,000. If this figure is compared to a monomeric molecular weight of 9,934 (calculated from Table I), then it appears that HTa elutes from the DNA as a tetramer. The yield of HTa in Fractions A plus B was about 50%, and the protein appeared to be about 95% homogeneous as judged by gel electrophoresis in sodium dodecyl sulfate (Fig. 1). The protein in each peak was further purified to apparent homogeneity as described under “Materials and Methods.”

Amino Acid Analysis—The compositions of the proteins that were further purified from Fractions A and B (Fig. 1; see above) are given in Table I. The major detectable difference in the compositions of the two fractions was the presence of about 0.5 residue of methionine/mol of protein in Peak A, but not in Peak B. In all other respects, Fractions A and B appeared to contain the same protein (see below).

Absorption Spectrum of HTa—HTa is unusual in that, due to the absence of tryptophan and the low content of tyrosine (Table I), the phenylalanine residues make a major contribution to the absorption spectrum (Fig. 2). Phenylalanine has 3

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**Table II**

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<sup>a</sup> The phenylthiodyantoinas of these residues were identified by gas-liquid chromatography.

<sup>b</sup> Based on 270 nmol of protein.

<sup>c</sup> Identified on a Beckman 121 analyzer after acid hydrolysis of the phenylthiodyantoinas. SnCl₄ was present during the hydrolysis for those indicated by Footnotes d and e.

<sup>d</sup> Alanine is a major product when the phenylthiodyantoin of serine is acid-hydrolyzed in the presence of SnCl₄.

<sup>e</sup> α-Phenylthiodyantoin (α-ABA) is a major product when the phenylthiodyantoin of threonine is acid-hydrolyzed in the presence of SnCl₄.

<sup>f</sup> This fraction gave a positive test for arginine with the phenanthroquinone reagent.

<sup>g</sup> This residue was later found to be glutamine (14).
several weak absorption maxima in the 240 to 270 nm region (23), but normally these are not observed due to the relatively stronger absorption of tyrosine and tryptophan in this region.

*Manual Edman Degradation of HTa*- The proteins purified from Fractions A and B (Fig. 1) were subjected to two steps of the manual Edman degradation with the results given in Table I. The protein in Fraction A gave 2 different residues at each step, whereas the protein in Fraction B gave only 1 residue at each step. One of the residues found at each step of the Edman degradation on Fraction A was the same as found for that step on Fraction B (valine at Step 1, glycine at Step 2). The other residues found for Fraction A (methionine at Step 1, valine at Step 2) strongly suggest that Fraction A contains two forms of HTa:

Met-Val-Gly-

Val-Gly-

with the second form probably being the same as the one found in Fraction B.

In different preparations of the protein, the relative amounts of the two forms of the protein were variable. *Automated Edman Degradation of HTa*- Enough of the 89-residue form (NH\textsubscript{2}-terminal Val-Gly-) of the protein (Fraction B) was obtained to make two successive automated Edman degradations for 47 steps. The results are given in Table II and provide a continuous sequence for the first 47 residues of the molecule. Of the 4 serines in this region of the sequence, only 1 (residue 4) was identified as the phenylthiohydantoin. The other 3 phenylthiohydantoins had undergone secondary reactions (as is often found) and were assigned as serine residues by the identification of alanine as the product of acid hydrolysis in the presence of SnCl\textsubscript{2} (20). The 3 threonine residues were identified both as the phenylthiohydantoins and as \( \epsilon \)-amino butyric acid, the product of acid hydrolysis in the presence of SnCl\textsubscript{2} (20). Of the 5 lysines, 2 (residues 26 and 41) could be identified only after acid hydrolysis, as was also the case for glycine residue 46.

*Hydrolysis of HTa with Carboxypeptidases*- As judged by electrophoresis at pH 1.3, carboxypeptidase A released only glutamine and tyrosine from HTa. Hydrolysis of HTa with both carboxypeptidases A and B for 3 h released the following residues: Gln (1.25), Lys (0.90), Tyr (0.43), Ile (0.44), Ser (0.31), Ala (0.13), and Arg (0.12). These results suggest the presence of more than 1 residue of glutamine (and probably more than 1 residue of lysine) near the COOH terminus, in accord with the sequence studies (14).

**DISCUSSION**

The results described above show that a lysine-rich, histone-like protein can be isolated from *T. acidophilum* in two forms; these contain 89 and 90 residues respectively, and seem to differ only by the presence or absence of an NH\textsubscript{2}-terminal methionine residue. Since the protein, as it eluted from DNA-cellulose, was apparently tetrameric, and in Peak A it contained an average of only 0.5 residue of methionine/mol of protein, it appears likely that each tetramer in this fraction was a heterotypic complex of the two forms of HTa. In contrast, in Peak B, the protein eluted as a homotypic tetramer entirely lacking methionine.

The NH\textsubscript{2}-terminal methionyl residue present in some of the molecules of HTa might be the initiator residue for protein synthesis. In this case, the 89-residue form of HTa should be a product derived from the 90-residue form by proteolytic processing. In support of this interpretation, Fraction A, which contained the NH\textsubscript{2}-terminal methionine, has been shown to disappear from older stationary phase cultures (data not shown). Thus, it appears that in *T. acidophilum*, as in other archaeobacteria (11), protein synthesis may be initiated with unformylated methionine.

From the studies on HTa described previously (5, 6, 9) and those described above, it is clear that HTa resembles eukaryotic histones in several respects. It is a small protein with 22% of its residues being basic, compared to 23 to 26% for the four core histones (12). The lysine:arginine ratio of HTa is 2.3 (Table I), which is similar to the ratio of 2.5 for calf histone 2B (12). HTa lacks tryptophan, cysteine (or cystine), and histidine, and the 89-residue form lacks methionine. No eukaryotic histone has been reported to contain tryptophan, and four of the five major histones lack cysteine (or cystine) in most species (12).

HTa differs from eukaryotic histones (8, 12, 13) in several ways. HTa (89 or 90 residues) is smaller than the smallest eukaryotic histone, H4, and contains 5 phenylalanine residues (Table I), compared to 1 to 2 for most eukaryotic histones (histone 3 has 4 residues of phenylalanine). This high phenylalanine content, coupled with the absence of tryptophan and the presence of only 1 residue of tyrosine, gives HTa a very characteristic absorption spectrum (Fig. 2). The first 45 residues of HTa contain only 1 basic residue cluster (2 residues) and have a net charge of +3 (Table II). In contrast, the NH\textsubscript{2}-terminal region of histones 2A, 2B, 3, and 4 are highly basic (net charge = +16 for H4) and contain several basic residue clusters (up to 5 residues). Whereas histones are thought to have specific roles in the regulation of gene expression, there is no evidence that HTa functions in this manner (6). This is possibly why we found no evidence for the post-synthetic modifications that are found in the eukaryotic histones (12, 13). The primary function of HTa appears to be in stabilizing the DNA against thermal denaturation, and it apparently accomplishes this in the form of a simple tetrameric complex. This is in contrast to eukaryotic chromatin, where several different types of histone are combined to create each nucleosome particle.

In the accompanying article (14), we present the studies that led to the elucidation of the complete amino acid sequence of HTa and its comparison with the sequences of histones and other DNA-binding proteins.

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**References**

Histone-like Protein (HTa) from T. acidophilum. I