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Maize Histone H1: A Partial Structural Characterization†

Carolyn Katovich Hurley* and John T. Stout

ABSTRACT: The first H1 histone from a plant, *Zea mays*, has been characterized and partially sequenced. The maize H1 molecule shares many sequence features with rabbit and sea urchin H1 and chicken H5. The central hydrophobic region of the protein exhibits sequence microheterogeneity, indicating the presence of multiple H1 proteins in maize. The cause of the genetically controlled electrophoretic variation in the major H1 subfraction of maize has been localized to the carboxy-terminal region of the molecule.

Histone H1 plays an important role in the organization of nucleosomes into higher order structures (Baldwin et al., 1975; Finch & Klug, 1976; Müller et al., 1978). The existence of variability in the H1 fraction of animals and plants suggests that this role may be regulatory in addition to structural. Even the H1 fraction from a single tissue consists of several subfractions which differ slightly in molecular weight and amino acid sequence (Panyim & Chalkley, 1971; Kinkade & Cole, 1966); the number, amount, and species of H1 subfractions vary from tissue to tissue within an organism (Bustin & Cole, 1968) and during development (Ruderman et al., 1974). Intraspecific variation (maize) (Stout & Phillips, 1973) and interspecific variation (Panyim et al., 1971; Spiker, 1975) also exist. These observations suggest that the several species of H1 have specific roles in gene control. Such control could be the result of differences in the primary structures of the several H1 subfractions. This paper examines the extent of H1 variation and presents the first detailed structural analysis of the H1 histones from a plant. The paper compares the subfractions of maize H1 to each other and to animal H1. It also localizes the cause of the genetically controlled electrophoretic variation of maize H1a observed by Stout & Phillips (1973) to the carboxy-terminal region of the H1a molecule.

Materials and Methods

Chromatin and histone were isolated from immature tassels of *Zea mays* as described previously (Stout & Hurley, 1977; Stout & Phillips, 1973). One gram of total histone, preincubated for 1 h at room temperature in 7 M guanidine hydrochloride and 0.1 M Tris base to decrease protein aggregation, was fractionated on a 5 × 270 cm Bio-Gel P100 column in 0.01 N HCl and 1 mM sodium azide (Sommer & Chalkley, 1974).

Amino acid compositions were determined from 24-h 4 N methanesulfonic acid and 6 N HCl hydrolysates (Simpson et al., 1976; Gibson et al., 1971) by using a Durrum D500 amino acid analyzer.

The preparative steps were assessed by electrophoresis in acetic acid, 6.25 M urea, and 15% polyacrylamide slab gels (Hurley, 1977). Size characterizations were carried out on 15 and 20% Laemmli (1970) sodium dodecyl sulfate (NaDodSO$_4$) gels by using calf thymus histones as molecular weight standards.

Cyanogen bromide (CNBr[1]) cleavages were performed by adding the solid CNBr to an equal weight of protein at a

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* Address correspondence to this author at the Department of Microbiology, University of Texas Health Science Center, Dallas, TX 75235.

[1] Abbreviations used: CNBr, cyanogen bromide; NBS, *N*-bromosuccinimide.

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concentration of 10 mg of protein per mL of 70% formic acid. The reaction mixture was incubated at room temperature for 24 h, diluted 10-fold with H_2O, and lyophilized. The CNBr fragments were separated on a Sephadex G-75 column in 0.01 N HCl and 1 mM sodium azide. An aliquot containing the major CNBr peptides was radiolabeled with ^{125}I by the chloramine-T method (Greenwood et al., 1963). The tyrosines labeled by this procedure were detected during Edman degradation by monitoring the radioactivity in aliquots of the butyl chloride fractions.

N-Bromosuccinimide (NBS) cleavages were performed by adding NBS to a 1 mg/mL solution of H1 in 50% acetic acid to give a final concentration of 0.09 mg of NBS per mg of protein. The reaction mixture was incubated at room temperature for 2 h; an equal amount of fresh NBS was added and the incubation continued for two additional hours. The mixture was diluted 10-fold with H_2O and lyophilized. The peptides were preincubated in 7 M guanidine hydrochloride and 0.1 M Tris solution as described above and chromatographed on a Sephadex G-100 column in 0.01 N HCl and 1 mM sodium azide.

Chymotryptic cleavages were performed by adding the enzyme, dissolved in 1 mM HCl, to protein (1 mg of protein per 0.3 mL of 0.1 M ammonium bicarbonate) to a concentration of 1:5000 enzyme/substrate by weight. The digestion mixture was incubated at room temperature for 1 h, followed by acidification and lyophilization.

Proteins and peptides were sequenced in an Edman–Begg sequenator (Edman & Begg, 1967) (Illinois Tool Works, Illitron Division, Chicago, IL) along with 1 mg of Braunitzer's reagent III (Braunitzer et al., 1971) and a radiolabeled internal standard (McKean & Smith, 1974). Thiazolinones were identified by amino acid analysis after back hydrolysis with either HI or NaOH plus sodium dithionite (Smithies et al., 1971), using a Durrum D500 amino acid analyzer. The data were corrected for background and out-of-step degradations (Smithies et al., 1971). Portions of the thiazolinones were also converted to phenylthiohydantoins (PTH's) by heating at 80 °C for 10 min in 1 N HCl. PTH derivatives were identified by thin-layer chromatography on silica gel plates with consecutive development in chloroform and chloroform–methanol (9:1) in the same dimension.

The Sankoff (1972) modification of the Needleman & Wunsch (1970) computer program was used to detect protein homologies.

Results

Gel Electrophoresis. Maize and vertebrate histones are compared in an acetic acid–urea polyacrylamide slab gel in Figure 1. Histones H3 and H4 show similar electrophoretic mobilities in both species. The H2 fractions of maize migrate as several bands and exhibit lower electrophoretic mobilities than the H2 fractions of calf. Maize H1 is also electrophoretically slower than its vertebrate counterpart and consists of several subfractions which vary slightly in mobility between inbred lines of maize. The major H1 subfraction, H1a, carries an electrophoretic variation, inherited as if controlled by a single Mendelian locus, which was examined in an earlier paper (Stout & Phillips, 1973). The alleles which control the variation have been mapped to a locus on chromosome 1 (Stout & Kermicle, 1979).

Molecular Weight. The H1 fraction of maize was isolated from total histone by P100 chromatography and its molecular weight determined by $NaDodSO_4$ gel electrophoresis. The H1 fraction exhibits three to four major subfractions in $NaDodSO_4$ gels. The apparent molecular weights of these subfractions, which vary slightly depending on the inbred lines tested, range from 24 400 to 22 100 (data not shown). The molecular weights are significantly greater than those found for calf thymus H1 (22 000 and 21 000) (Panyim & Chalkley, 1971).

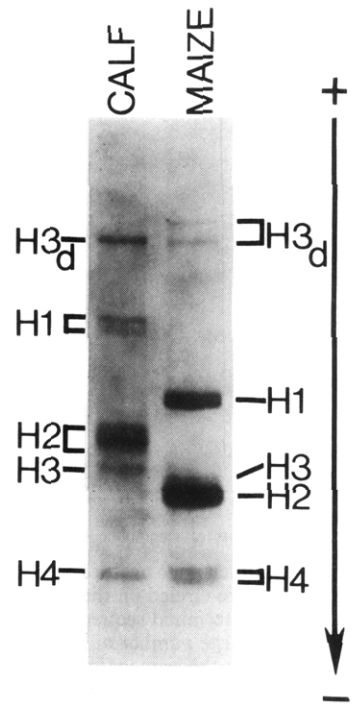

FIGURE 1: Acid–urea gel electrophoresis of maize and calf thymus histones. The individual histone fractions are indicated.

Table I: Amino Acid Compositions[a]

	total H1		amino-terminal region,[b]	carboxy-terminal region[c]	
	maize[d]	rabbit[e]	maize[d]	maize[f]	rabbit[g]
Asx	3.0	2.2	3.6	1.3	0.9
Thr	4.6	4.3	6.5	3.8	0.9
Ser	4.1	5.3	2.5	3.1	4.4
Glx	5.1	3.6	11.9	1.6	0.9
Pro	12.2	11.1	18.2	18.6	15.8
Gly	3.3	6.8	0.9	3.1	4.4
Ala	28.2	26.6	29.9	31.9	31.6
Val	3.9	5.0	4.8	2.5	2.6
Met	0.3	0.0	1.4[h]	0	0.0
Ile	1.1	0.9	0.2	0.3	0.0
Leu	4.1	4.5	1.8	2.1	0.9
Tyr	1.1	0.4	1.4	0	0.0
Phe	0.8	0.4	0	0.2	0.0
His	0.8	0.0	2.1	0	0.0
Lys	24.5	27.3	12.7	29.2	37.7
Trp	0	0.0	0	0	0.0
Arg	2.6	1.6	1.6	2.0	0.0
basic/acidic	3.4	5.0	1.1	10.7	20.9

[a] Because the amino acid compositions (in mole percent) were determined on a mixture of peptides representing the homologous regions of the different maize H1 subfractions, the values presented are not whole-number values. [b] Composition of the small molecular weight CNBr peptides. [c] Composition of the high molecular weight NBS peptides. [d] Average of two hydrolyses from each of three inbred lines. The compositions from the different maize lines are indistinguishable. [e] Bustin & Cole (1969). [f] Average of two compositions from each of two inbred lines. The compositions from the two lines are indistinguishable. [g] Residues 107–220 (Hsiang & Cole, 1975; Hsiang et al., 1975). [h] Methionine in the form of homoserine and homoserine lactone.

Amino Acid Composition. The amino acid composition of maize H1 is presented in Table I along with values for rabbit H1 taken from the literature (Bustin & Cole, 1969). In maize,

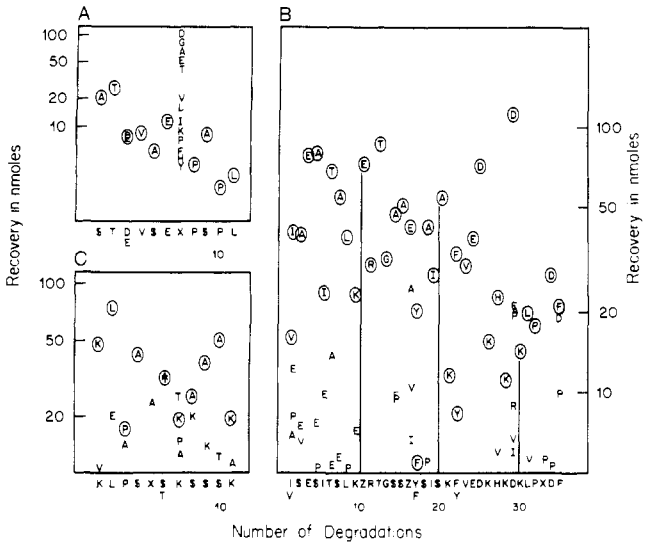

FIGURE 2: Semilog plots of the amino acid sequence data. The amino acid residues at each step are circled on the graph and are written along the axis. (A) Amino-terminal sequence of H1 isolated from the inbred line Wf9. The large number of residues at position 7 is the result of a washout from the sequenator cup. (B) Amino acid sequence of the high molecular weight CNBr peptides isolated from the inbred line Wf9. (C) Amino acid sequence of the high molecular weight NBS peptides isolated from the inbred line Mo17. $ indicates alanine or serine.

the three most abundant amino acids are alanine (28.2%), lysine (24.5%), and proline (12.2%), as they are in rabbit thymus H1, although the relative amounts are somewhat different in the rabbit (26.6, 27.3, and 11.1%, respectively).

Amino-Terminal Sequence. Maize histone H1 (106–167 nmol) was subjected to sequential Edman degradation. In three attempts, the vast majority of the H1 molecules failed to sequence, suggesting the presence of modified, presumably acetylated, amino termini as reported for H1's of other species (Phillips, 1963). A small amount of sequence data was, however, obtained corresponding to ~6% of the number of nanomoles loaded on the sequenator. Some of these data are presented in Figure 2A and in the summary of the sequence data shown in Figure 5. The sequence, although not identical with that observed for the amino-terminal residues of rabbit and trout H1, shows more than coincidental homologies with them, suggesting that a small proportion of the H1 molecules in maize are not blocked.

Cyanogen Bromide Cleavage. Cyanogen bromide was used to cleave maize H1 at the single methionine shown to be present by amino acid composition, and the reaction mixture was chromatographed on Sephadex G-75. Two peaks were obtained; the first, peak A, contained several high molecular weight peptides (as observed by $NaDodSO_4$ gel electrophoresis) together with some uncleaved H1. The second, peak B, contained the small molecular weight peptides produced by the cleavage.

The amino acid composition of the peak B peptides is shown in Table I. The presence of methionine in the form of homoserine and homoserine lactone indicates that peak B contains the amino-terminal and/or internal peptides. An attempt to sequence the peak B peptides indicated that the majority of the peptides contain blocked amino termini; however, a small amount of the peptide pool, ~2%, was unblocked as in uncleaved H1 and gave the same sequence. This suggests that peak B contains the amino-terminal peptide(s) of H1.

The amino acid composition of peak B (Table I) shows that the ratio of basic amino acids to potentially acidic amino acids is 1.1:1 so that this region may not be as basic as its counterpart in other H1's [rabbit H1, 2.5:1 (Jones et al., 1974)]. However, the disparity in length between this region in maize H1 (20–25 residues as calculated from the difference between the molecular weight of uncleaved H1 and that of the high molecular weight cyanogen bromide peptides described below) and the homologous region from rabbit H1 (44 residues) precludes any detailed comparisons.

The high molecular weight peptides produced by the cyanogen bromide cleavage, isolated by G-75 chromatography in peak A, have an apparent molecular weight range of 21 500 ± 1200 (as determined by $NaDodSO_4$ gel electrophoresis). These high molecular weights indicate that the methionine being cleaved is near one end of the H1 molecule. These peptides were sequenced as a group. Figure 2B presents the data from one of the sequencing experiments using the inbred line Wf9. A single major sequence is found, indicating that all of the major peptides produced by the cyanogen bromide cleavage of unfractionated H1 are from homologous regions of the different H1 subfractions. The sequence given in Figure 2B and summarized in Figure 5 shows that this sequenced region of 36 amino acids is hydrophobic, having 11 hydrophobic residues, with a net charge of ≥0 (7 basic residues and 7 possible acidic residues). The sequences from two other maize inbred lines, W23 and Mo17, were found to be almost identical with that presented in Figure 2B with microheterogeneities at the same position. (The H1 patterns from all three maize inbred lines are electrophoretically different from one another in acid–urea gels.) In general, this region appears to be well conserved in all three inbred lines of maize examined. The only difference was at position 5 in Figure 2B: in Wf9, valine is not found at this position and the alternative residue, isoleucine, is present in decreased yield as if the position is heterogeneous; however, no second residue could be found. Microheterogeneities, consisting of conservative amino acid substitutions, appear at several positions in the sequence, indicating differences between or within the H1 subfractions. In particular, microheterogeneity was detected at positions 1 (isoleucine/valine), 5 (isoleucine/valine), 17 (tyrosine/phenylalanine), and 22 (phenylalanine/tyrosine).

A sequence comparison of the maize H1 sequence with the rabbit H1 amino acid sequence aligns the methionine being cleaved by the cyanogen bromide with a leucine at position 44 in rabbit H1 (Figure 5). This alignment also agrees with the presence of an unblocked amino terminus in the maize peak A peptides. In addition, the major cyanogen bromide peptides extend to the carboxy terminus of H1 since the peptides contain all of the region included in the major carboxy-terminal NBS peptides (discussed below).

N-Bromosuccinimide and Chymotryptic Cleavage. The susceptible tyrosines of maize H1 were cleaved with *N*-bromosuccinimide, and the resultant peptides are shown in Figure 3. The gel compares the cleavage products from two maize inbred lines, Wf9 and Mo17, which each carry different forms of the H1a variation. All of the H1 molecules have been cleaved in the reaction, producing a set of peptides which range in apparent molecular weight from 18 500 to 17 400 (data not shown). Limited cleavage with chymotrypsin produces the same set of peptides. These peptides are contained within the high molecular weight CNBr peptides, as determined by double digests—CNBr followed by chymotrypsin and vice versa. These major NBS peptides differ in mobility within an inbred line and between lines in the same way as do the uncleaved H1 molecules so that many of the factors causing the mobility differences in the uncleaved H1 molecules must

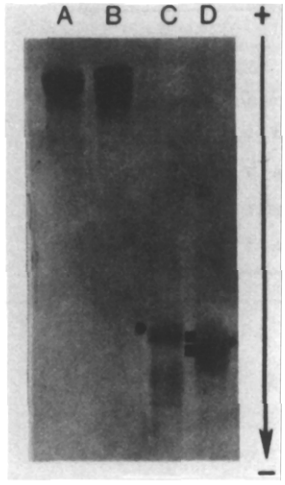

FIGURE 3: Acid–urea gel electrophoresis. (Track A) H1 from the inbred line Mo17. (Track B) H1 from the inbred line Wf9. (Track C) An NBS digest of Wf9 H1. (Track D) An NBS digest of Mo17 H1. The solid squares indicate the major H1a-derived peptides; the solid circles indicate the minor H1a-derived fraction. Uncleaved H1a has migrated 7.2 cm from the origin; the H1a-derived peptide has migrated 13.5 cm.

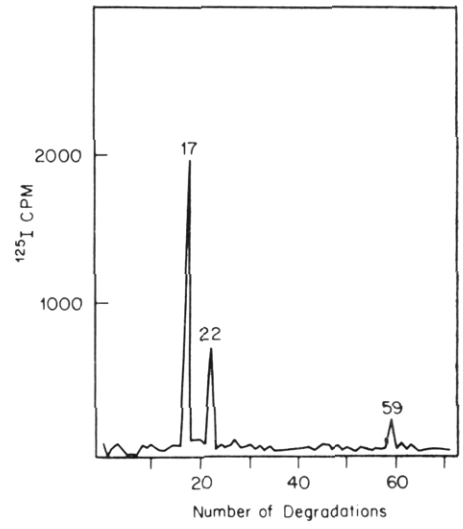

FIGURE 4: Linear plot of the sequence position vs. the counts per minute for ^{125}I-labeled high molecular weight H1 CNBr peptides from the inbred line Wf9. Tyrosines are found at positions 17, 22, and 59.

be due to structural features located in this region of the molecule. (The smaller peptides produced by the NBS cleavage do not appear in the gel.)

For the purpose of localization of the tyrosine residues giving rise to the NBS peptides, the high molecular weight CNBr peptides were labeled with ^{125}I and sequenced. Each sequence step was monitored for radioactivity, and the resultant data are presented in Figure 4. There are three tyrosines in the first 70 residues of the large CNBr peptide. The first two tyrosines, at positions 17 and 22, were also detected in the chemical sequence (Figure 2B). The third tyrosine, at position 59, corresponds in position to the phenylalanine found at position 106 in rabbit H1 (Figure 5).

Further Characterization of the N-Bromosuccinimide Peptides. The H1 peptides produced by an NBS cleavage were fractionated by G-100 column chromatography into six peaks, and the peptides in the first four were characterized by amino acid sequencing and/or composition. The major set of peptides, shown in Figure 3, cochromatographed in the first peak from the G-100 column and were characterized as a group. A single amino acid sequence was obtained (Figure 2C), indicating that these NBS peptides are produced by cleavage of the several H1 subfractions at homologous positions. The sequences obtained correspond to those expected if the peptides arose from cleavage of the tyrosine located at a position homologous to the phenylalanine located at position 106 in the rabbit H1 sequence, i.e., at position 59 in the large CNBr peptide. The quality of the sequence data from these peptides was, in general, very poor, and only short sequences could be obtained. Possibly the subfractions of H1 have slightly different sequences in this region and/or out-of-step sequences are produced by poor cleavage at the proline residues which are sometimes difficult to cleave during the Edman degradation. These usually minor sequencing problems are compounded by the repetitive nature of the amino acid sequence in this region of the H1 molecule (Hsiang & Cole, 1975; Hsiang et al., 1975) which produces a high background of alanine, lysine, and proline and obscures the sequence results.

The NBS peptides obtained by cleavage of the tyrosine at position 59 in the high molecular weight CNBr peptide extend to the carboxy terminus of H1, since they contain no tyrosine lactone, the NBS cleavage product of tyrosine (Table I). The composition of this carboxy-terminal portion of maize H1 is similar to the composition of the comparable carboxy-terminal region of rabbit H1, also presented in Table I. It is highly alanine, lysine, and proline rich with 80% of this region being accounted for by these three residues.

The remaining NBS peptides isolated by G-100 chromatography were partially sequenced and corroborated sections of the major CNBr peptide sequence. Some of the peptides exhibited microheterogeneity, as did the cyanogen bromide sequence. One of the NBS peptide sequences extended the CNBr sequence several steps amino-terminal to the methionine (Figure 5). For the purpose of determining if all of the tyrosine residues had been accounted for, an unfractionated NBS digest of maize H1 was sequenced and the four expected sequences arising from cleavage at the tyrosines at −3 (in relationship to the methionine at 0), 17, 22, and 59 could account for all the residues detected.

Localization of the H1a Electrophoretic Variation. The protein cleavages allow the localization of the genetically controlled electrophoretic variation of maize H1a to a particular region of the H1a molecule. For example, the inbred lines Wf9 and Mo17 exhibit different forms of this variation in that the major H1 subfraction of Mo17 (H1a) migrates more rapidly in acid–urea gels than does Wf9 H1a. The most prominent NBS cleavage product, indicated by a solid square in Figure 3, exhibits the same variation as the intact H1a proteins. This suggests that the peptide is derived from H1a and localizes the genetically controlled electrophoretic variation to the carboxy-terminal region of the H1 molecule. When the NBS peptides from two lines containing different forms of H1a are examined by $NaDodSO_4$ gel electrophoresis, a small difference in mobility, equivalent to 100–200 in molecular weight, is observed between variant H1a-derived peptides (data not shown).

There appears to be no further differences in other regions of the H1a molecules which contribute to the electrophoretic variation since the amino acid sequence of the central region of the molecule showed no major sequence differences between the variant inbred lines. In addition, an examination of the amino-terminal region of H1 (isolated by G-75 chromatography following CNBr cleavage) by acid–urea gel electrophoresis and thin-layer electrophoresis failed to show any

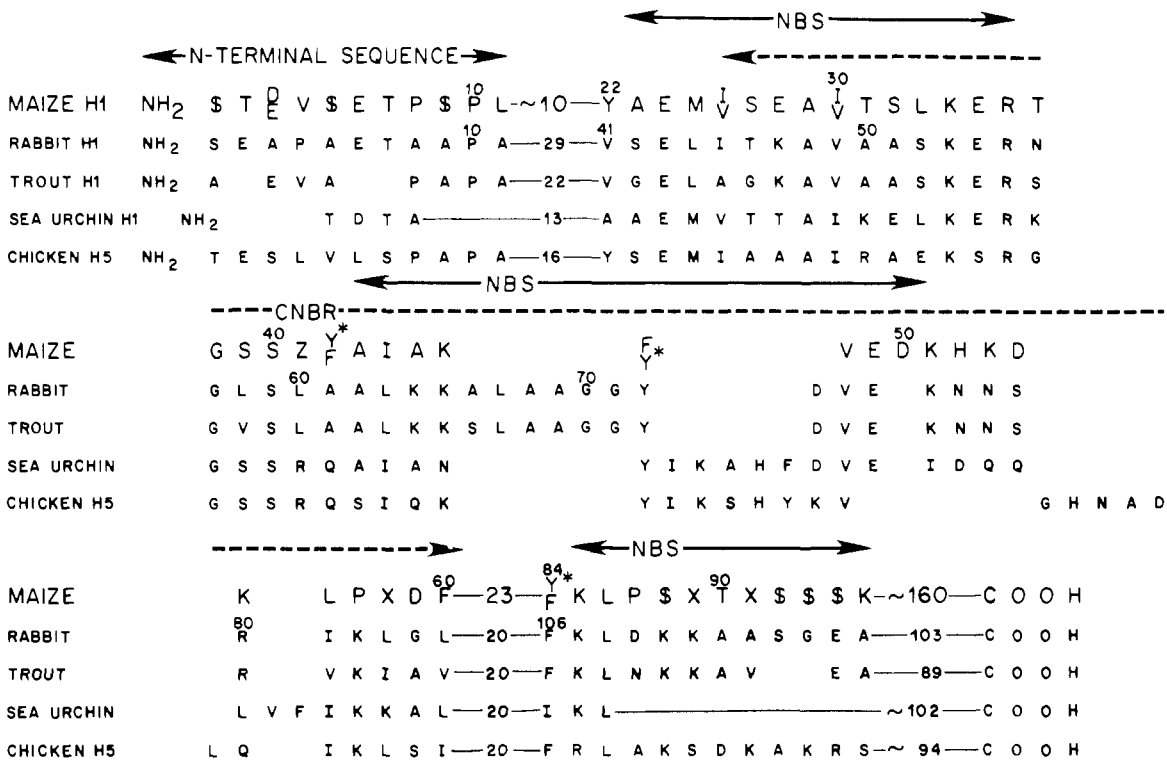

FIGURE 5: Summary of the maize H1 sequence data compared to the sequence data from vertebrate H1's, an invertebrate H1, and chicken H5. The maize sequence is a compilation of three to four separate sequenator runs on each region. The alignments were made in part by using a computer program to detect protein homology. Regions of sequence not listed or not determined are indicated by the solid lines. The numbers indicate the lengths of these regions. The numbering of the maize sequence is only approximate. An asterisk indicates that the residue was labeled with ^{125}I. Rabbit H1 data are from Jones et al. (1974), Hsiang & Cole (1975), and Hsiang et al. (1975), trout H1 data are from MacLeod et al. (1977), sea urchin H1 data are from Schaffner et al. (1978), and chicken H5 data are from Sautiere et al. (1976).

differences in the peptides isolated from the maize lines (data not presented).

Of particular interest in understanding the architecture of the maize H1 molecules in comparison to those of other species is the presence in the NBS digestion products of a minor band (indicated by a solid circle in Figure 3) migrating slightly slower than the major H1a-derived band. This band, which is present in the limit digest, exhibits the same type of electrophoretic variation as the major H1a-derived band. The simplest explanation for the origin of this peptide is that the H1a fraction itself contains two closely related proteins, one in greater abundance than the other. The difference in these two proteins is that the less abundant would have the sequence ---Y-F----- and the more abundant ---F-Y-----. The tyrosine (Y) in the more abundant species is located at position 84 in the maize H1 sequence (Figure 5). The phenylalanine (F), located amino-terminal to the tyrosine, has not been localized within the molecule; however, it is probably within a few residues of position 84 since the two H1a-derived NBS peptides migrate closely in the gel. Cleavage of the more abundant protein with *N*-bromosuccinimide (cleavage only at tyrosine) would produce the shorter peptide. Cleavage of the less abundant protein would produce a small amount of the longer and, thus, slower migrating peptide. Cleavage of either with chymotrypsin (cleavage at tyrosine and phenylalanine) would yield only the shorter peptide. As predicted by this hypothesis, the minor H1a-derived peptide was virtually absent in chymotryptic digests of H1, and amino acid compositions of a pool containing both of the variant NBS peptides show the presence of a small amount of phenylalanine (see Table I). In the H1 molecules from other species, there is a phenylalanine residue at the position corresponding to the tyrosine of the more abundant maize H1 (position 59 in the major cyanogen bromide peptide). These data provide strong evidence for the existence of at least two slightly different genes for the H1a histones, both carrying the inbred line-specific variation.

Discussion

The first histone H1 from plants has been characterized, and ~25% of the protein has been sequenced. Maize H1 is very similar to animal H1 in the general organization of the molecule including the hydrophobic central region and the basic, repetitive carboxy-terminal region. The increased molecular weight of maize H1 coupled with its decreased positive charge as compared to vertebrate H1 accounts for the decreased mobility of the plant protein in acid–urea gels. Figure 5 summarizes the available amino acid sequence data from maize H1 and compares them to the data from vertebrate H1 (trout and rabbit), invertebrate H1 (sea urchin), and chicken histone H5.

The amino acid sequence of the central hydrophobic region of the maize H1 protein (residues 41–106 in rabbit), determined mainly from the major CNBr peptides, is homologous to animal H1's and H5 as determined by a computer program designed to detect protein homologies (Sankoff, 1972; Needleman & Wunsch, 1970). [The maize H1 sequence was not homologous to any other histone sequence tested—H4 (pea), H3 (pea), H2A (trout and calf), and H2B (trout and calf).] This conservation of sequence in a region which may be the site of interaction between H1 and other molecules (Singer & Singer, 1976; Chapman et al., 1976; Boulikas et al., 1980) indicates the importance of the region. The extent of amino acid homology differs within the segment. The first 25 residues (in Figure 5, positions 22–46 in maize) are highly conserved in all species; this is followed by a region of low interspecific homology in which several gaps have to be inserted in order

to align the sequences. Although the maize data are not complete in the area, the final 26 residues of the hydrophobic region (positions 81–106 in rabbit H1) are highly conserved between animal H1's. The close homology found between the hydrophobic region of maize H1 and chicken H5 supports the idea that H1 and H5 could have arisen from a common ancestral gene [see also Yaguchi et al. (1977)]. The present similarity in sequence also implies the retention of common functions by these two histone fractions.

It is interesting to note that the length of the hydrophobic segment is highly conserved. Its length is an invariant 66 residues long in rabbit, trout, and sea urchin H1 and chicken H5 and is only slightly shorter in maize (63 residues from residue 22 to residue 84 in Figure 5). This conservation in length, as compared to the widely varying lengths of the carboxy-terminal and amino-terminal regions of these molecules, suggests the importance of this feature to the correct functioning of the molecule.

Studies in other organisms have shown that the histone genes are present in multiple copies (Wilson & Melli, 1977; Jacob et al., 1976). The maize H1 sequencing data, which show the presence of microheterogeneities in the protein sequence, establish that in maize there are several different H1 genes. The sequencing data do not permit an accurate estimate of this number; however, some lower limits can be given. There are at least two different genes for histone H1a as judged by the data presented under Results. Judging by the qualitative and quantitative nature of all of the microheterogeneity, there may be up to seven different H1 genes. Further sequence data would be likely to increase the upper limit as more heterogeneity is found.

Unlike the histone genes in other organisms which have been shown to be clustered (Schaffner et al., 1976; Cohn et al., 1976; Lifton et al., 1978), the maize H1 genes appear to be located on different chromosomes (Stout & Phillips, 1973; Stout & Kermicle, 1979). A clearly defined genetic basis for histone H1a electrophoretic variations has been demonstrated with the genetic locus determining these variations being on a different chromosome from the loci determining the electrophoretic behavior of the other H1 subfractions. Our present work extends these findings in that it provides additional evidence that the H1a structural variation, used to map the H1a genes, is due to a change in the H1a primary structure. The cause of the electrophoretic variation has been localized to a particular region of the H1a molecule and appears to be associated with a small molecular weight difference. Unfortunately, the difference is located in the basic, repetitive carboxy-terminal region of H1 which occupies 60% of the molecule. This part of the molecule has proved difficult to sequence, and this difficulty has so far prevented us from identifying the cause of the H1a variation.

The present study has shown that the H1 proteins of maize provide a wide repertoire of molecules through which the cell could exert different types of control via structural changes in chromatin. The complexity of the H1 system is not likely to be resolved solely by protein structural studies. The need to isolate by molecular cloning techniques and to characterize the DNA corresponding to these genes is evident. The present amino acid sequence data should provide sufficient structural information to permit the identification of clones of maize H1 DNA.

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Investigation of the Structure of Metallothioneins by Proton Nuclear Magnetic Resonance Spectroscopy†

Milan Vašák,* Alphonse Galdes, H. Allen O. Hill,* Jeremias H. R. Kägi, Ian Bremner, and Brian W. Young

ABSTRACT: The proton nuclear magnetic resonance spectra of metallothioneins from horse, human, and sheep livers were investigated. The spectra of the metallothioneins from the three species are similar as are the two isoproteins from any one species. The spectra indicate that metallothioneins possess a well-defined tertiary structure. Zinc(II) and cadmium(II) ions induce similar but not identical tertiary structures. Confirmatory evidence was obtained for the involvement of cysteine residues in metal binding, but no evidence was obtained for the involvement of any other amino acid residue in metal binding. The apoprotein thionein was found to exist essentially in a random-coil conformation with perhaps some residual segmental structure.

Metallothionein is a widely occurring cysteine- and metal-rich protein which was first isolated from the equine renal cortex by Margoshes & Vallee (1957). Other proteins possessing similar properties were subsequently isolated from kidney, liver, and certain other parenchymatous organs of a wide variety of animal species and recently also from a eucaryotic microorganism (Kojima & Kägi, 1978; Lerch, 1979). One of the best characterized metallothioneins is that isolated from equine kidney and liver. The native protein consists of a single polypeptide chain with a molecular weight of 6100 (Kojima et al., 1976). The high metal content [7 g-atoms of Zn(II) and/or Cd(II) per mol of protein] together with the high cysteine content (33%) and the total absence of aromatic amino acids and histidine makes this protein very unusual (Kägi et al., 1974). The biosynthesis of the protein occurs in the liver, kidney, and intestinal wall and is largely accelerated by the administration of metal ions, for example, zinc and cadmium. Consequently, it has been suggested that the protein is involved in metal metabolism, homeostasis, or detoxification (Kojima & Kägi, 1978). Spectroscopic and complexometric titration data have led to the suggestion that the apoprotein (thionein) possesses equivalent and independent binding sites, each containing three cysteinyl residues (Kägi & Vallee, 1961). Furthermore, the sequence shows a distinct clustering of the 20 cysteinyl residues. Within the protein chain the cysteinyl residues occur 7 times in Cys-X-Cys sequences. These sequences have been suggested to be the primary metal binding sites (Kojima et al., 1976). This hypothesis is supported by recent dark field electron microscopy measurements, which have shown that partially denatured metallothionein has a pearl chain structure with six to seven metal ion centers positioned equidistantly along the polypeptide string (Fiskin et al., 1977). The primary structure also reveals that seven of the eight seryl residues occur in -Ser-Cys- sequences.

Table I: Amino Acid Composition of Ovine Metallothioneins 1 and 2

	metallothionein 1[a]	metallothionein 2[a]
cysteine[b]	17.9	19.3
aspartic acid	3.3	3.7
methionine[c]	1.2	1.1
threonine	2.5	2.2
serine	8.8	7.4
glutamic acid	1.3	2.1
glycine	6.9	5.4
alanine	6.1	7.4
isoleucine	0.4	
lysine	7.7	10.0
valine	1.9	2.1
arginine		
proline	*d*	2.1
total	60.1	62.8

[a] Number of residues per M_r 6000. [b] Measured as cysteic acid. [c] Measured as methionine sulfone. [d] Not estimated.

In this paper we report ^{1}H NMR[1] data for metallothioneins isolated from man, horse, and sheep. ^{2}H exchange as well as pH and temperature studies has been used to obtain information about the tertiary structure of the proteins. The results allow tentative conclusions about the function of seryl residues in these proteins. A preliminary account of some of this work has been published (Galdes et al., 1978a,b).

Experimental Section

Materials. Equine liver metallothioneins 1A and 1B (Kojima et al., 1976; Kojima & Kägi, 1978), human liver metallothioneins 1 and 2 (Buhler & Kägi, 1974), and ovine liver metallothioneins 1 and 2 (Bremner & Davies, 1975; Bremner & Marshall, 1974; Bremner et al., 1977) were isolated by a

† From the Biochemistry Department, University of Zurich, 8032 Zurich, Switzerland (M.V. and J.H.R.K.), the Inorganic Chemistry Laboratory, University of Oxford, Oxford OX1 3QR, England, United Kingdom (A.G. and H.A.O.H.), and the Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB Scotland, United Kingdom (I.B. and B.W.Y.). *Received September 17, 1979.* This work was supported by a Rhodes Fellowship (to A.G.), an E.M.B.O. Fellowship (M.V.), and Swiss National Foundation Grant No. 3.125-0.77. It constitutes part of the program of the Oxford Enzyme Group of which H.A.O.H. is a member.

[1] Abbreviations used: NMR, nuclear magnetic resonance; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate.

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