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The primary structure of a minor isoform (H1.2) of histone H1 from the nematode *Caenorhabditis elegans*

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The complete amino acid sequence of a minor isoform (H1.2) of histone H1 from the nematode *Caenorhabditis elegans* was determined. The amino acid chain consists of 190 residues and has a blocked *N*-terminus. Histone subtype H1.2 is 17 residues shorter than the major isoform H1.1, mainly as the result of deletions of short peptide fragments. Considerable divergence from isoform H1.1 has occurred in the *N*-terminal domain and the very *C*-terminus of the molecule, but the central globular domain and most of the *C*-terminal domain, including two potential phosphorylation sites, have been well conserved. Secondary-structure predictions for both H1 isoforms reveal a high potential for helix formation in the *N*-terminal region 1–33 of isoform H1.1 whereas the corresponding region in isoform H1.2 has low probability of being found in α-helix. No major differences in secondary structure are predicted for other parts of both H1 subtypes. The aberrant conformation of isoform H1.2 may be indicative of a significantly different function.

INTRODUCTION

The histone H1 family of *Caenorhabditis elegans* comprises at least two isohistones, which we have designated H1.1 and H1.2 (Vanfleteren & Van Beeumen, 1983; Vanfleteren *et al.*, 1988). The former subtype has an apparent molecular mass of 20 kDa and its primary structure has been determined (Vanfleteren *et al.*, 1988). H1.2 is a minor isoform with an apparent molecular mass of 18.5 kDa. In the present paper we describe the isolation and the sequence determination of histone H1.2.

EXPERIMENTAL

Nematode growth

The wild-type strain of *C. elegans* var. Bristol (N2) was used in this study. Harvests from several mass cultures were combined until about 2 kg (wet wt.) of nematode tissue was obtained. Typically 40–60 g of nematodes were raised in sterile culture conditions in a few hundreds of large (175 cm^2 growth area) tissue-culture flasks containing 75 ml of 3% (w/v) yeast extract, 3% (w/v) soy peptone and 0.5 mg of haemoglobin/ml [a stock solution of 5% (w/v) haemoglobin in 0.1 M-KOH was autoclaved for 10 min to generate a sterile water-soluble growth-promoting peptide mixture]. At harvest the culture medium was poured into a large vessel and the worms were allowed to settle overnight at 4 °C. Most of the culture medium was then aspirated. The remainder was centrifuged at 4000 *g* for 10 min. The pelleted nematodes were washed once more with S buffer (0.1 M-NaCl/50 mM-potassium phosphate buffer, pH 6.0) and used as an inoculum for further cultivation in a xenic medium containing 1% (w/v) plasmolysed yeast cells (Protibel; Bel Industries, Paris, France) and 40 μg of chloramphenicol/ml in S buffer. Xenic cultures were grown in flat trays made of poly(vinyl chloride) (60 cm × 40 cm × 5 cm), each containing 1.2 litres of culture medium. They were agitated on a gyratory shaker at 100 rev./min. Nematode biomass increased about 10-fold in 1 week in this medium. At harvest, the culture medium was filtered through 25 μm-mesh gauze that was stretched over a large-aperture stainless-steel sieve. Adult worms were retained on the gauze along with a background of juveniles and clumps of plasmolysed yeast cells. The retained material was rinsed with large amounts of S buffer and then suspended in several litres of the same buffer, and the adult worms were allowed to settle at room temperature for 20–30 min. The supernatant, which contained plasmolysed yeast cells and juvenile worms, was aspirated and discarded. This treatment was repeated several times, and finally the settled worms were cleaned by flotation on 30% (w/w) sucrose (2000 *g* for 7 min). The nematode caps were aspirated and resuspended in 0.1 M-NaCl, distributed over a 25 μm-aperture stainless-steel sieve and rinsed with excess 0.1 M-NaCl to remove sucrose. The retained worms were finally resuspended in a minimal volume of cold 0.1 M-NaCl and centrifuged at 2000 *g* for 10 min in graduated centrifuge tubes (Macrolon; Brandt, Wertheim, Germany). The supernatant was aspirated and the worm pellet was suspended in an equal volume of homogenizing buffer (0.25 M-sucrose/5 mM-$CaCl_2$/50 mM-Tris/HCl buffer, pH 7.4). The nematode slurry was next dripped into liquid N_2 and the frozen nematode nuggets were stored at −70 °C.

Abbreviation used: C, percentage concentration of bisacrylamide relative to T, the total percentage concentration of acrylamide and bisacrylamide.

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Preparation of histone H1

At all stages of the procedure, warming up of the preparations above 4 °C was carefully avoided. Typically, 500 g of frozen nematode beads were crushed in a pre-cooled (−30 °C) 3.8-litre (1-U.S.-gallon) container of a Waring Blendor, which was operated at the low-speed position for 30–60 s until a fine dust was obtained. Immediately after thawing phenylmethanesulphonyl fluoride was added at 1 mM final concentration. It was diluted from a stock solution containing 100 mM-phenylmethanesulphonyl fluoride in propan-1-ol/propan-2-ol (4:1, v/v) mixture. The preparation was next homogenized at full speed for 2 min. The container was cooled, if necessary, and the mixture was blended for 2 min more. The volume of the homogenate was increased to 2.4 litres with homogenizing buffer, and fresh amounts of phenylmethanesulphonyl fluoride and 2-mercaptoethanol were added at 1 mM and 1% (v/v) final concentrations respectively. Nematode fragments, unbroken cells, large cell fragments and nuclei were pelleted at 4000 ***g*** for 10 min. The pellets were suspended in 250 ml of homogenizing buffer containing 0.5% Nonidet P-40 and blended at low speed for 2 min. The volume of the homogenate was diluted to 1 litre with homogenizing buffer containing 0.5% Nonidet P-40, and a fresh amount of phenylmethanesulphonyl fluoride was again added at 0.1 mM final concentration. Stirring was continued for 30 min. Large debris was sedimented at 40 ***g*** for 10 min, and the supernatant, which contained the nuclei, was kept on ice. The pellets were re-extracted in homogenizing buffer and centrifuged again at 40 ***g***. This procedure was repeated once more and the pooled supernatants were centrifuged at 4000 ***g*** for 15 min. The pelleted nuclei were washed once with 0.14 M-NaCl/10 mM-Tris/HCl buffer, pH 7.4 (7000 ***g*** for 10 min), resuspended in 10 vol. of 5% (v/v) $HClO_4$, and blended in the 1-litre container of the Waring Blendor at low speed for 2 min. The mixture was further stirred for 1 h to allow complete extraction of $HClO_4$-soluble protein and centrifuged at 7000 ***g*** for 10 min. The supernatant was clarified by filtration through a no.-4-porosity sintered-glass funnel. Protein was next precipitated from the clear filtrate by the addition of Reinecke salt (ammonium tetrathiocyanodiammonochromate) (Lindh & Brantmark, 1965) and pelleted at 7000 ***g*** for 15 min. Pellets from the successive preparations were pooled, washed with acetone containing 60 mM-HCl and twice with acetone only, and dried under reduced pressure. After redissolution in 0.35 M-HCl, histone H1 protein was selectively precipitated by adding 3.5 vol. of acetone and sedimented at 2000 ***g*** for 15 min. The pellet was washed with acetone containing 60 mM-HCl and twice with acetone. It was finally dried under reduced pressure, yielding approx. 220 mg of acetone-dried powder.

Purification of histone H1.2

Histone H1.2 was purified on 19 preparative SDS/PAGE (Laemmli, 1970) gels (15% T, 2.6% C) measuring 0.4 cm × 16 cm × 18 cm. Mercaptoacetic acid was added to the upper buffer at 0.1 mM final concentration to scavenge oxidative components present in the gel matrix. After completion of the electrophoretic run the gels were stained with Coomassie Brilliant Blue R-250 for 2–3 h and destained in 10% (v/v) acetic acid/10% (v/v) ethanol until the protein bands became clearly visible. The staining and destaining solutions contained 1% (v/v) 2-mercaptoethanol. Bands containing isoform H1.2 (Fig. 1, track a) were excised and stored at −30 °C.

The excised bands were minced and transferred to the gel-loading reservoir of an enlarged Hunkapiller elution cell (Hunkapiller *et al.*, 1983), and covered with elution buffer. Both collecting and gel-loading reservoirs of the cell were bounded by a benzoylated dialysis membrane (2000 Da cut-off). Elution buffer was 0.1 M-acetic acid containing 0.3% (w/v) cetyltrimethylammonium bromide and 0.2% (v/v) thiodiethanol in the gel-loading reservoir of the elution cell and in the electrode chamber connected to the positive electrode. The sample-collecting reservoir and the buffer chamber connected with the negative electrode contained 0.1 M-acetic acid and 0.2% (v/v) thiodiethanol but no cetyltrimethylammonium bromide. Protein was eluted at a constant current of 15 mA. Buffer was withdrawn from the sample-collection reservoir after 6 h of electrophoretic elution and stored in the cold; fresh buffer was then added and elution was continued overnight. All buffer was then carefully removed from the sample-collection reservoir and combined with the first harvest in a 50 ml conical centrifuge tube (Falcon; Beckton Dickinson, Lincoln Park, NJ, U.S.A.). Buffer was evaporated in a Savant SVC 200H Speed Vac concentrator (Savant Instruments, Hicksville, NY, U.S.A.). The vacuum pump was protected by two glass traps immersed in liquid N_2. Coomassie Brilliant Blue dye, cetyltrimethylammonium bromide and thiodiethanol were extracted three times from the dry pellets with ethanol/methanol (1:1, v/v) mixture containing 10 mM-H_2SO_4. The protein precipitate was washed once more with acetone and dried. The yield was approx. 18 mg of acetone-dried powder.

Generation and separation of peptides

A sample containing 3.6 mg of acetone-dried powder (nominal amount approx. 200 nmol) was dissolved in 500 μl of 50 mM-acetic acid/pyridine buffer, pH 4.0, and digested with *Staphylococcus aureus* V8 proteinase (EC 3.4.21.19; Miles Laboratories) at an enzyme/substrate ratio of 1:50 (w/w) overnight (16 h) at 37 °C. One-half amount of fresh enzyme was next added and digestion was continued for 24 h.

A second sample containing 3.6 mg of acetone-dried powder was dissolved in 2% (v/v) formic acid and heated at 106 °C for 4 h (limited acid hydrolysis; Inglis, 1983).

Both digests were evaporated to dryness, dissolved in Laemmli (1970) sample buffer and loaded on preparative 1.5 mm-thick, 24 cm-wide and 25 cm-long small-pore Tricine/SDS/PAGE (16.5% T, 2.6% C; Schägger & Von Jagow, 1987) gels. Mercaptoacetic acid was added to the upper buffer at 0.1 mM final concentration. The electrophoretic conditions were 45 mA/gel for 16 h at 20 °C. Peptide bands were stained with Coomassie Brilliant Blue R-250 and destained in the presence of 1% 2-mercaptoethanol as outlined above. Peptide bands generated from histone H1.2 by limited acid hydrolysis were labelled AH-A to AH-P (Fig. 1, track b), and those obtained after digestion with *S. aureus* V8 proteinase were labelled Sa-A to Sa-L (Fig. 1, track c), according to their decreasing apparent molecular mass. The peptide bands were excised and eluted by shaking in 70% (v/v) formic acid for 24 h at ambient temperature. Coomassie Brilliant Blue dye was extracted from the dried (Speed

Vac concentrator) samples with a mixture of 17.5 vol. of acetone, 1 vol. of acetic acid, 1 vol. of triethylamine and 0.5 vol. of water, and dried again.

The remainder of the peptide fractions Sa-E, Sa-F and AH-I that was left after automated Edman degradation was pooled, yielding about 10 nmol of *C*-terminal peptide fragments, and digested with 0.1 nmol of chymotrypsin (EC 3.4.21.1; Worthington Corp.) in 0.1 M-HCO_3, pH 8.3 for 4 h at 37 °C. The peptide mixture was next separated by reversed-phase h.p.l.c. on a Poly F column (6.2 mm × 80 mm; Dupont de Nemours, Willmington, DE, U.S.A.) with a linear gradient of 0–60% (v/v) acetonitrile in 0.1% (v/v) trifluoroacetic acid in 60 min at a flow rate of 1 ml/min. The peaks were designated C_1 to C_{10} according to their increasing retention times (results not shown).

A third sample, containing approx. 4 mg of histone H1.2 acetone-dried powder, was dissolved in 50 mM-sodium phosphate buffer, pH 8.15, and digested with 6 μg of endoproteinase Asp-N (sequencing grade; Boehringer Mannheim) at 37 °C for 36 h. The resulting peptides were separated by reversed-phase h.p.l.c. on a Vydac C4 column (4.6 mm × 250 mm). The peaks were numbered pD_1 to pD_{52} according to their increasing retention times (Fig. 2).

Peak pD_{16}, which contained the *C*-terminal peptide fragment (approx. 2 nmol) of histone H1.2 was subcleaved with 50 pmol of thermolysin (EC 3.4.24.4; Boehringer Mannheim) for 2 h at ambient temperature in the presence of 20% (v/v) propan-2-ol and low Ca^{2+} (0.3 mM) to restrict the number of cleavable sites, as recommended by Welinder (1988). The resultant peptide mixture was separated on a small-bore PTC C_{18} column (Applied Biosystems, Foster City, CA, U.S.A.). The peaks were numbered $pD_{16}Th_1$ to $pD_{16}Th_{21}$ according to their increasing retention times (Fig. 3*a*).

Approx. 400 pmol of peptide peak pD_{31}, which contained the *N*-terminal peptide fragment, was subcleaved with *S. aureus* V8 proteinase for 16 h at 35 °C in 0.2 M-sodium phosphate buffer, pH 8.6, at an enzyme/substrate ratio of 1:20 (on a molar basis). The released peptides were separated on the small-bore PTC C_{18} column and numbered $pD_{31}Sa_1$ to $pD_{31}Sa_{15}$ according to their increasing retention times (Fig. 3*b*). A second sample of peak pD_{31}, containing approx. 600 pmol of peptide, was subcleaved with 20 pmol of trypsin (EC 3.4.21.4; Worthington Corp.) in 0.1 M-NH_4HCO_3 for 4 h at 37 °C. The resulting peptide fragments were also separated on the small-bore PTC C_{18} column and numbered $pD_{31}T_1$ to $pD_{31}T_{40}$ as they emerged from the column (Fig. 3*c*).

Hydropathy estimation and secondary-structure prediction

Hydropathy plots were calculated with the HYDROP program with several averaging windows (five up to 17). The hydropathy parameters of Kyte & Doolittle (1982) were used. Secondary-structure prediction was performed with the CHUFAS program using the parameters as described by Chou & Fasman (1978). Both programs were written by Peter A. Stockwell, Department of Biochemistry, University of Otago, Dunedin, New Zealand, and run on a VAX-VMS computer.

Automated sequence analysis

We used a pulsed-liquid model 477A Sequenator equipped with an on-line 120A phenylthiohydantoin analyser (Applied Biosystems). The peptides were applied on to glass-fibre discs that had been coated with 3 mg of Polybrene and subjected to two Edman degradation cycles before application of the samples.

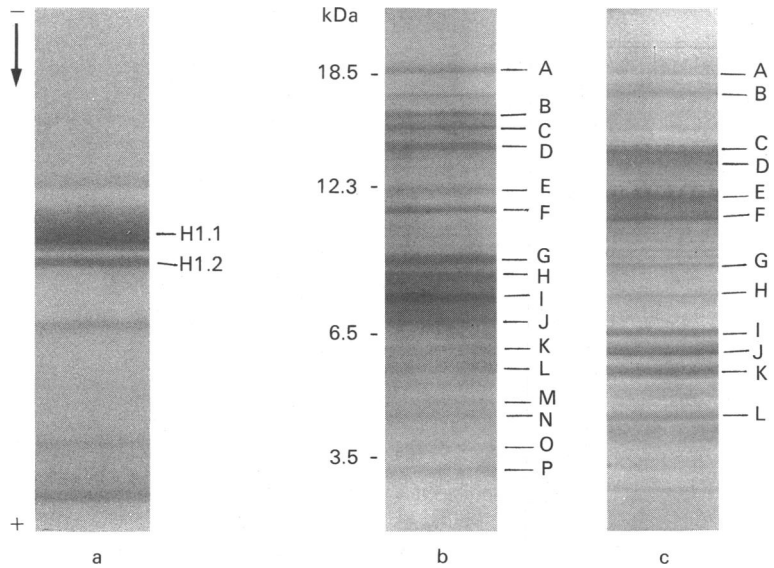

Fig. 1. Purification of intact histone H1.2 and cleavage fragments thereof by preparative electrophoresis

Track a, preparative SDS/PAGE (15% T, 2.6% C) of crude histone H1; tracks b and c, preparative Tricine/SDS/PAGE (16.5% T, 2.6% C) of peptides generated from histone H1.2 by limited acid hydrolysis (track b) and digestion with *S. aureus* V8 proteinase (track c). The migration of molecular-mass markers on the Tricine gels is shown on the left of track b: 18.5 kDa, intact histone H1.2; 12.3 kDa, cytochrome *c*; 6.5 kDa, aprotinin; 3.5 kDa, insulin B-chain. The peptide bands were labelled AH-A to AH-P (track b) and Sa-A to Sa-L (track c) according to their decreasing appararent molecular mass.

RESULTS AND DISCUSSION

Peptide isolation

We have chosen the Tricine/SDS/PAGE method of Schägger & Von Jagow (1987) for the separation of peptides generated from histone H1.2 by limited acid hydrolysis and digestion with *S. aureus* V8 proteinase in acetate buffer, pH 4, as we expected that these treatments would yield large peptides, comparable with those released from histone H1.1 after the same treatments (Vanfleteren *et al.*, 1988). Large peptides are particularly well suited for automated sequence analysis. Much too frequently, however, they are badly separated by reversed-phase h.p.l.c. As shown by the photographs depicted in Fig. 1, the Tricine/SDS/PAGE system separated peptides in the 18500 Da (intact histone H1.2)–3500 Da range effectively. High-molecular-mass peptides result from incomplete cleavage.

Sequence analysis of the peptide bands represented in Fig. 1 showed that they were derived from both intact isoform H1.2 and a trimmed form of isoform H1.1 commencing at Ala-19. For example, peptide AH-I was the *C*-terminal peptide Lys-149–Ala-190 of histone H1.2, whereas the slightly larger peptide AH-H turned out to be exactly the same region of histone H1.1 (Lys-160–Ala-207), peptide Sa-E was the *C*-terminal part of histone H1.2 commencing at Lys-113 whereas peptide Sa-C was the larger counterpart of histone H1.1, and so on (Fig. 5).

The cleavage of histone H1.1 at the carboxy group of Lys-18 appears to have occurred with quite high specificity, since no evidence for any cleavage at the *C*-terminal side of Lys-16 or Lys-21 was found. Possibly, proteolytic degradation has occurred in the course of the process of the extraction and purification of histone H1 protein, in spite of our efforts to prevent proteolysis. Alternatively, trimmed isoform H1.1 may have resulted from processing *in vivo*.

Sequence evidence

Much like the major form H1.1, histone subtype H1.2 has a blocked serine residue at the *N*-terminus, which could be partially unblocked by prolonged digestion with *S. aureus* V8 proteinase. This peculiar ability of at least some batches of *S. aureus* V8 proteinase to unblock *N*-acetylserine has also been reported by others (Martinage *et al.*, 1983; Wouters-Tyrou *et al.*, 1981, 1982), and since *N*-acetylserine is a common *N*-terminal residue of H1 protein we assume that both H1 subtypes of *C. elegans* are blocked by acetylation.

Extended runs in the Sequenator of major peptides released after cleavage of intact histone H1.2 by limited acid hydrolysis or digestion with *S. aureus* V8 proteinase soon elucidated most of the amino acid sequence. Problems arose, however, in the *N*- and *C*-terminal regions of the molecule. The *C*-terminal peptide AH-I could not be sequenced up to its *C*-terminus, as it was rapidly lost from the Polybrene filter. We then first digested the pooled peptide fractions Sa-E, Sa-F and AH-I, all of which contained the *C*-terminal part of histone H1.2, with chymotrypsin, mainly because the sequence run of peptide AH-I gave a rather ambiguous indication of a leucine residue at position 180 of the polypeptide chain. In histone H1.1 the analogous residue is Ile-192 (Fig. 5). This treatment yielded no peptide that was useful in elucidating the *C*-terminus of histone H1.2. However,

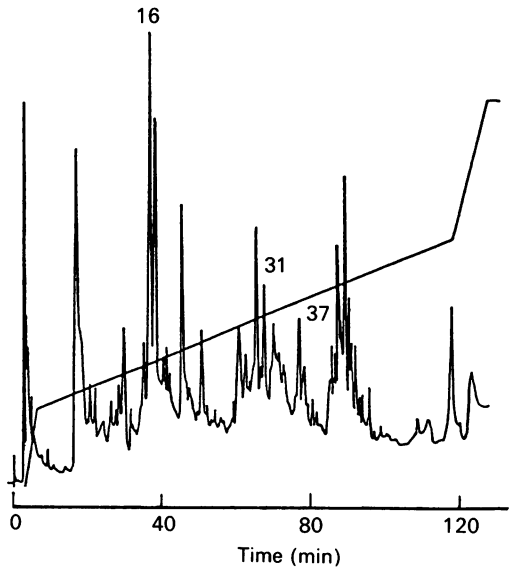

Fig. 2. Reversed-phase h.p.l.c. of peptides produced by digestion of histone H1.2 with endoproteinase Asp-N

A peptide mixture representing approx. 100 nmol of protein was resolved on a Vydac C4 column (4.6 mm × 250 mm) with the following gradient of acetonitrile in 0.1 % trifluoroacetic acid at a flow rate of 1 ml/min: 0–15 % (v/v) in 5 min, 15–40 % (v/v) in 120 min, and 40–60 % (v/v) in 10 min. Absorbance at 220 nm (0.64 A_{220} unit full scale) was monitored. Peaks were numbered pD_1 to pD_{52} according to their increasing retention times.

two small peptide peaks (C_8 and C_9) emerged from the column that were apparently derived from a minor *N*-terminal peptide present among peptides Sa-E, Sa-F, and AH-I and provided new sequence information in that region of the molecule.

The *C*-terminus of histone H1.2 was eventually elucidated by using a separate digest of the protein with endoproteinase Asp-N. This strategy was based on our previous work on histone H1.1 (Vanfleteren *et al.*, 1988), which had shown that the *C*-terminal peptide from *S. aureus* V8 proteinase digestion emerged from a Vydac C4 reversed-phase column as a well-resolved peak at relatively low acetonitrile concentration. Actually we used the endoproteinase Asp-N hoping to find the shorter peptide commencing with the ultimate aspartic acid residue in the sequence. This was peptide pD_{16} (Fig. 2; peptide pD_{17} was the longer but analogous peptide from histone H1.1). This peptide was then further subcleaved with thermolysin in the presence of 20 % propan-2-ol and low Ca^{2+} to generate a smaller *C*-terminal peptide, $pD_{16}Th_{15}$, which enabled the ultimate elucidation of the *C*-terminal amino acid sequence of histone H1.2.

Further screening of the peptides from proteinase Asp-N digestion revealed that peptide peak pD_{31} comprised a major peptide commencing at Glu-7 of histone H1.2 (Fig. 4) and a minor fraction commencing at Gln-85 (apparently cleaved after partial deamidation of this residue; Fig. 5) of histone H1.1 as well. One portion of peptide fraction pD_{31} was then cleaved extensively with *S. aureus* V8 proteinase so that the contaminating histone H1.1 peptide would be cut into smaller peptide fragments. As expected, a highly pure peptide fragment representing the *N*-terminal region of histone H1.2 was resolved by

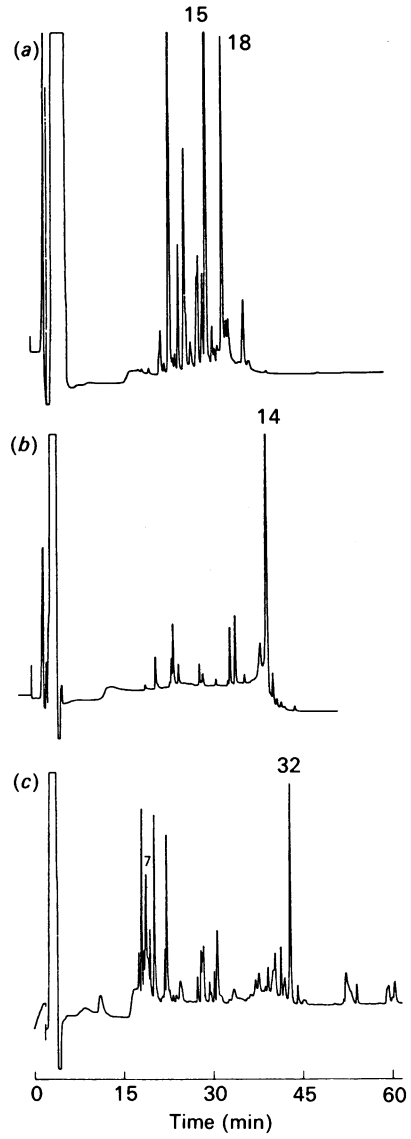

Fig. 3. H.p.l.c. map of the peptides released after subcleavage of particular endopeptidase-Asp-N-digest peptide fragments

(*a*) Peptides released after digestion of peptide pD_{16} (2 nmol) with thermolysin in the presence of 20% (v/v) propan-2-ol and low Ca^{2+}; (*b*) digestion of peptide pD_{31} (400 pmol) with *S. aureus* V8 proteinase; (*c*) subcleavage of peptide pD_{31} (600 pmol) with trypsin. The peptide mixtures were resolved on a small-bore PTC C_{18} column (2.1 mm × 220 mm) with the following gradient of acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 200 μl/min: 0–28% (v/v) in 60 min, 28–42% (v/v) in 10 min, and 42–70% (v/v) in 5 min. Absorbances were measured at 220 nm [0.2 A_{220} unit full scale (*a*) or 0.3 A_{220} unit full scale (*b* and *c*)]. Peaks were numbered $pD_{16}Th_1$ to $pD_{16}Th_{21}$ (*a*), $pD_{31}Sa_1$ to $pD_{31}Sa_{15}$ (*b*) and $pD_{31}T_1$ to $pD_{31}T_{40}$ (*c*) according to their increasing retention times (only peptide fragments that enabled the final elucidation of the amino acid sequence of histone H1.2 are actually indicated).

reversed-phase h.p.l.c. on a small-bore C_{18} column (peptide $pD_{31}Sa_{14}$; Fig. 3*b*). Unfortunately, we were unable to obtain sequence information of this peptide up to its *C*-terminal residue, as it was rapidly lost from the filter.

A second portion of peptide fraction pD_{31} was then subdigested with trypsin, resulting in much smaller peptide fragments, two of which (peptides $pD_{31}T_7$ and $pD_{31}T_{32}$) were useful in eventually elucidating the complete amino acid sequence of histone H1.2.

We were surprised to detect a methionine residue (position 43) in histone H1.2. Both histone H1.1 and histone H1.2 were found on a diagonal in acetic acid/urea/Triton X-100/PAGE and acetic acid/urea/PAGE two-dimensional gels, showing little or no affinity for the detergent (results not shown). On the other hand it is well known that methionine may bind Triton X-100 avidly, even to the extent that artificial isoforms produced by partial oxidation of methionine residues can be separated by acetic acid/urea/Triton X-100/PAGE (Zweidler, 1978). We have therefore checked whether any of the phenylthiohydantoin derivatives of modified residues such as ε-acetyl-lysine, ε-methyl-lysine, ε-dimethyl-lysine, phosphoserine and phosphothreonine would be co-eluted from the column with methionine phenylthiohydantoin derivative, but none so did. We therefore assume that the residue identified at position 43 in histone H1.2 is truly methionine and that its affinity for Triton X-100 is masked somehow by the neighbouring amino acid residues.

Proteinase-specificity

We have observed several unexpected cleavages of the polypeptide chain in the course of this study, e.g. an Ala–Ala cleavage by chymotrypsin and a Tyr–Lys cleavage by *S. aureus* V8 proteinase (Fig. 4). The cleavage of the Tyr-70–Lys-71 (Fig. 4) peptide bond in histone H1.2 by *S. aureus* V8 proteinase is remarkable. We have never observed this kind of cleavage before, nor did we find any evidence for it to occur after-Tyr-70 of histone H1.1 or after Tyr-65 of histone H1.2 (Fig. 5). One possible explanation would hold that Tyr-70 in histone H1.2 has been modified post-translationally in such a way (phosphorylation of the phenolic hydroxy function?) that it is recognized by the enzyme.

Another important observation concerns the specificity of the novel 'endoproteinase Asp-N', which has become commercially available only recently. This enzyme was claimed to be highly specific for Xaa–Asp bonds. However, we found that Xaa–Glu bonds were cleaved as well, though with lower efficiency.

Sequence comparison with histone isoform H1.1

A comparison of both H1 subtypes (Fig. 5) has shown that the central globular domain (His-37–Lys-113) has been well conserved. The *N*-terminal region Ser-1–Ala-36 has strongly diverged. In particular, several alanine residues in histone H1.1 seem to have been replaced by the more hydrophylic amino acids threonine and serine, and the net amount of positive charges in this region has decreased from 7 in histone H1.1 to 4 in histone H1.2. Several deletions occur in the *C*-terminal domain (Ala-114–*C*-terminus) and the very *C*-terminal region has diverged appreciably. Yet, as a whole, the *C*-terminal domain of histone H1.2 has preserved its typical character, including both potential *O*-phosphorylation sites. The amino acid chain of histone H1.2 is 17 residues shorter than that of histone H1.1.

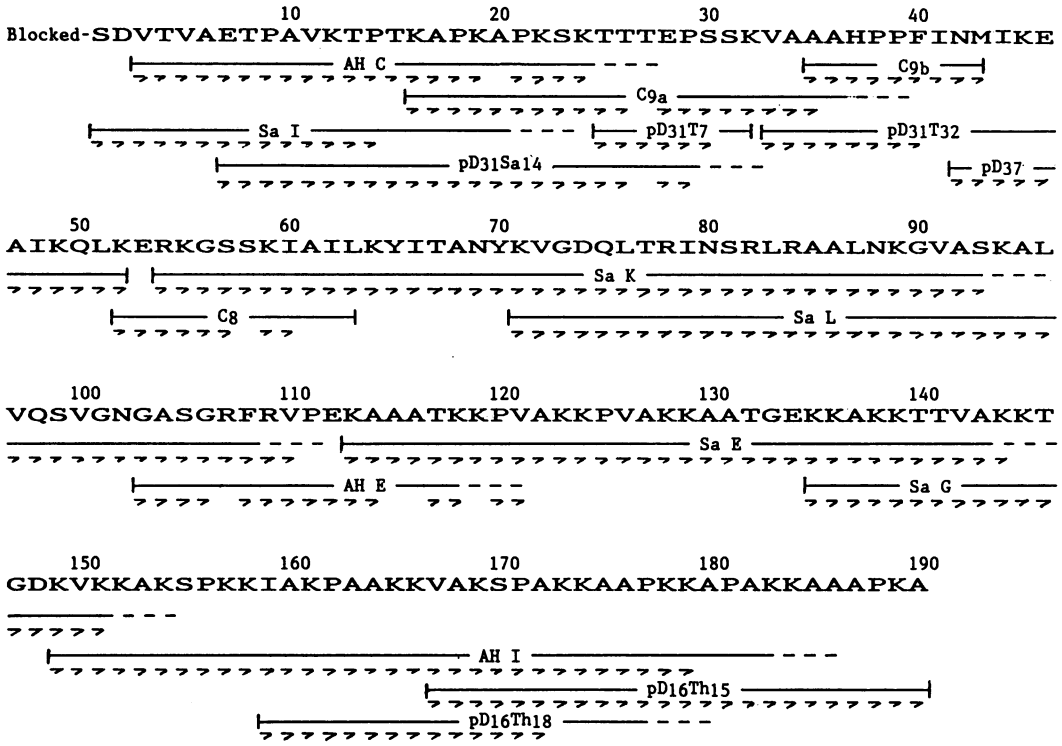

Fig. 4. Primary structure of histone H1.2 from *C. elegans*

Automated sequence analysis was carried out on peptides generated by acid hydrolysis (AH), by digestion with *S. aureus* V8 proteinase (Sa) and by digestion with endoproteinase Asp-N (pD). Peptide pD_{16} was further subcleaved with thermolysin ($pD_{16}Th$). A portion of peptide pD_{31} was cleaved with *S. aureus* V8 proteinase ($pD_{31}Sa$), and a second sample was subdigested with trypsin ($pD_{31}T$). Peptides C_8 and C_9 were resolved as minor peaks when a chymotrypsin digest of the pooled peptide bands AH-I, Sa-F and SA-E was separated by reversed-phase h.p.l.c. on a Poly F (Dupont) column. Unambiguous identification of amino acid residues is shown as ⇜⇝.

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Blocked-S D V T V A E T P A V K T P T K A P K A P K S K T T T E P S S K V A A A H P P F I N M I K E A I K Q
                          10                  20                  30                  40                  50
Blocked-S D S A V V A A A V E P K V P K A K A A K A A K P T K V A K A K A P V A H P P Y I N T I K E A I K Q

L K E R K G S S K I A I L K Y I T A N Y K V G D Q L T R I N S R L R A A L N K G V A S R A L V Q S V G N G A
              60                  70                  80                  90                  100
L K D R K G A S K Q A I L K F I S Q N Y K L G D N V I Q I N A H L R Q A L K R G V T S K A L V Q A K G S G A

S G R F R V P E K A A A T K K P V A - - - - - - - K K P V A K K A A T G E K K A K K T T V A K - - - - K T G
      110                 120                 130                 140                 150
N G R F R V P E K A A A A K K P A A A K K P A A A K K P A A A K K A T G E K K A K K P A A A K P K K A A T G

D - K V K K A K S P K K I A K P A A K K V A K S P A K K A A P K K A P A K K A A A P K A - - - - -
 160                 170                 180                 190                 200
D K K V K K A K S P K K V A K P A A K K V A K S P A K K A A P K K I A K P A A K K A A K P A A K A
```

Fig. 5. Comparison of the sequences of the histone isoforms H1.1 (lower lines) and H1.2 (upper lines) from *C. elegans*

Amino acid changes are marked by **bold** capitals; - indicates a deletion. Boxed sequences are potential *O*-phosphorylation sites.

Secondary-structure predictions for histone isoforms H1.1 and H1.2

An important question concerns the possible specific function of both H1 subtypes. The hydropathy profiles (results not shown) for histones H1.1 and H1.2 are very similar and not indicative of a different function of both isoforms. Secondary-structure predictions (Fig. 6) also reveal great similarity in the polypeptide backbones from about residue 40 up to the respective *C*-termini. Notably the *C*-terminal domains of both isoforms have a high potential for α-helix formation, and it has been proposed that it is this structured form that may bind DNA (Clark *et al.*, 1988). A high α-helix potential is also predicted for

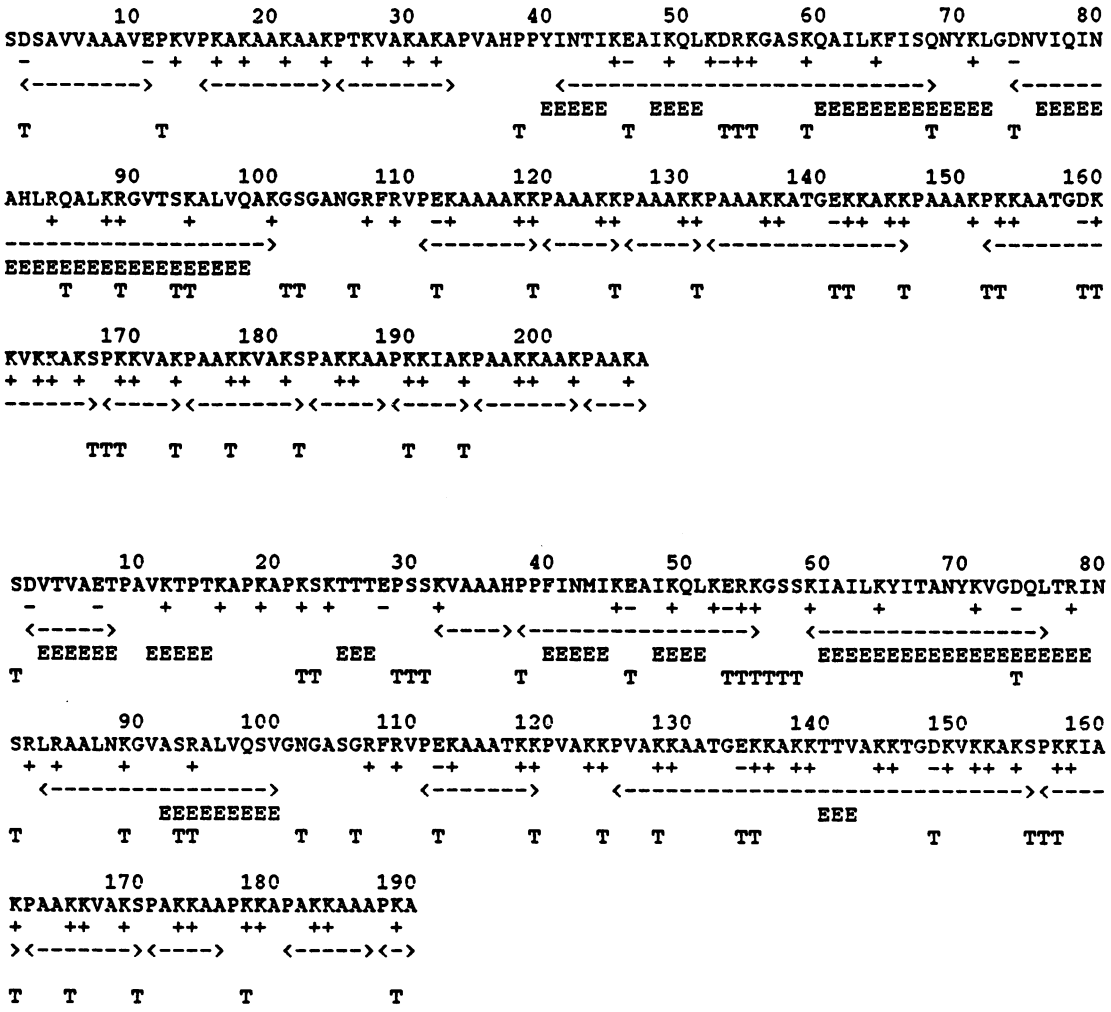

Fig. 6. Secondary-structure predictions for histone isoforms H1.1 (upper lines) and H1.2 (lower lines)

←----→, α-Helix; EEE..., β-sheet; T, turn; unassigned, coil.

the *N*-terminal residues 1–33 of histone H1.1. However, no such structure is predicted for this region in histone H1.2, where random-coil and perhaps some β-sheet formation appear more probable. Possibly this difference in secondary structure reflects a different function of histone H1.2.

We have also compared the two-dimensional Triton/acetic acid/urea/PAGE and acetic acid/urea/PAGE (Bonner *et al.*, 1980) gel pattern of whole *C. elegans* histone prepared from eggs, gravid worms and post-reproductive senescent nematodes to ascertain whether changes occur as a function of the developmental stage of the worms (units not shown). No such changes were observed in the H1 histone region. Thus, if histone H1.2 does have a significantly different function, it would appear to be tissue-specific rather than stage-specific. Further studies with selective antibodies directed against the H1.2 isoform may provide new insights.

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