Structural differences between somatic H2B and testis-specific TH2B histones of the rat[1]

T. Yongvanich and J. Svasti

Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400 (Thailand), 22 August 1983

Summary. Testis-specific histone TH2B from rat testis showed a very similar tryptic peptide map to somatic H2B from rat liver, indicating a common evolutionary origin. However, 5 peptide differences were detected between the 2 proteins, and the amino acid compositions of these distinctive peptides were determined.

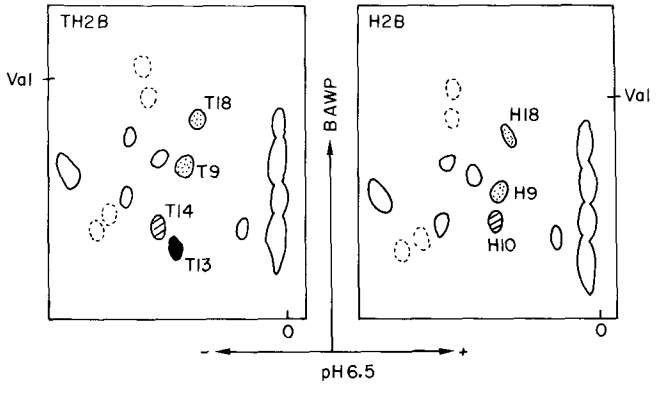
Mammalian male germ cells contain testis-specific histones TH1-XB (or H1t), TH2B and TH2A, absent from somatic cells[2-5]. Peptide map comparisons between testis-specific histones and their somatic counterparts have been reported for rat TH2A/H2A[5], and for rat TH2B/H2B and human TH2B/H2B[6]. In this paper, we report more detailed studies comparing rat TH2B and H2B, where an additional peptide difference was detected by peptide mapping, and 2 more differences were detected by amino acid analysis.

Materials and methods. Nuclear basic proteins were isolated from rat liver and rat testes by acid extraction of purified nuclei[7] and were subjected to chemical fractionation[8,9] to yield H2B (from liver) and H2B/TH2B (from testis). Separation of TH2B was performed by covalent chromatography on an organo-mercurial Sepharose column as described[10]. The purity of rat H2B and TH2B fractions was checked by electrophoresis in 15% polyacrylamide gels containing 2.5 M urea, 0.9 N acetic acid[11] or 0.4% Triton X-100, 2.5 M urea, 0.9 N acetic acid[12]. Purified rat liver H2B and rat testis TH2B were reduced with dithiothreitol, alkylated with iodo-[^{14}C]acetic acid, and subjected to tryptic peptide mapping on paper as earlier described[6], except that peptides were stained overnight with 0.03% ninhydrin in acetone instead of cadmium-ninhydrin. Selected peptides were eluted with 0.5% acetic acid, dried down, hydrolysed with 6 N HCl under vacuum at 105 °C for 20–24 h, and analysed on an LKB4101 amino acid analyser.

Results and discussion. The peptide maps obtained in the present study (fig.) were similar to those previously described[6], with TH2B and H2B showing obvious similarities, suggesting a common evolutionary origin. Of the 16–18 major peptide spots, attention will only be paid to those showing peptide differences between the 2 proteins. As noted earlier[6], TH2B contains 1 radioactive half-cystine containing peptide T13 (shown with dark shading) which was absent from H2B, and lacks 1 non-radioactive peptide H10 (shown with hatched lines) present in H2B. However, the present study also revealed a hitherto undetected difference, namely the presence of peptide T14 in TH2B and its absence in H2B. This different result could be due to the fact that prolonged (overnight) staining with ninhydrin was used here, instead of the brief (15 min) staining with cadmium-ninhydrin used earlier[6]. The use of dilute ninhydrin also enabled amino acid analysis to be performed, revealing the presence of 2 pairs of peptides T9/H9 and T18/H18 (shown with dots) of similar mobilities in TH2B and H2B, but of different composition.

The amino acid compositions of selected peptides from TH2B and H2B are shown in the table. Unfortunatley, the use of dilute ninhydrin staining causes partial destruction of the N-terminal amino acid and of lysine[13], making sequence determination difficult. Comparison of the composition of peptide H9 with the amino acid sequence of calf thymus H2B[14] suggests that it is the C-terminal tryptic peptide of rat liver H2B. Peptide T9, of similar mobility, has a similar composition but contains 1 extra threonine and isoleucine, and shows a lower content of tyrosine which is expected to be N-terminal in H9. Peptide H18 has a composition similar to residues 87–92 of calf thymus H2B[14]: -Ser-Thr-Ile-Thr-Ser-Arg. Peptide T18, apparently in a similar position in the map (fig.) but of slightly different electrophoretic mobility (table), has a similar composition but suprisingly contains two additional histidine residues.

3 peptides were present in one protein and absent in the other. Of these, peptide H10, found in H2B, which gave a consistently low yield of lysine on repeated analysis (probably due to destruction by ninhydrin), had high contents of alanine and proline, reminiscent of the N-terminal peptide in calf thymus H2B[14]. Unfortunately, it was not possible to locate peptides T13 and T14, found only in TH2B, by comparison with the



Tryptic peptide maps of reduced and ^{14}C-carboxymethylated rat testis TH2B and rat liver H2B. Peptides were fractionated by paper electrophoresis at pH 6.5 in the first dimension, followed by chromatography (BAWP) in the second dimension. Peptide differences between TH2B and H2B are marked as follows: dotted peptides (▨) were present in both proteins; hatched peptides (▨)were present in one protein but not the other; darkened peptide (■) was radioactive. The origin is indicated by O, and the marker valine by Val. Peptides shown in broken lines were present in lower yield.

Amino acid compositions of peptides distinguishing rat testis TH2B and rat liver H2B[a]

Amino acid	Peptide T9	H9	T18	H18	H10	T13	T14
Asp/CMCys[b]						0.6(1)	0.9(1)
Thr	2.3(2)	1.2(1)	1.9(2)	1.7(2)			1.4(1)
Ser	2.2(2)	2.2(2)	1.4(1–2)	1.2(1–2)	0.9(1)	0.8(1)	1.3(1)
Glu			0.4(–)	0.4(–)		1.4(1)	3.2(3)
Pro					3.9(4)	0.5(?)	
Gly					0.4(–)	1.7(2)	2.1(2)
Ala					3.9(4)	0.5(?)	1.7(2)
Val							0.6(1)
Ile	0.8(1)		1.1(1)	1.0(1)			
Tyr	0.4(?)	0.6(1)					
Lys	1.0(1)	0.8(1)			0.5(1)	0.9(1)	1.0(1)
His			2.0(2)				
Arg			1.0(1)	1.0(1)		1.1(1)	1.0(1)
Residues[c]	6–7	5	7–8	5–6	10	9–11	13
$m_{6.5}$[d]	–0.29	–0.30	–0.32	–0.26	–0.31	–0.31	–0.38
Yield[e]	19	13	17	28	12	5	10

[a] Data given as residues/mole peptide: values of 0.3 or less omitted; [b] Asp or CMCys not distinguishable on analyzer trace; [c] Sum of nearest integers; [d] Electrophorectic mobility at pH 6.5 relative to aspartic acid; [e] Yield of peptide in nmoles.

calf thymus H2B sequence. Both peptides contained carboxymethylcysteine (CM-Cys) or aspartic acid, which were indistinguishable in our amino acid analyzer system: peptide T13 probably contained CM-Cys since it was radioactive, while the non-radioactive peptide T14 probably contained aspartic acid. The amino acid analyses shown here clearly indicate the presence of structural differences between rat TH2B and rat H2B, which must be of genetic origin rather than due to post-translational modifications. Some of these differences, e.g. peptide H10, probably involve the highly variable N-terminal region. However, other differences, e.g. peptides H9/T9 and H18/T18 involve the C-terminal portion, which generally shows little variation in H2Bs of different species[15].

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Low-molecular-weight proteinase inhibitor in human plasma inhibiting papain and trypsin activity

I. Takiuchi, Y. Sei, H. Takagi and Y. Sagawa

Department of Dermatology, Showa University Fujigaoka Hospital, 1-30 Fujigaoka, Midoriku, Yokohama 227 (Japan), 18 November 1983

Summary. A low-molecular-weight proteinase inhibitor was isolated from human plasma on the basis of its papain-inhibiting capacity. The isolated fraction demonstrated inhibitory activity against papain and trypsin activity and the molecular weight was estimated to be approximately 3200 by gel-filtration.

Human plasma contains several proteinase inhibitors[1], but except for α_2-macroglobulin[2] and antithrombin III[3], there is no proteinase inhibitor which has inhibitory activity against plural proteinase groups such as cysteine, serine, carboxyl and metallo proteinases. We describe here the purification of a low-molecular-weight proteinase inhibitor from human plasma on the basis of its papain-inhibiting capacity using casein as a substrate. The isolated fraction demonstrated inhibitory activity not only against papain but also against trypsin.

Materials and methods. 45 ml of plasma obtained from a healthy human was eluted by diafiltration through an Amicon YM 5 membrane. The filtrate was then concentrated 3-fold by the use of an Amicon YM 2 membrane and chromatographed on a Sephadex G-25 column. Fractions number 90–99 with inhibitory activity against papain were combined and passed through 3 g of CM Cellulofine (Seikagaku Kogyo, Tokyo) which had been equilibrated with 28 mM phosphate buffer, pH 7.2. The eluate was then applied to DEAE Cellulofine (Seikagaku Kogyo) that had previously been equilibrated with the same buffer. The column (1 × 5 cm) was washed with equilibrating buffer and eluted with a linear gradient formed from 100 ml of equilibration buffer and 100 ml of 0.2 M NaCl containing same buffer, at a flow rate of 13.5 ml/h (3 ml per tube). The samples with inhibitory activity were combined and con-

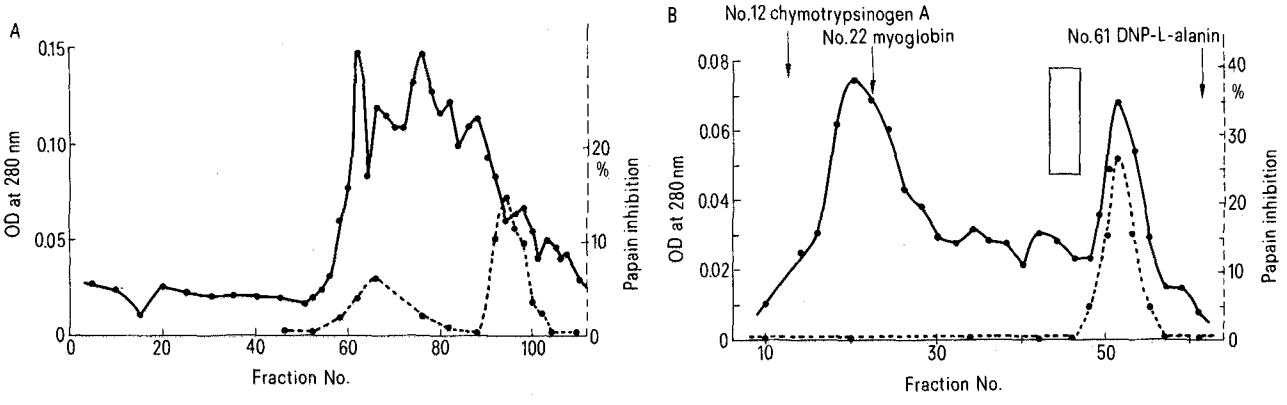

Figure 1. Gelfiltration of the low-molecular-weight proteinase inhibitor. *A* Plasma diafiltrate which was concentrated was applied on a column (2.64 × 90 cm) of Sephadex G-25 (Pharmacia Fine Chemicals) which had been equilibrated with 28 mM phosphate buffer, pH 7.2 and eluted with the same buffer at a flow rate of 13.5 ml/h. Fractions (6 ml) were collected and assayed for the inhibition against papain using casein as a substrate. *B* The sample that was partially purified by diafiltration, gelfiltration, CM Cellulofine and DEAE Cellulofine was applied on a column (1.9 × 85 cm) of Sephadex G-25 that had been equilibrated with the same buffer and eluted with equilibrating buffer at a flow rate of 13.5 ml/h. Fractions (3 ml) were collected and assayed.