THE PRIMARY STRUCTURE OF HISTONE H2B FROM BROWN TROUT (*SALMO TRUTTA*) TESTES

A. KOOTSTRA and G. S. BAILEY
Department of Biochemistry, University of Otago, Dunedin, New Zealand

Received 5 June 1976
Revised version received 9 July 1976

1. Introduction

Histones are basic proteins associated with the DNA of eukaryotic cells, and appear to have a fundamental role in the organisation of the chromosomes of higher organisms [1–3]. Comparative primary sequence information is available for most of the major histones (for recent review see [3]). However the complete primary structure of histone H2B is known only for calf thymus [4].

Here we present the primary sequence of histone H2B isolated from brown trout (*Salmo trutta*) testes. Comparison of the primary structure of histone H2B between trout testes and calf thymus indicates that the trout histone H2B differs by deletion of two residues, and substitution of seven other residues. All observed changes except one have occurred in the N-terminal half of histone H2B.

2. Materials and methods

Histone H2B was initially extracted from whole testes by a modification of the method of Johns [5], and purified to homogeneity by gel filtration on Bio-Gel P10 and Sephadex G-100. Fragments were produced by treatment of unmodified or maleylated H2B with trypsin, thermolysin, cyanogen bromide (CNBr), and *N*-bromosuccinimide (NBS).

Sequence analyses were carried out entirely by automated degradation in a Beckman 890C sequencer, and all residues were detected by gas liquid chromatography [6], thin-layer chromatography [7] and amino acid analysis after hydrolysis of the phenylthiohydantoins [8]. Details of the above methods and results will be published elsewhere.

3. Results

Hydrolysis of histone H2B for 24, 48 and 72 h revealed the following amino acid composition: $Lys_{20(19)}$, His_3, Arg_8, Asp_5, Thr_9, $Ser_{13(14)}$, Glu_9, Pro_5, Gly_9, Ala_{12}, Val_8, Met_2, Ile_7, Leu_6, Tyr_5, Phe_2.

The complete amino acid sequence of histone H2B as shown in fig.1 was deduced from automated sequence analysis and compositional analysis of (a) the whole protein, (b) CNBr and NBS fragments, (c) thermolysin peptide Th-1, (d) maleylated and demaleylated tryptic peptides.

All individual peptides were aligned by overlap, with the exception of MT7 and MT8, whose relative positions were aligned by homology with the calf histone sequence. MT9 is the only peptide lacking arginine and must be C-terminal. The relative positions of dMT9c and dMT9d are established since NBS treatment of MT9 gives only 2 peptides, one of which is Thr-Ser-Ser-Lys, which must be C-terminal.

A partial sequence of the first 22 residues of H2B from rainbow trout (*Salmo gairdneri*) has been previously published [9]; those residues which were positively identified are identical to the brown trout sequence.

North-Holland Publishing Company – Amsterdam

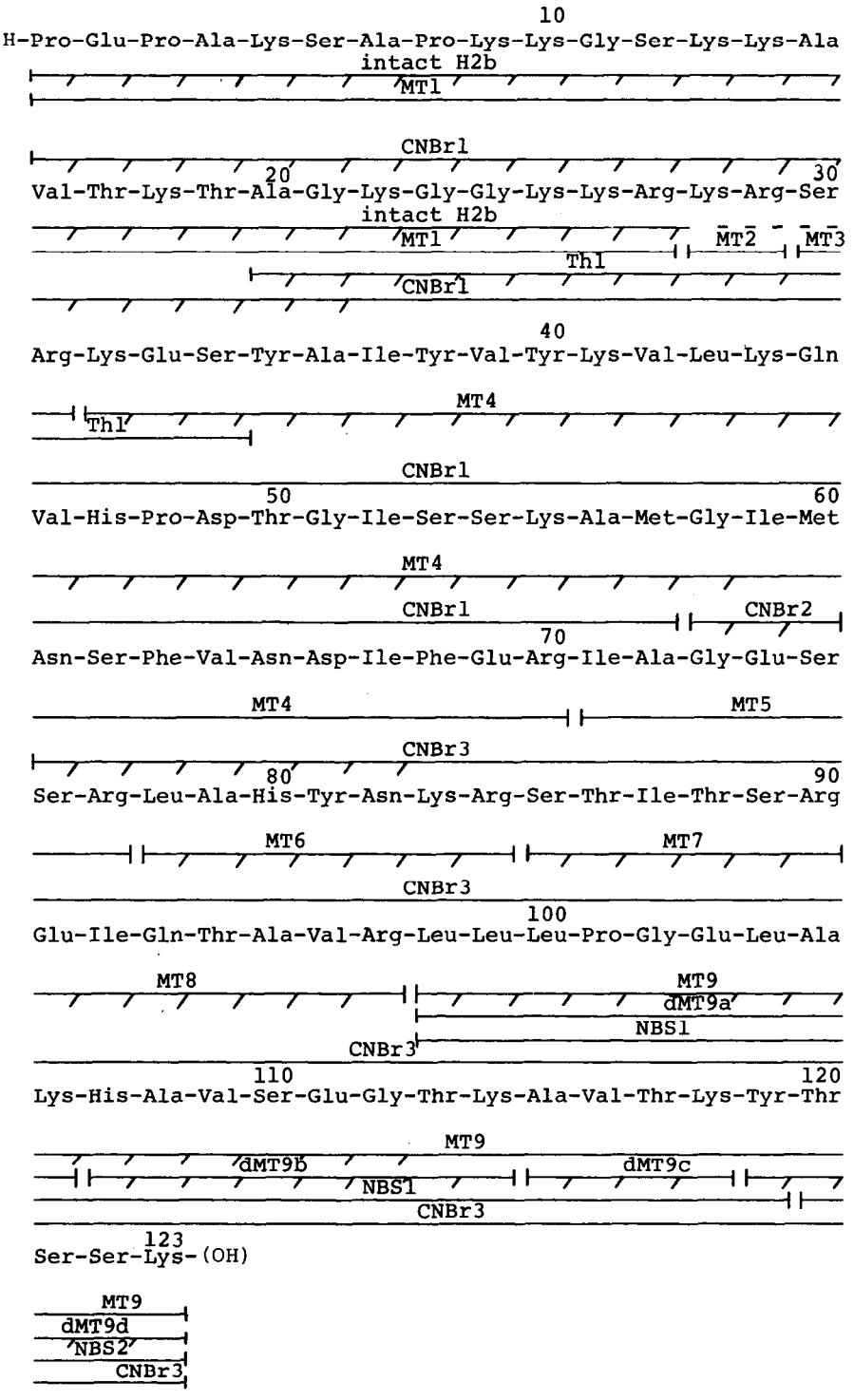

Fig.1. The amino acid sequence of histone H2B isolated from trout (*Salmo trutta*) testis. Residues identified directly from sequence analysis are shown as ——/ / /——

4. Discussion

The observed sequence differences between calf histone H2B and the trout histone H2B could have arisen through several different pathways. Assuming that substitutions occur more frequently than insertions or deletions, the calf histone H2B differs from that of the trout histone by the insertion of -Ala-Pro- after Pro #8 in trout, and the substitutions of Ala for Thr #19, Gln for Ala #20, Lys for Gly #21, Asp for Gly #23, Ser for Ala #36, Val for Ile #37, and Ala for trout Ser #75. Considering each difference as a single mutational event the calculated rate of change for histone H2B is 0.16 residues/100 residues per 10^7 years. This rate is identical to that shown by histone H2A [10] and offers further evidence for the pair-wise behaviour of these two histones within the subunit structure of chromatin [1].

However all of the above changes except one have occurred in the amino terminal 37 residues of H2B, which is in striking contrast to the highly conserved sequence of this region in histones H2A, H3 and H4. In fact two of these changes alter the spacing of the basic residues, and the replacement of alanyl residue with bulky glutamine side chain and that of glycyl residue with charged lysine or aspartic side chain have to be considered as non-conservative changes. Therefore, while the amino terminal region of histone H2B has been implicated in intimate binding to the DNA [4,11,12] within the subunit structure of chromatin, models for the mode of binding in which the exact placement of basic residues and/or the nature of the side chains of non-basic residues are critical (e.g., helical arrangements) [13] should be examined closely for this histone.

Acknowledgements

The authors wish to thank Mrs D. B. Gillett for valuable technical assistance. This work was supported by grants from the Medical Research Council of New Zealand and the New Zealand Grants Committee Research Fund.

References

[1] Thomas, J. and Kornberg, R. D. (1975) FEBS Lett. 58. 353–358.
[2] Oudet, P., Gross-Bellard, M. and Chambon, P. (1975) Cell 4, 281–300.
[3] Elgin, S. C. R. and Weintraub, H. (1975) Ann. Review Biochem. 726–774.
[4] Iwai, K., Hayashi, H. and Ishikawa, K. (1972) J. Biochem. 72, 357–367.
[5] Johns, E. W. (1964) Biochem. J. 92, 55–59.
[6] Pisano, J. J., Bronzert, T; J. and Brewer, H. B., Jr. (1972) Anal. Biochem. 45, 43–59.
[7] Summer, M. R., Smythers, G. W. and Oroszlan, S. (1973) Anal. Biochem. 53, 624–628.
[8] Smithies, O., Gibson, D., Fanning, E. M., Goodfliesh, R. R. H., Gillman, J. G. and Ballantyne, D. L. (1971) Biochem. 10, 4912–4921.
[9] Candido, E. P. M. and Dixon, G. H. (1972) Proc. Natl. Acad. Sci. USA 69, 2015–2019.
[10] Bailey, G. S. and Dixon, G. H. (1973) J. Biol. Chem. 15, 5463–5472.
[11] Bradbury, E. M. and Rattle, H. W. E. (1972) Eur. J. Biochem. 27, 270.
[12] Weintraub, H. (1975) Proc. Natl. Acad. Sci. USA 72, 1212–1216.
[13] Sung, M. T. and Dixon, G. H. (1970) Proc. Natl. Acad. Sci. USA 69, 1616–1623.