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ABSTRACT

Mammalian testes contain a testis-specific histone TH2B, absent from somatic cells. Several techniques for the purification of TH2B from rat testes and for assessing the purity of such preparation of TH2B have been tested. In terms of analytical techniques, polyacrylamide gel electrophoresis in 2.5M urea, 0.4% triton X-100, 0.9N acetic acid is capable of resolving TH2B from all other rat testis histones. In addition, the determination of N-terminal residue by the dansyl procedure was considered to be useful in detecting small amounts of H3 contamination in preparation of TH2B.

Hydroxylapatite chromatography combined with covalent chromatography employing PCMB-AE-Sepharose was found to be effective for TH2B-isolation. In this procedure, sheared chromatin fragments have been bound to hydroxylapatite columns and histones sequentially stripped off by stepwise elution with increasing NaCl concentrations, so that a fraction containing only TH2B, H2B and H2A could be obtained. Subsequent covalent chromatography using PCMB-AE-Sepharose could specifically isolate TH2B from this fraction making use of its half-cystine residue. The TH2B preparation obtained is of high purity as judged by the single band observed on triton-acid-urea polyacrylamide

gel and by the fact that only DNS-Pro could be detected upon dansylation of the N-terminus of the protein indicating no H3 contamination.

Comparison of the structures of rat testis TH2B and somatic H2B from rat liver by peptide mapping on SDS polyacrylamide gel and high voltage paper electrophoresis indicated some difference in structures of these two proteins.