THE NON-HISTONE PROTEINS OF CHROMATIN

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1. Introduction

Previous work from our laboratory has shown that many cytoplasmic and nuclear sap proteins can be non-specifically adsorbed to chromatin during saline (0.14 M NaCl) washing [1]. It was also shown that many of these adsorbed proteins could be removed from the chromatin using 0.35 M NaCl. We have now to report that if this procedure is carried out prior to the procedure for preparing non-histone chromatin proteins, based on that described by Wang [2] the yield from calf thymus is zero.

Further, if care is taken to prepare soluble calf thymus deoxyribonucleoprotein (DNP) [3] free of contamination from suspended material then no "alkali soluble" non-histone nuclear protein can be obtained from calf thymus, following the procedure of Wang et al. [4] as modified by Busch and Steele [5].

2. Methods and results

2.1. *Experiment 1.*

Calf thymus (100 g) was blended with 1 litre of 0.14 M NaCl pH 7.0 for 2 min in an M.S.E. Atomix at top speed and the solution filtered through 4 layers of surgical gauze. The chromatin was recovered from the filtrate by centrifuging at 1000 *g* for 20 min, and washed 5 more times in a similar manner.

The sediment was then divided into 2 equal portions and one portion washed 6 times, using 200 ml of 0.35 M NaCl pH 7.0 and blending in the Atomix for 30 sec at half speed. (This procedure does not reduce the yield of DNA or histone.) The 2 portions were then treated separately but identically as follows. The sediments from the last wash were blended in 150 ml of 0.14 M NaCl and 150 ml of 2 M NaCl pH 7.0 added. The solutions were then blended for 5 min at half speed in an M.S.E. Atomix, stirred for 1 hr and centrifuged at 40,000 *g* for 30 min. The supernatants obtained were then diluted by the addition of 600 ml of water at pH 7.0. This decrease in the ionic strength causes the recombination of the DNA and the histones, and the complex was then removed by centrifugation. The remaining supernatants were clarified by filtering through a number 4 sintered glass funnel and any protein in solution recovered by the addition of trichloroacetic acid to 10%. 30 mg of protein was obtained from the chromatin which had received the usual saline washing, but the yield was zero from the chromatin which had received the additional washing in 0.35 M NaCl. Polyacrylamide gel electrophoresis in low pH gel [6] showed that the proteins obtained by this procedure (i.e. without the preliminary washing in 0.35 M NaCl) are similar to the proteins removed from the chromatin with 0.35 M NaCl. It appears therefore that this preliminary washing in 0.35 M NaCl removes the non-histone proteins which would normally be obtained by procedures similar to that described by Wang [2]. The amount of non-histone protein extracted by 0.35 M NaCl varies from 5% to 10% of the histone content.

2.2. *Experiment 2*

A solution of DNP was prepared by a method derived from the methods of Zubay and Doty [3] and Garrett [7] as follows. Calf thymus (40 g) was

blended in 500 ml of 0.075 M NaCl, 0.024 M EDTA pH 8.0 for 45 sec at full speed in an M.S.E. Atomix blender. The solution was filtered through 4 layers of surgical gauze and the chromatin recovered from the filtrate by centrifugation at 1,000 *g* for 20 min and washed 4 times more in a similar manner. It was then washed 6 times with 0.35 M NaCl, .001 M EDTA pH 8.0 and finally with .075 M NaCl, .025 M EDTA pH 8.0 in a similar manner. The chromatin (containing 0.5–0.7 g DNA) was then blended in 1 litre of double-distilled water (to which had been added 0.75 ml of N-NaOH) for 30 sec at half speed in the Atomix and then stirred rapidly with a propeller stirrer for 2 hr. 3.5 ml of 0.2 M phosphate buffer pH 7.0 was then added and the solution stirred for 30 min. To remove undissolved matter the solution was centrifuged at 35,000 *g* for 1 hr. The supernatant which contained over 95% of the original DNA was made 2 M with respect to NaCl and stirred for 30 min. The clear viscous solution was centrifuged for 2 hr at 35,000 *g*. No sediment was obtained showing that DNP prepared in this manner is completely dissociated and dissolved in 2 M NaCl. It also indicated that the "alkali soluble" proteins are in fact derived from undissolved matter suspended in the chromatin when it is dissolved in low ionic strength solutions.

3. Discussion

Preliminary experiments on the purified deoxyribonucleoprotein prepared as described above (i.e. after 0.35 M NaCl extraction and removal of "alkali soluble" proteins) show that the non-histone protein present is approx. 3% of the histone content. Because of the considerable interest shown in these proteins recently and their possible implication in derepression mechanisms [8] it is extremely important to ensure that the proteins described as non-histone chromosomal proteins are indeed so. We do not know if all the 0.35 M NaCl extracted proteins are cytoplasmic contaminants or if some are of chromosomal origin but we feel that the content of non-histone chromosomal protein is considerably less than the values given in previous reports [9–11], which indicate that 25–30% of the calf thymus chromatin protein is non-histone.

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