ANTIGEN-INDUCED CHANGES IN LYMPHOID CELL HISTONES

IV. Changes in Solubility of Isolated Chromatin

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ABSTRACT

In this study we have examined the solubility of deoxyribonucleoprotein (DNP) isolated from control and antigen-affected thymocytes. 2-M sodium chloride extracts containing the DNP of rat thymus glands were serially diluted. A comparison was made of the effect of dilution on fiber formation in the control and test series. Fiber formation is usually complete for the control material at a salt concentration between 0.63 and 0.57 M. The test material shows some fiber formation within this range. However, a significant portion of the DNP is precipitated at dilutions of 0.54–0.48 M. Ammoniacal silver (A–S) stains the control fibers a characteristic yellowish color. With the test material, those fibers formed within the control range tended to be stained yellowish brown by A–S, whereas those formed only after greater dilution stained blackish. These data, coupled with our previous observations on altered A–S staining, clearly demonstrate an antigen-induced physical and/or chemical alteration of the histone or histone–DNA complex of lymphoid cell chromatin.

INTRODUCTION

In vivo or in vitro exposure to antigen causes an acute transitory change in the ammoniacal silver (A–S) stainability of lymphoid cell nuclei. This alteration is also demonstrable in chromatin fibers isolated from such nuclei. However, no associated change was demonstrated in the amount of histones. The A–S staining and disc electropherograms of the isolated histones are similar in the control and test material. Furthermore, prolonged pretreatment with formalin before A–S staining yields a control type of staining in antigen-affected nuclei and DNP fibers. These various data were interpreted *in toto* as as indicating that antigens cause a change in histone–DNA binding (1).

If antigens truly alter histone–DNA binding, physio-chemical properties other than the A–S staining might also be changed. Since the formation of histone–DNA complexes alters the solubility of the reactants, it occurred to us that antigen-induced alterations in binding might change the solubility. This paper reports on alterations in the solubility of chromatin obtained from from antigen-affected thymocytes.

MATERIALS AND METHODS

Thymus glands were removed from 21-day-old weanling Lewis rats of the same litter. The separated lobes of three or four glands were placed in a tube containing 2 ml of 0.16 M NaCl plus 0.2 ml of tetanus toxoid diluted 1:10 with 0.16 M NaCl. A matched number of lobes was placed in another tube containing only the saline solution. The tubes were incubated for 2 hr at 37°C with constant agitation. The thymus tissue was then rinsed with cold (0–4°C) 0.16 M NaCl and then homogenized and extracted

in 25 ml of cold 2 M NaCl for 1 hr. Constant magnetic stirring was maintained during the extraction period. All phases of the extraction and isolation procedures were performed in the cold (0–4°C). After the extraction period the homogenate was centrifuged for 20 min at 17,000 *g*, and the supernate was decanted. The supernate was diluted with cold distilled water to a final molarity of 0.16 M. Such dilution caused the appearance of DNP fibers which were collected on a glass rod, rinsed in cold 0.16 M NaCl, and then dissolved in 2 M NaCl. The latter solution was then centrifuged as before, and the clear supernate was collected.

Fibers were prepared from the DNP solution by the following procedures.

(*a*) Approximately 0.25 ml of the DNP solution was added to a large excess (100–125 ml) of 0.16 M NaCl solution. The fibers that formed after mixing were taken up on a microscope slide and fixed while wet with 10% acetate-neutralized formalin (2 g sodium acetate per 100 ml of 10% formalin).

(*b*) An aliquot of the 2 M NaCl solution of DNP was subjected to stepwise dilution with distilled water. Fibers that formed upon each successive dilution were removed with a wooden applicator stick, rinsed in 0.16 M NaCl, then transferred to glass slides, and fixed in 10% acetate-neutralized formalin. At each successive dilution, the prominence of the fiber formation and the viscosity of the solution was qualitatively noted and a value from 0 to 4 plus was assigned in relation to the amount of fiber formation upon dilution to 0.16 M.

The formalin-fixed fibers were stained with ammoniacal silver (A–S) after exposure to the formalin for 1, 24, and 72 hr (2). In all instances the fibers from the control and antigen-exposed thymus glands were fixed and stained together.

RESULTS

Fiber Formation

Dilution of the DNP in 2 M NaCl to a final concentration of 0.40 M NaCl caused the formation of a fibrous gel in both the control and the antigen-affected material. This gel readily adhered to a wooden applicator stick and could be removed with ease from the mother liquor. Further dilution of the 0.4 M NaCl solution failed to elicit additional fiber formation although a faint turbidity tended to appear and persist from dilution of 0.4 through 0.16 M NaCl.

When the control 2 M NaCl solution is diluted in a more gradual fashion, the solution becomes highly viscous and traces of fibers are seen at between 0.80 and 0.67 M. The formation of well-defined fibers seems to take place all at once at a given dilution, although the exact dilution at which this occurs is somewhat variable. In four separate experiments in which all conditions were alike, the main fiber-forming point fell between 0.62 and 0.57 M for control thymocytes. In no case did further dilution result in the formation of additional fibers, and after removal of the fibers the viscosity remained similar to that of water. In antigen-affected DNP a variable quantity of fibers is formed within the control range. Such fibers accounted for one-half to a mere trace of the total number of fibers. The remainder came out of solution upon further dilution, e.g. in some preparations fiber formation in antigen-affected material was not complete until 0.48 M.

The fibers formed at various dilutions were redissolved in aliquot volumes of 2 M NaCl for spectrophotometric analysis. The relative amount of material precipitated at the various dilutions was estimated from the absorbance of these solutions at 2300 and 2600 A.

A–S Staining

Fibers which are formed by the addition of just enough distilled water to bring all of the fibers out of solution stain like those formed by addition of enough water to bring the dilution to that of physiological saline (0.16 M). That is, the control fibers stain yellowish whereas the antigen-affected samples stain brownish black. However, differences are found in the staining of the test fibers formed at different dilutions, namely those formed in the control range stain like the control while those formed only after greater dilution are stained black.

COMMENTS

The present study has confirmed our previous observations that antigens alter the A–S stainability of thymocyte DNP. Since the altered staining occurs without demonstrable change in the isolated histones, we had suggested that the change in A–S staining reflects a change in histone-DNA binding.

The ability of salt solutions of high ionic strength to solubilize DNP is apparently dependent upon neutralization of the attractive forces between the DNA and histones (3). Upon dilution the histone and DNA recombine and precipitate out of solution in the form of fibers. To what extent the recombination duplicates the native state is

not clear. However, under similar conditions the type of recombination should be reproducible. On the other hand, alterations in binding tendency might influence the recombination and subsequently the fiber formation. A change in the solubility characteristics of DNP is suggestive of a change in the histone–DNA binding. Since the data clearly demonstrate a change in the solubility characteristic of antigen-affected chromatin, we interpret these findings as supporting our previous suggestion that antigens induce a change in histone–DNA binding properties.

It is also pertinent to note that those fractions of the antigen-affected DNP which exhibit solubility similar to that of the control DNP also stain similarly with A–S. In contrast, the fraction which differs from the control in solubility also exhibits the most striking difference in A–S staining.

Taken together, the altered A–S staining and the altered solubility leave little doubt that antigens induce alterations in the chromatin of lymphoid cells. The findings are of particular interest since this system utilizes a physiological stimulus demonstrated to produce the same highly selective changes in lymphoid cells both in vivo and in vitro. The data suggest that the histone–DNA bindings may provide an environmentally sensitive mechanism whereby external stimuli may be translated into altered DNA function. It should also be recalled that A–S staining differentiates various cell types and tends to be altered in a characteristic fashion in cancer cells (4). Data will be presented elsewhere which indicate that the DNP isolated from such cells stains like the nuclear chromatin *in situ*. Furthermore, the isolated DNP exhibits distinctive solubility characteristics. A more complete understanding of the molecular basis of the observed variations in nuclear chromatin might provide new approaches to the investigation of cells in normal and pathologic states.

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