STRUCTURAL CHANGES IN CHROMATIN UNDER THE INFLUENCE OF PHYTOHEMAGGLUTININ DURING ITS POSSIBLE CONTACT IN THE CELL

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Interaction between phytohemagglutinin (PHA) and the DNP-systems of chromatin was investigated by a combined thermo-mechanical method. The character of action of PHA was shown to depend on its concentration. Small doses (of the order of 0.0001%) disturb the structure of DNP-systems and weaken intermolecular interaction in a similar way to small doses of radiation. The action of high PHA concentrations (0.003%) leads to stabilization of the structure. The two effects of PHA are dependent on the state of the intermolecular bonds, which varied at different stages of cell function.

The mechanisms triggering mitosis constitute a vital problem in modern biology, but one in which progress so far has been extremely limited. This is due primarily to technical difficulties in the approach to the study of the initimate mechanisms by which the living cell works. An important step forward in the solution of this problem was the discovery of compounds which can initiate cell division (mitogens). The mitogen which has received most study is phytohemagglutining (PHA). At the same time, the identity of the object in the cell on which PHA acts to stimulate the onset of mitosis is not yet known. A number of hypotheses have been put forward. The authors of one hypothesis [1] suggest that PHA modifies the permeability of cell membranes, so that the balance of the intracellular components is disturbed, and it is this, according to these workers, which acts as the stimulus for mitosis. Another similar point of view [5] links the initiation of mitosis under the influence of PHA with its action on mechanisms of the cell membrane controlling division. According to yet another view the action of PHA resembles that of an antigen [8, 13].

However, regardless of which view is more correct, it should be emphasized that initiation of mitosis under the action of PHA is always accompanied by changes (activation or derepression) of the cell chromatin. This is also manifested as activation of RNA synthesis [6, 12], as acetylation of histones [15], and as intensified interaction of luminescent compounds with the chromatin, staining the DNA in it [10]. The question thus arises whether stimulation of mitosis is the result of direct contact between PHA or other mutagens with the chromatin of the cell nucleus. This view, expressed indirectly, crops up in a number of papers [9].

In the investigation described below an attempt was made to discover the possible effect of PHA directly on the supermolecular DNP-systems of chromatin isolated from cells. The thermomechanical method of investigation of supermolecular DNP-systems suggested by the writers previously [3], unlike other methods of DNP investigation, yields information not only on physicochemical or functional changes taking place in DNP, but also on local modifications in the systems analyzed, including the number and character of intermolecular interaction in them. These alter the method of supermolecular organization of the DNP-systems of the chromatin, and this is yet another way whereby the functional activity of the genome is controlled.

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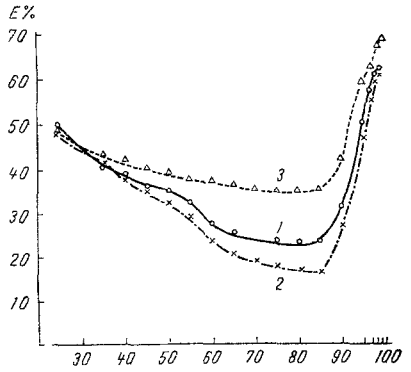

Fig. 1

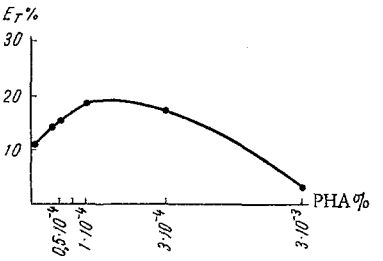

Fig. 2

Fig. 1. Relative extensibility of DNP-fibers as functions of temperature in 0.14 M NaCl solution (1), in 0.14 M NaCl + 0.0001% PHA (2), and in 0.14 M NaCl solution + 0.003% PHA (3). DNP concentration 0.3 mg/ml, N/P = 4.2, $[\eta]$ =32 dl/g. Abscissa, temperature (in °C); ordinate, relative extensibility of DNP-fibers (in %).

Fig. 2. Amplitude of flow of DNP-fibers as a function of PHA concentration in the medium DNP concentration 0.4 mg/ml, N/P =4.0, $[\eta]$ =48 dl/g. Abscissa, PHA concentration in medium (in %); ordinate, amplitude of flow of DNP-fibers (in %).

EXPERIMENTAL METHOD

Supermolecular DNP-systems obtained from calf thymus DNP isolated in 0.7 M NaCl solution were investigated. The protein and DNA concentrations were determined by the methods of Lowry et al. [11] and Spirin [2] respectively. The intrinsic viscosity of the preparations was determined with a three-ball low-gradient viscosimeter of the Ostwald type with gradients of β =50 sec^{-1}, β =35 sec^{-1}, and β =23 sec^{-1} respectively relative to water. Supermolecular DNP-systems were obtained and investigated as described previously [4]. PHA (Difco) was used.

The action of PHA was judged from changes in the thermomechanical indices of the supermolecular DNP-systems (fibers) in medium containing PHA compared with those in a control medium (physiological NaCl solutions, μ =0.14, without PHA). The temperature of the medium was maintained with an accuracy of ±0.05° C. The relative extensibility (E%) of the DNP-fiber was calculated from the ratio:

$$E = \frac{l_t - l_0}{l_0} \times 100\%,$$

where l_0 is the length of the fiber at the moment when it was obtained, before relaxation, and l_t the length of the fiber at a specific temperature. The amplitude of flow (displacement of the centers of gravity of the molecules – irreversible deformation) was determined from the ratio:

$$E_T = \frac{l_{25°\,r} - l_{60°}}{l_{25°\,r}} \times 100\%,$$

where $l_{25°\,r}$ is the length of the DNP fiber after relaxation at 25° C and $l_{60°}$ is the length of the DNP fiber at 60° C, the temperature of the maximum of flow.

EXPERIMENTAL RESULTS

Results showing the relative change in extensibility of the DNP-systems with temperature are shown in Fig. 1. The effect of PHA on the amplitude of flow of these systems as a function of concentration is shown in Fig. 2. The significant action of PHA on the structural parameters of the DNP-systems is visible in Fig. 1. This action, as Fig. 2 shows, is obviously biphasic in character, depending on the PHA concen-

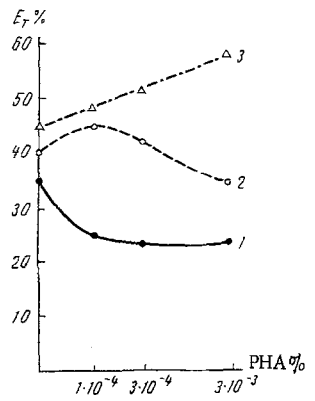

Fig. 3. Amplitude of flow of DNP-fibers as a function of DNP concentration: 1) 0.45 mg/ml; 2) 0.4 mg/ml; 3) 0.37 mg/ml; N/P = 4.0, $[\eta]$ = 48 dl/g. Abscissa, PHA concentration in medium (in %). Ordinate, amplitude of flow of DNP-fibers (in %).

tration. Low concentrations of the mitogen (0.00005-0.0001%) induce a sharp flow of DNP, while high concentrations of PHA (around 0.003%) have the opposite effect and cause stabilization of the system.

The flow of supermolecular DNP-systems is known to be associated with disturbance of intermolecular interactions either through direct rupture of intermolecular bonds or (secondary effect) through dissociation of the protein component of the DNP-complex, the donor of the intermolecular bonds. In the system under examination the first mechanism is considered to be predominant. This conclusion is based, in particular, on the relative numbers of PHA and DNP molecules in the system. With a PHA concentration of 0.0001% and a DNP concentration of 0.03% in the fiber (taking the molecular weight of DNP as 20×10^6), this gives approximately 1 PHA molecular weight 30×10^3) per molecule of DNP. At the same time, individual macromolecules in supermolecular DNP-systems are linked [4] by a very infrequent system of intermolecular bonds (not more than 10 per DNP molecule). With a molar ratio of PHA:DNP = 1:1 it is difficult to imagine that any significant dissociation of the protein component takes place. However, disturbance of a very few intermolecular bonds is substantially reflected in the properties of the supermolecular system for their very existence is due to single intermolecular contacts. This situation is highly reminiscent of the reaction of DNP to small doses of ionizing radiation: a dramatic change in the system as a whole although injury (solitary) to its components is slight [4]. The effect of small concentrations of PHA can evidently be explained on these grounds. With an increase in the PHA concentrations in the reaction mixture the DNP-system begins to react in the opposite way: by stabilization. In this case, an increase in the PHA concentration evidently leads to some degree of deproteinization of the complex. The molecules of the completely dissociated or merely labilized protein themselves give an additional number of intermolecular contacts, thus giving rise to stabilization of the DNP-system as a whole. This hypothesis is confirmed by investigation of the action of PHA on DNP-systems obtained from solutions with different DNP concentrations. As Fig. 3 shows, the action of low PHA concentrations on DNP in higher concentrations (equivalent to an increase in the number of intermolecular bonds in the DNP-system) leads to a decrease in the flow i.e., to stabilization of the DNP-systems. This is only natural because the number of intermolecular contacts in the system in that case is now sufficiently large.

With a DNP concentration of 0.45 mg/ml a decrease in the amplitude of flow is observed under the influence of both large and small PHA concentrations, and the stabilizing action increases with an increase in the PHA concentration.

With a DNP concentration of 0.4 mg/ml, PHA acts in two ways: the flow of the fibers is increased by PHA in low concentrations while the DNP-structure is stabilized by high concentrations. With a further decrease in the DNP concentration, instead of stabilization, PHA induces flow of the DNP-systems, and this increases with an increase in the PHA concentration. The action of PHA on DNP-systems is thus complex in character: besides its obvious deproteinizing effect it simultaneously induces cross-linking of the DNP-structure. Whichever action is manifested is evidently dependent on the state of the intermolecular bonds of the DNP-structure and these, in turn, depend on the DNP composition, which varies dynamically during cell function. If the number of intermolecular bonds is insufficient, the deproteinizing action is stronger than the cross-linking. If the number of intermolecular contacts is considerable, even a low PHA concentration is sufficient to stabilize the DNP-system.

These results agree to a certain extent with those of other workers who demonstrated differences in the viability of the cell cultures of the lymphocytes [14] and in the level of RNA synthesis [7] during changes in the PHA concentration in the medium. Although the results are interpreted by the authors cited from different standpoints from those used in this paper, it is important to emphasize that on the basis of their results the mitogenic action of PHA can be regarded as either activation or repression of the chromatin.

The experiments described above thus indicate that, in principle, it is possible to modify the supermolecular systems of the DNP of the chromatin and, consequently, its functional activity. If direct contact

between PHA and chromatin is possible in the cell it is very probable that the modification of the chromatin observed in these experiments may be a decisive factor in the analysis of the trigger mechanisms of mitosis during the action of mitogens.

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