Changes in the Macromolecular Structure of DNA-RNA-Protein Complexes Induced by Gel-Filtration on Sephadex G-25

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Changes in the Macromolecular Structure of DNA-RNA-Protein Complexes Induced by Gel-Filtration on Sephadex G-25 cause a disruption of the macromolecular structure in nucleoproteins. The effect of these shearing forces can be observed both in electron micrographs and by electrophoretic analysis. The decrease of the molecular weight and also the changes in the electronegative charge/molecular weight ratio cause an increase of the electrophoretic mobility of the gel-filtered highly polymerized DNA and of the RNA complexed to it compared with the not gel-filtered nucleoprotein.

Certain macromolecular alterations induced in mice spleen nucleoproteins by gel-filtration on Sephadex G-25 are being described in this work.

Nucleoproteins (NP) extracted from mice spleen with sodium lauryl sulphate 5% in ethanol 45°, by a method described in a previous paper, contained RNA and DNA in a ratio of 66.3 ± 11.1 per cent.

An important part of this RNA (30%) showed resistance to RNase and hence was complexed to DNA. Approximately 50% of the complexed RNA were bound to DNA by a protein susceptible to trypsin treatment; the remainder was bound to DNA by certain other bonds — so far unidentified — but apparently stronger than hydrogen bonds (thermal denaturation failed to split such RNA fractions from the complex and to sensitize them against RNase).

The agarose gel electrophoresis of mice spleen NP revealed the presence of two fractions, both with anode migration (fig. 1c).

![Fig. 1. Agarose gel electrophoresis of mice spleen NP.](image)

1 Gr. Ghyka, Cl. Dumitrescu, and C. David, Rev. roum. Biochem. 6, no. 2, 125 [1969].
One fraction representing the major component migrated very slowly. The second one representing a minor component migrated fast (14 mm/hour) and disappeared almost completely after RNase treatment of the NP (final concentration of the RNase – 100 μg/ml; 2 hours incubation at 37 °C) (fig. 1 d).

Hence, the DNA-RNA-protein complex represented the slow moving fraction, the rapidly moving one consisted almost exclusively of free RNA susceptible to RNase treatment.

The above data are in close agreement with previous results of studies on the nucleoprotein extracted from rabbit spleen by a similar method 2. NP gel-filtration on Sephadex G-25 columns showed an elution pattern with a single peak (fig. 2) formed by DNA, RNA and protein. If this peak was concentrated and then investigated electrophoretically it revealed the presence of a single fraction (fig. 1 b) which migrated more rapidly (14 – 15 mm/hour) than the slow-moving fraction of the not gel-filtered NP. RNase treatment of gel-filtered NP did not affect the migration velocity of this single fraction, which fact demonstrated its resistance to RNase.

Electron microscopic examination of the mice spleen nucleoprotein (negatively stained with 1% phosphotungstic acid, 30,000 ×) revealed a network-like structure built up by large fibers of different sizes which were randomly crossed in the microscopic field of view (fig. 3 a)*.

These large fibers were formed by juxtaposed filamentous, long, and thin macromolecules of similar thickness.

Some round, small corpuscles appeared randomly scattered throughout the network’s meshes or attached to the fibers.

The electron micrograph of the RNase treated NP (final concentration of RNase 100 μg/ml; 2 hours’ incubation at 37 °C) (fig. 3 b) was very similar to that obtained for untreated NP.

Hence, the large fiber network seemed to be mainly formed by an aggregation of the DNA-protein-RNA complex macromolecules.

Electron microscopic examination of the NP filtered over Sephadex G-25 (negatively stained with 1% phosphotungstic acid, 30,000 ×) revealed a different aspect (fig. 4 a). The former network made up by large fibers was replaced by a homogenous background of compact masses of thin fibrils arranged unevenly.

Here and there occasional gaps appeared on this background. The large fibers forming the network in the NP not gel-filtered, disappeared in such preparations. The thin fibrils seemed to be disrupted into shorter fragments and the former joints connecting them to the large fibers seemed to be split, too, which accounts for such compact background. The network meshes appearing in the not gel-filtered NP were further reduced to a few small circular gaps. The small round corpuscles appearing in the network meshes and on the large fibers in the not gel-filtered NP were no longer detectable here.

The electron micrograph of the gel-filtered NP treated with RNase (fig. 4 b) was rather similar to that obtained for untreated gel-filtered NP. Here again, a similar background formed by compact masses of thin fibrils could be observed; within the gaps, circular aggregations of fibrillar material were observable. Thus, the fibrillar material of such preparations seems to be formed mainly by DNA-RNA-protein complex macromolecules.

Hence, changes in the electrophoretic pattern and the electron micrographs of gel-filtered NP as compared to not gel-filtered NP would evidence the occurrence of some alterations in the macromolecular structure of the DNA-RNA-protein complexes induced exclusively by gel-filtration.

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3 W. McIndoe and H. N. Munro, Biochim. biophysica Acta [Amsterdam] 134, 458 [1967].

* Figs. 3 a, b and 4 a, b see table 354 a.
The above mentioned modifications might be caused by:

a) Some changes of the osmotic pressure during gel-filtration or NP concentration against poly-vinylpyrrolidon (P.V.P.).

b) Exclusion of certain ions or of compounds of small molecular-weight by gel-filtration.

c) Adsorption to the Sephadex grains of some nucleic acid or protein fractions playing the role of linkers.

d) Hydrodynamic shearing forces appearing during gel-filtration.

a) To investigate effects caused by the osmotic pressure in NP preparations an electrophoretical analysis of an NP sample diluted in 0.01 acetate buffer, pH 6.8 and then concentrated 45 fold against P.V.P. was made. The sample failed to reveal any modifications in the electrophoretic pattern which fact indicated that the above described macromolecular alterations of the gel-filtered NP were not induced by changes in the osmotic pressure.

b) The possibility of macromolecular damages induced by gel-filtration and followed by exclusion of some ions or of compounds of small molecular weight seems unlikely since the 24 – 48 hours' dialysis of the NP against distilled water, prior to the electron microscopic observation, failed to cause modifications the macromolecular aggregation of preparations whether previously gel-filtered or not.

c) To investigate whether some nucleic acid or protein fractions were adsorbed to the Sephadex G-25 gel during filtration and hence not eluted, the following experiment was carried out:

After gel-filtration the gel was washed for 1 hour with a solution of 1% NaOH to elute the nucleic acids or proteins eventually adsorbed to the gel particles. The gel was then centrifuged and in the supernatant the optical density was measured at 260 and 280 mμ.

The blank consisted of a NaOH solution used to wash another gel not employed in NP gel-filtration. The optical density showed no increase neither at 260 mμ nor at 280 mμ, thus demonstrating that neither nucleic acid nor protein fractions were selectively adsorbed to the Sephadex grains.

d) The effect of the hydrodynamic shearing forces on the NP macromolecular alterations was investigated in the following experiments: Two NP samples were gently pipetted onto the Sephadex G-25 gel, previously equilibrated with acetate buffer, in the same amounts as during gel-filtration. The two samples were kept at room temperature for 2 hours, one being stirred by a rotatory magnetic stirrer for 15' to produce hydrodynamic shearing forces.

The two samples were then filtered very gently through Büchner funnels, the gel well-washed with acetate buffer, the filtrate concentrated against P.V.P. and electrophoretically analyzed. The stirred sample in which hydrodynamic shearing forces had appeared, showed an electrophoretic pattern very similar to that of the gel-filtered NP (fig. 1 b).

The NP macromolecules were disrupted into smaller fragments rather similar in length (the unique electrophoretic fraction was relatively narrow and well defined). The other not-stirred sample showed an electrophoretic pattern with a single very broad fraction (fig. 1 a), which suggests that part of the NP macromolecules were disrupted nevertheless into smaller fragments variable in length during the manipulations. The majority of these macromolecules, however, had a molecular weight similar to that of the DNA-RNA-protein complexes found in the not gel-filtered NP.

Disruption of the macromolecules occurred even during very gentle manipulation which fact proved the high susceptibility of the DNA-protein-RNA complexes to hydrodynamic shearing forces.

Hence, during gel-filtration the hydrodynamic shearing forces seem to produce most of the alterations of the aggregation state of DNA-protein-RNA complexes. The nucleic acid macromolecules with high molecular weight, much above 200,000 \(^1\) are excluded by the gel column, i.e. shearing forces do not arise by passage of the nucleic acid macromolecules through the Sephadex G-25 grains.

The effect of these forces is observable in electron micrographs as well as by electrophoretic analysis. In the latter, a decrease of the molecular weight and also changes in the electronegative charge per molecular weight cause an increase of the electrophoretic mobility of DNA-protein-RNA complexes.

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