PHYSICAL PROPERTIES OF THYMUS NUCLEOPROTEIN

by

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In the numerous studies which have been made of the desoxyribonucleoproteins, extracted from tissues, an extremely varied behaviour has been reported. This is due in part to the effects of the extraction procedures on the properties of the material. Much of the early work on thymus nucleoprotein utilised the solubility in molar saline as a method of extraction. However, it is now generally recognised that in this solvent the nucleoprotein is largely dissociated into nucleic acid and histone. The reconstitution of the nucleoprotein which occurs on dilution can hardly be expected to reproduce the starting material. For this reason several recent investigations have been based on an alternative extraction procedure which makes use of salt solutions of low ionic strength, and the physical properties of the material obtained have been studied in water or in salt solutions containing less than 0.02 *M* NaCl. Using this method of preparation PETERMAN AND LAMB[1], GAJDUSEK[2], STERN *et al.*[3] and ALLGÉN[8], have reported obtaining highly viscous solutions of nucleoprotein. On the other hand, STEINER[4] obtained by similar methods a readily soluble material of low viscosity. The experiments described in this paper were an attempt to resolve this contradiction. Briefly, it is found that when carefully prepared the nucleoprotein is obtained in the form of a gel that cannot be completely dispersed by any mild means and is completely precipitated in 0.01 *M* NaCl. Enzymic degradation produces a material which is readily soluble and is not precipitated by 0.015 *M* NaCl.

EXPERIMENTAL

Preparation of nucleoprotein

Thymus from freshly killed calves was cleaned, frozen, minced and homogenised in a Waring Blender with 0.15 *M* Sodium chloride solution to give a thick suspension. This suspension was centrifuged for 40 minutes at 2200 r.p.m. on the International Refrigerated Centrifuge. The supernate was discarded, and the sediment was resuspended in 0.15 *M* saline, and centrifuged for 30 minutes at 2200 r.p.m. The sediment obtained formed two fairly distinct layers. The top layer consisting mainly of nucleoprotein was collected and the heavier, fibrous cell debris which formed the lower layer was discarded. The collected sediment was again homogenised in 0.15 *M* saline, and centrifuged for 30 minutes at 2200 r.p.m. All but the lowest layer of the sediment was resuspended and re-centrifuged for 20 minutes. This washing process was continued from three to seven times. The final product was homogenised in water and dialysed free of the last traces of salt.

In some preliminary investigations the initial homogenate was allowed to stand overnight before the centrifugation to increase the yield, but in later experiments the final nucleoprotein was obtained within 5 hours of obtaining the tissue. The nitrogen to phosphorus ratios of the preparations used in this investigation (seven in all) lay between 4.0 and 4.8.

Properties of the nucleoprotein

The nucleoprotein obtained by the above procedure was a thick, gel-like, opalescent material, usually containing a very small amount of suspended fibrous matter. The gel

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usually contained 0.5 to 1.0% nucleoprotein. It has generally been reported that the nucleoprotein is soluble in NaCl solution up to a concentration of 0.02 *M* (*e.g.* FRICK[7], STEINER[4]). Our nucleoprotein gels were precipitated when dialysed against 0.01 *M* NaCl, even at low concentrations of nucleoprotein. For this reason the experiments to be reported have all been made with solutions containing only the small amount of salt left after 16 hours stirred dialysis against water.

Sedimentation behaviour

The sedimentation characteristics of this material have been investigated using the Spinco Ultracentrifuge. In the majority of cases the sedimentation diagram shows two components (Fig. 1). The faster component with which the turbidity of the solution is associated is characterised by a sharp peak which shows little spreading with time. The slower component shows normal spreading as it moves down the cell but has an asymmetric peak. The relative concentration of the two components varies in different preparations. In a few cases the slow component has not been present at all, but in the majority of cases it is present in small relative amounts (Fig. 1), while in a few cases a relatively high concentration has been found (Fig. 2) and in one preparation the faster component was absent. The sedimentation constants have been calculated in the usual manner.

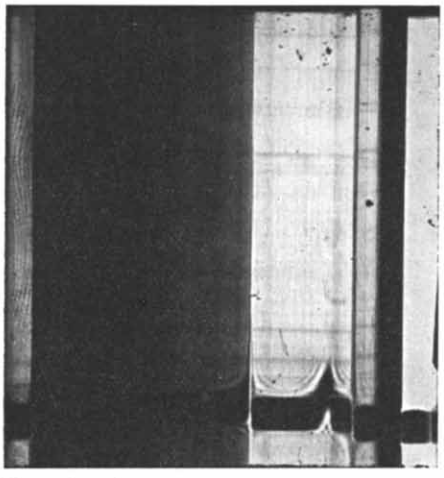

Fig. 1. Sedimentation diagram of nucleoprotein showing gel boundary and small concentration slow component.

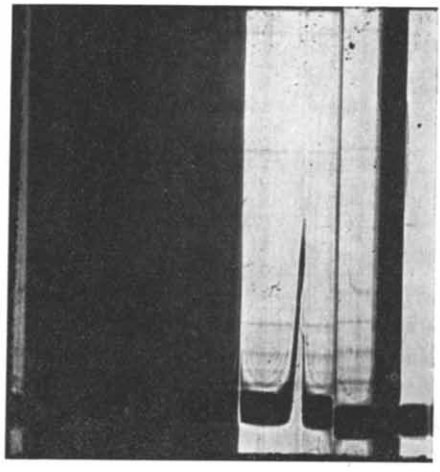

Fig. 2. Gel and large concentration of slow component.

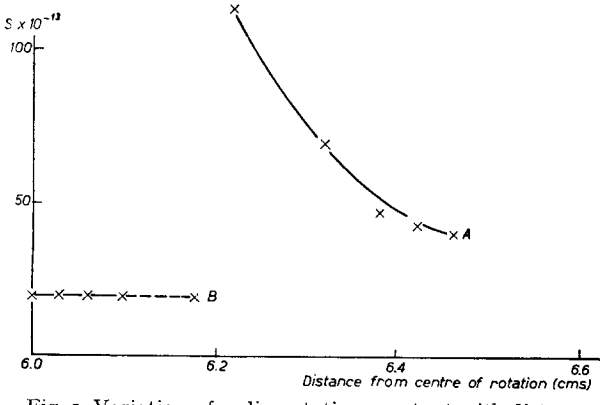

Fig. 3. Variation of sedimentation constant with distance down the cell. A. gel boundary. B. slow component.

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The sedimentation constant of the slow component is the same at all points along the cell. The sedimentation constant of the faster component has been calculated from successive pairs of measurements and, as shown in Fig. 3, its value falls as the boundary moves down the cell.

The slower component

The sedimentation constants of the slow component range from 7–60 *S*. There does not appear to be any correlation between the sedimentation constant and concentration as is shown by the results for one preparation of nucleoprotein given in Table I.

TABLE I

	$S_{20} \cdot 10^{-13}$
1. Original gel-like material	19.3
2. Original gel-like material diluted × 2	31.7
3. Original gel-like material diluted × 20	33.9
4. Solution (2) stirred for 10 hours	50.3
5. Supernate from (1) after centrifuging off the gel phase	57.7
6. Supernate from (3) after centrifuging off the gel phase	14.6
7. Solution (1) precipitated and redissolved	14.7
8. Solution (1), diluted, precipitated and redissolved	37.2

It is clear from these results that the material giving rise to the slower boundary is extremely variable in either molecular size or shape.

Solutions of nucleoprotein have been tested after keeping them at 4° C for several days. In general it has been found that the concentration of the slow component increases with time and the corresponding sedimentation constant decreases. The gel-like nature of the material is slowly lost. This degradation process is accelerated when the solutions are kept at room temperature. These experiments suggest that the slow component is produced by the enzymic degradation of the gel phase. Somewhat similar observations have been made by ALLGÉN[8], who observed a decrease of the apparent molecular weight of the nucleoprotein with time. Other experiments corroborate this suggestion. For example, with a preparation containing only a small concentration of the slow component repeated precipitation does not increase the concentration. Conversely, when a preparation containing a large concentration of slow component is precipitated and redissolved twice the concentration is not reduced. In the later preparations, when the extraction procedure was completed in one day and the number of washings with 0.14 *M* NaCl was increased from four to seven, in all but one case the concentration of slow component was very small. In the anomalous preparation an attempt to achieve maximum yield of nucleoprotein had been made, which meant that a considerable amount of fibrous debris along with the nucleoprotein had been collected from the sediment after centrifuging in the first few stages. This preparation had a low N/P ratio of 3.8 and contained no gel phase at all. This suggests that the enzymes responsible for the degradation are occluded to some extent on the fibrous material.

In one preparation, the slow component was isolated by centrifuging off the gel phase. Analysis of the freeze-dried material gave a nitrogen/phosphorus ratio of 4.5.

The solution of the slow component obtained could be readily diluted and was not precipitated in 0.015 *M* NaCl. In the presence of the salt, a much more symmetrical

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boundary was obtained in the ultracentrifuge. In the absence of salt, the solution of the slow component has a fairly high viscosity and shows shear dependence as shown in Fig. 4. In the presence of 0.01 *M* NaCl the viscosity is independent of the rate of shear and is reduced considerably.

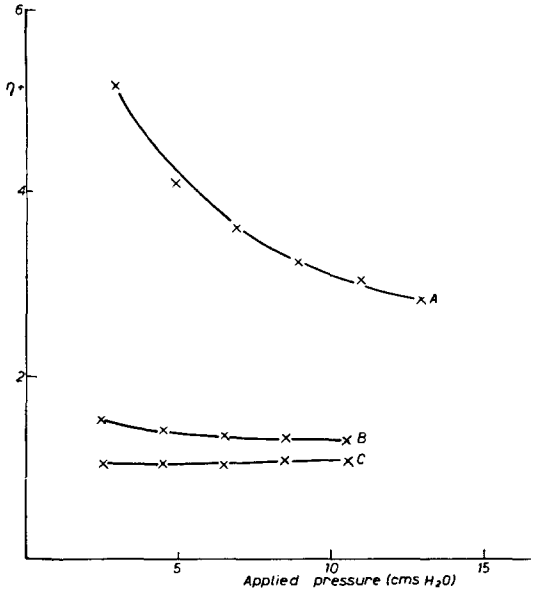

Fig. 4. Variation of viscosity with applied pressure.
A. 0.03% solution of nucleic acid in water.
B. 0.03% solution of nucleoprotein slow component in water.
C. 0.03% solution of nucleoprotein slow component in 0.01 *M* NaCl.

The faster component

When the initial, opalescent, gel-like nucleoprotein is diluted it disperses only with difficulty. If stirred for a sufficiently long time, the gel swells to fill all the volume. When such a solution is examined in the ultracentrifuge the boundary of the faster component moves away from the meniscus as a sharp peak followed later by the peak of the slow component (see Fig. 1). However, when the stirring has not been sufficiently prolonged, although the peak of the slow component moves away from the meniscus normally, the peak of the faster component is formed in the middle of the cell, beginning as a broad peak and gradually becoming sharper (Fig. 5). This behaviour is clearly due to the presence of undispersed islands of gel in the solution which sediment down to the bottom of the cell to form a separate gel phase. The same phenomenon is exhibited at very low concentrations of nucleoprotein; even after prolonged stirring the faster boundary is formed in the middle of the cell. This suggests that the gel is not completely dispersed by dilution. The island structure of the gel probably exists in the more concentrated solutions since even after prolonged stirring the solutions appear lumpy when poured. The difficulty of obtaining homogeneous solutions precludes the possibility of accurate viscosity measurements. Measurements that have been made with a solution

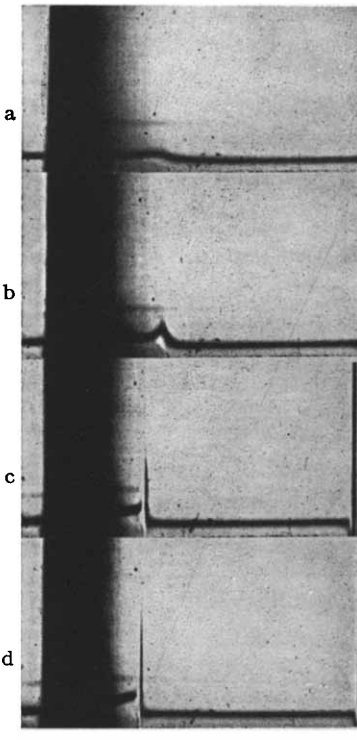

Fig. 5a, b, c, d. Formation of the gel boundary in the middle of the cell. Direction of sedimentation right to left.

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containing 0.1% of nucleoprotein indicate that the viscosity is markedly shear dependent and that the solutions exhibit thixotropy.

Attempts have been made to disperse the gel but these have not proved successful. Experiments with hydrogen bond breaking agents such as urea and phenol were rather inconclusive. When dialysed against *M* urea for 16 hours the gel structure is not lost but a certain amount of polydisperse material is associated with the slow component. Dialysing out the urea results in the precipitation of the nucleoprotein. 0.5 *M* and *M* phenol precipitates the nucleoprotein.

DISCUSSION

It has been found that preparations of nucleoprotein prepared by washing with dilute salt solutions and water extraction contain two components, one of which is a gel and the other consists of dispersed particles. The proportions of these two substances differ very considerably, but it is possible to obtain the nucleoprotein mainly in the gel form. It appears from the experiments reported that the dispersed material is formed from the gel-like material by a degradation process, which is probably due to enzymes. These observations do much to resolve the contradictory statements of previous investigators. It is obvious that the product obtained by some procedures has been mainly the gel-constituent, while others have yielded the dispersed material. It is evident that the extensive clarification procedures which some investigators have used will have had the effect of removing the gel-fraction, so that the physical constants obtained in such cases refer to the dispersed material. STEINER's[4] results, in particular, would seem to refer to a substance of this kind.

The main conclusion from our experiments is therefore that the nucleoprotein can be obtained in a highly aggregated state, which probably approximates more closely to its state in the living nucleus. In this state particle weight and size have very little meaning. Attempts to separate the particles and to break up this gel-like substance without damaging the native nucleoprotein have not been successful. The action of hydrogen bond breaking reagents is extensive, but probably considerable damage is caused to the protein as well as modification of the interparticle bonding.

The extremely variable sedimentation constants observed with the slow component are worthy of comment. Notwithstanding this wide variation in sedimentation rates the peaks obtained are always similar and are markedly asymmetric, the trailing edge being sharp. This is itself indicative of a considerable degree of molecular interaction and under these conditions the existence of a sharp peak is not evidence of monodispersity. This is confirmed by the fact that in dilute solutions much more spreading is observed. The filtering action of the gel in its various degrees of swelling probably also contributes to the variation of the properties of the soluble material.

Finally, we must enquire if it is possible to draw any conclusions about the nature of the nucleoprotein gel. Since the protein is almost completely dissociated from the nucleic acid in concentrated salt solutions it is necessary to suppose that the bond between the protein and the nucleic acid is largely electrostatic. Possibly some degree of hydrogen bonding could not be excluded, as a supplementary factor, although it may not be strong enough to hold a large amount of the protein when the electrostatic bonds are broken. It may be supposed therefore that the bonding occurs between the basic groups of the protein (arginine or lysine) and the primary phosphate groups of

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the nucleic acid, giving a bond of the type $> P\bar{O}_4 H_3\overset{+}{N} -$. According to the measurements of VENDRELY AND VENDRELY[5] there is in calf thymus nuclei approximately 1 arginine residue to 3 phosphate groups. There is also present in the histone a number of lysine residues almost equal to the number of arginine residues so that on the assumption that all the arginine and lysine present are combined with nucleic acid, it is possible that two phosphate groups out of three are combined with basic groups of the histone. On the other hand, the basic amino acids only comprise approximately 20% of the total amino-acid residues in the histone[9], so that room has to be found for four other residues for each one combined with phosphate. This gives a great deal of latitude in possible arrangements of the nucleic acid and protein. It is to be noted that the X-ray diffraction patterns of RILEY AND ARNDT[6] suggest that the nucleic acid and histone are combined in "blocks" rather than finely intertwined. There are two main possible structures for the nucleoprotein gel, if we suppose it to be a mass of interlinked nucleoprotein particles. We could suppose each nucleoprotein particle to be a distinct combination of nucleic acid with a certain amount of the histone. The gel would then be formed by the occurrence of bonds between these particles. There are many possibilities for the formation of hydrogen bonds between the histones, and it is also possible for electrostatic bonds to be formed, since the histone contains acidic groups (glutamic and aspartic) in amounts almost equal to the basic groups. On the other hand it is possible that some of the histone chains are attached to and so unite two or more nucleic acid particles. On the whole the extremely tenacious nature of the gel-like structure perhaps supports the second hypothesis. The fact that the gel is degraded into a non-gel like material by enzyme action on the whole supports the second hypothesis, in that proteolytic action on the histone connecting links would clearly break up the gel.

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SUMMARY

1. Nucleoprotein prepared by washing with dilute salt solution and extracting with water can be obtained mainly in the form of a highly aggregated gel-like material.

2. The dispersed nucleoprotein obtained by several previous investigators is probably formed by enzymic degradation of the gel-like material.

RÉSUMÉ

1. Une nucléoprotéine préparée par lavage avec une solution diluée et extraction à l'eau se présente principalement sous forme d'un produit gélifié fortement aggrégé.

2. La nucléoprotéine dispersée obtenue antérieurement par divers auteurs résulte probablement d'une dégradation enzymatique du produit gélifié.

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ZUSAMMENFASSUNG

1. Durch Waschen mit verdünnter Salzlösung und Extraktion mit Wasser dargestelltes Nucleoprotein wird hauptsächlich in Form eines hochaggregierten, gelähnlichen Materials erhalten.

2. Das bei mehreren früheren Untersuchungen erhaltene dispergierte Nucleoprotein wird wahrscheinlich durch enzymatischen Abbau des gelähnlichen Materials erhalten.

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