DESOXYRIBOSENUCLEOPROTEIN FROM BOVINE SPLEEN

by

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INTRODUCTION

Since it has become obvious through the work of HUISKAMP[1], CARTER AND HALL[2], and MIRSKY AND POLLISTER[3] that earlier drastic methods of nucleoprotein extraction from mammalian tissues decidedly altered the desoxyribosenucleic acid protein complexes from the state in which they existed within the chromosomes, impetus has been given to the study of the physical and chemical properties of desoxyribosenucleoproteins in a state as close as possible to that in which they exist *in vivo*. This paper deals with the study of some of the physical and chemical properties of two desoxyribosenucleoprotein preparations obtained from bovine spleen by two different methods of extraction. Special attention has been devoted to the electrophoretic behaviour of these proteins.

The term *chromosin*, suggested by MIRSKY AND POLLISTER[4], will be used to designate the preparation obtained by 1 *M* NaCl extraction of splenic tissue. Distilled water has been used as the solvent in the second method of nucleoprotein extraction.

EXPERIMENTAL

The moving boundary technique of TISELIUS as modified by LONGSWORTH was used in the electrophoretic studies. The Schlieren scanning optical system was employed throughout. Both rising and descending boundaries have been observed, but mobility data is reported for descending boundaries only. Except where otherwise stated, the mobility as calculated from the rising boundary was slightly greater than that calculated from the descending boundary. Protein concentration was kept at 0.6% (based on N-analysis and N-Factor of 7.50).

1 *M NaCl Preparations (Chromosin)*

Chromosin has been prepared from calf spleen by the method of MIRSKY AND POLLISTER[3,4] involving exhaustive extraction of minced fresh spleen tissue with 0.14 *M* NaCl and subsequent extraction of the nucleoprotein from the residue in 1.0 *M* NaCl solution.

The chromosin was reprecipitated five times from 0.14 *M* NaCl and redissolved in 1 *M* NaCl with clarification of the 1 *M* NaCl solutions by high speed centrifugation at 30 000 g. Electrophoretic study of this material has revealed it to be a multiple component system. Among the peaks in the electrophoretic patterns, three major heterogeneous components can be identified, but resolution into distinct components is far more equivocal than for electrophoretic patterns of mammalian sera. This observation of the heterogeneity of 1 *M* NaCl solutions of Chromosin is in agreement with those of

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MIRSKY[5] and STERN AND DAVIS[6], who have shown 1 *M* NaCl Chromosin preparations to be mixtures of sodium thymonucleate and of histone chloride plus some non-histone protein.

Repeated reprecipitations (to a total of ten) of the above Chromosin preparation from the viscous 1 *M* NaCl solution by dilution to 0.14 *M* NaCl and solution in 1 *M* NaCl with high speed centrifugal clarification at 30 000 g failed to render the Chromosin preparations any less heterogeneous electrophoretically. Various preparations of 1 *M* NaCl Chromosin gave rather different electrophoretic patterns, but the general nature of the curves and the mobility values calculated therefrom are well illustrated by the representative runs below:

In barbital buffer, p_H 8.72 (0.1 *M* NaOH + 0.12 *M* barbital + 1.0 *M* NaCl) the various components migrated with mobilities between $-6.9 \cdot 10^{-5}$ and $+9.6 \cdot 10^{-5}$; and the three major components had approximate mobilities of $-6.9 \cdot 10^{-5}$, 0.0 and $+8 \cdot 10^{-5}$. In the rising boundary the peak corresponding to the $-6.9 \cdot 10^{-5}$ mobility component of the descending boundary was resolved into two distinct peaks.

In phosphate buffer, p_H 6.46 (0.027 *M* K_2HPO_4 + 0.019 *M* KH_2PO_4 + 0.9 *M* NaCl) the various components migrated with mobilities between $-6.3 \cdot 10^{-5}$ and $+2.4 \cdot 10^{-5}$; and the major components migrated with approximate mobilities of $-6.2 \cdot 10^{-5}$, $-4.6 \cdot 10^{-5}$ and $+2.3 \cdot 10^{-5}$, respectively.

It must be emphasized that these components are so heterogeneous that this resolution of the patterns is *very arbitrary*.

When the 1 *M* NaCl Chromosin preparation was precipitated from 0.14 *M* NaCl and redissolved in distilled water a much less viscous solution was obtained. The solution was clarified by centrifugation at 30 000 g. Electrophoretic analysis of this solution in phosphate buffer, p_H 7.06 and ionic strength 0.02, revealed only two components. The major component comprised 71% of the total and migrated with a mobility of $-11.8 \cdot 10^{-5}$. The other component comprised 29% of the total and migrated with a mobility of $-15.0 \cdot 10^{-5}$. Although still heterogeneous, the preparation was resolved into these two electrophoretic components in contrast to the very heterogeneous electrophoretic patterns obtained in the 1 *M* NaCl solution.

When the Chromosin was dissolved in distilled water, as above, and reprecipitated from 0.14 *M* NaCl by addition of 1 *M* NaCl and finally redissolved in distilled water and clarified by centrifugation, electrophoretic analysis then revealed only one relatively homogeneous, electrophoretic component with mobility of $-12.2 \cdot 10^{-5}$ in barbital buffer, p_H 8.50 and ionic strength 0.02.

Distilled Water Preparations

Because of the greater electrophoretic homogeneity of the preparations in distilled water solution, an attempt was made to extract the nucleoprotein from the spleen pulp with distilled water immediately after initial exhaustive extraction with 0.14 *M* NaCl. This is essentially the procedure used by STERN, GOLDSTEIN, WAGMAN, AND SCHRYVER[7] in preparing their Genoprotein T from calf thymus, except that no sodium arsenate has been used to inhibit the nucleodepolymerase in these preliminary studies.

After exhaustive extraction of minced calf spleen tissue, with 0.14 *M* NaCl the pulp was divided into two portions: one portion was extracted with 1 *M* NaCl and the other with distilled water. The Chromosin preparation in 1 *M* NaCl was reprecipitated and redissolved five times as described above. The distilled water preparation was precipitated by addition of 1 *M* NaCl to bring the NaCl concentration to 0.14 *M*, centrifuged down, and redissolved in distilled water. This was repeated five times, each

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distilled water solution being centrifuged clear at 30 000 g. All work was conducted in a 4° C cold room.

Electrophoretic study of this distilled water preparation revealed it to migrate as one peak over a wide p_H range. The 1 *M* NaCl Chromosin preparation revealed a multicomponent system on electrophoresis similar to the patterns described above for chromosin. The relative homogeneity of the water preparation with a single electrophoretic component was in marked contrast to the heterogeneity of the 1 *M* NaCl preparation.

The electrophoretic behavior of this water preparation was investigated throughout its soluble p_H range using univalent anionic buffers of ionic strength 0.02. Acetate, cacodylate, barbital, and glycine buffers were employed. Protein concentration was kept at 0.6% (based on N-analysis and a N-factor of 7.5). When dialyzed against acetate buffers of p_H 4.63 or lower, ionic strength 0.02, the protein precipitated. It redissolved when the p_H was raised by dialyzing against acetate buffer of p_H 5.61 and ionic strength 0.02. This precipitation was no longer reversible after 30 hours exposure to acetate buffer of p_H 4.63.

At higher p_H values the protein remained in solution up into the strongly alkaline range with glycine buffers, and even in alkali solutions with p_H above 12. In unbuffered acid solutions with p_H below 4 the protein promptly precipitated.

The protein migrated as a single component in all buffers in which it was soluble, from acetate buffers in the acid p_H range to the glycine buffer of p_H 10.13. In glycine buffer, p_H 10.66, ionic strength 0.02, alteration of the protein was evident from the separation of a faster-moving component in the descending boundary (representing 7% of the total protein) and of a slower-moving component in the rising boundary (representing 11% of the total protein). In four repetitions of this study the rising and descending boundaries demonstrated this dissimilar behaviour: a small fast-moving component in one and a small slow-moving component in the other. At higher p_H values (glycine buffer p_H 11.7) decomposition into a very heterogeneous system with many poorly resolvable electrophoretic peaks occurred. The mobilities of the many components of this alkali-altered protein complex ranged from $-4.26 \cdot 10^{-5}$ to $-15.9 \cdot 10^{-5}$.

Table I gives the mobility data for a fresh preparation of Nucleoprotein in distilled water from p_H 5.32 to 11.7. The data are plotted in Fig. 1. It should be noted that the phosphate buffer gives a mobility value lying far off the p_H-mobility curve obtained from data yielded in the univalent anionic buffers.

The distilled water nucleoprotein preparation may be precipitated by bringing to 0.14 *M* NaCl by the addition of 1 *M* NaCl and redissolved in 1 *M* NaCl. Electrophoretic patterns of this distilled water nucleoprotein

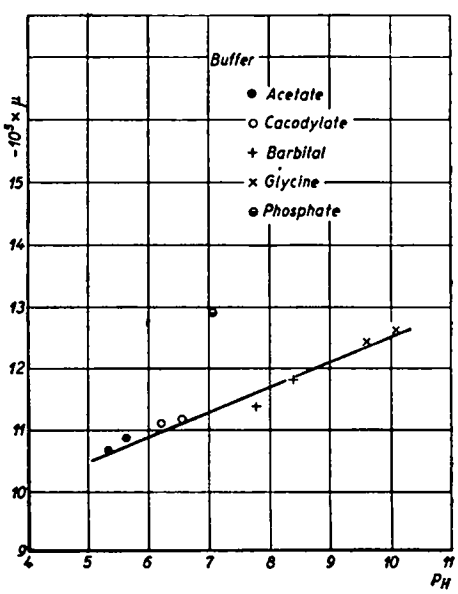

Fig. 1. p_H-Mobility Curve for a Distilled Water Nucleoprotein Preparation plotted from the data of Table I

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TABLE I

MOBILITY OF DISTILLED WATER NUCLEOPROTEIN PREPARATION AT VARIOUS H-ION CONCENTRATIONS

Buffer	pH	Mobility ($\cdot 10^{-5}$)
Acetate	5.32	10.7
	5.61	10.9
Cacodylate	6.19	11.1
	6.57	11.2
Barbital	7.78	11.4
	8.42	11.8
Glycine	9.61	12.4
	10.10	12.6
	10.60	12.2 major peak
		13.8 minor peak
	11.7	heterogeneous
		4.3–15.9
Phosphate	7.07	12.9
Nucleoprotein altered on standing for 2 weeks		
Acetate	4.48	12.9
Barbital	8.42	14.6

Protein Concentration: 0.6% (Based on N-analysis and N-factor of 7.5).
Ionic Strength of all buffer solutions: 0.02

preparation redissolved in 1 *M* NaCl are quite similar to the patterns obtained for Chromosin preparations extracted directly from spleen pulp, after 0.14 *M* NaCl solution washing, with 1 *M* NaCl. The following representative runs illustrate well the marked loss of homogeneity and the marked mobility changes that accompany the transfer of water nucleoprotein preparations to 1 *M* NaCl.

In barbital buffer, p_H 8.2 (0.1 *M* NaCl + 0.12 *M* barbital + 0.9 *M* NaCl) the various components migrate with mobilities between $-8.5 \cdot 10^{-5}$ and $+0.6 \cdot 10^{-5}$.

In phosphate buffer, p_H 6.47 (0.027 *M* K_2HPO_4 + 0.019 *M* KH_2PO_4 + 0.9 *M* NaCl) the various electrophoretic components migrate with mobilities between $-6.5 \cdot 10^{-5}$ and $+3.1 \cdot 10^{-5}$; and the three major components have approximate mobilities of $-6 \cdot 10^{-5}$, $-4 \cdot 10^{-5}$ and $+2 \cdot 10^{-5}$. The pattern is very similar in even its fine structure to the pattern of 1 *M* NaCl Chromosin preparations in the same buffer.

On standing in a refrigerator at 4° C preparations of the nucleoprotein in water were noted to be unstable and no longer yielded precipitates in 0.14 *M* NaCl. Some preparations stood for 6 weeks before this change in precipitability occurred, while other preparations changed within 10 days. This solubility change was also noted by STERN AND DAVIS[6] in their water-extracted nucleoprotein from calf thymus.

After this change in solubility occurred the mobility of the protein — which still migrates as a single component in an electric field — was found to be markedly increased (see Table I). The solubility was also altered in acid solutions. The protein was now soluble when dialyzed *vs.* acetate buffers down to p_H 4.48 but it still precipitated in

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dilute acid solutions of p_H 3.24 and in very dilute HCl (p_H 1.8). Before this alteration occurred, the protein was insoluble in acetate buffers of p_H 4.6 and lower.

ULTRAVIOLET ABSORPTION SPECTRA

The distilled water nucleoprotein ultraviolet absorption curve (using 0.01% protein solutions) showed an absorption maximum at 2595 Å and a minimum at 2350 Å. The curve was essentially similar to that obtained for the 1 *M* NaCl Chromosin preparations with maximum at 2590 Å and a minimum at 2375 Å.

The lyophilized nucleoprotein preparation redissolved in a 27 *N* H_2SO_4 solution gave an ultraviolet absorption curve identical with that of a commercial preparation of desoxyribose nucleic acid and differing from ribosenucleic acid preparations.

QUALITATIVE CHEMICAL TESTS

Chemical tests on the eight-times reprecipitated water nucleoprotein preparations to demonstrate the presence of protein were performed. Strongly positive reactions were obtained with the Biuret Reagent and with the MILLON's Reagent.

One of the water preparations of nucleoprotein contained 13.4% nitrogen and 3.4% phosphorus. These analyses were based upon dry weight obtained by lyophilization to constant weight, which does not, of course, remove all of the bound water. These values give a nitrogen-phosphorus ratio of 3.9.

DISCUSSION

Because of the evidence in the literature[4, 5, 6] that 1 *M* NaCl extractions of desoxyribosenucleoprotein from mammalian tissue yield a dissociated sodium thymonucleate and histone chloride and a non-histone protein, and because of the present electrophoretic observations of the marked heterogeneity of such Chromosin preparations, a water extraction has been employed, making use of the solubility of the nucleoprotein in solutions of low ionic strength. As in the preparation of Chromosin in 1 *M* NaCl, use is made of the insolubility of the nucleoprotein in 0.14 *M* NaCl to purify the protein by reprecipitation, and high speed centrifugal clarification of the water solutions is also employed.

STERN, GOLDSTEIN, WAGMAN AND SCHRYVER[7] have prepared a nucleoprotein from thymus by an essentially similar procedure, making use of sodium arsenate to inhibit the nucleodepolymerase activity in preparing their Genoprotein T, which they claim is probably the native desoxyribosenucleoprotein of this tissue. In the present work with spleen, no sodium arsenate enzyme inhibition has been used, and a relatively homogeneous water preparation has been obtained, which remains stable in the cold (4° C) for 2–6 weeks before the solubility changes—also noted by STERN AND DAVIS[6]—occur.

With different preparations reproducible p_H-mobility data have been obtained.

WINCKLE AND FRANCE[8] have studied Bovine Liver Nucleoprotein prepared according to MIRSKY AND POLLISTER in 1 *M* NaCl. They obtained heterogeneous patterns for these preparations in 1 *M* NaCl and more homogeneous patterns in phosphate buffers of ionic strength 0.02.

FRICK[9] found marked differences in electrophoretic patterns for different preparations

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of calf thymus nucleoprotein prepared according to MIRSKY AND POLLISTER (Chromosin). This was also observed in the Chromosin preparations from spleen in the present study. FRICK's mobility value for the main component averaged from all his data was $-7.9 \cdot 10^{-5}$ in phosphate buffer, p_H 6.3 ionic strength 0.1 for 1 *M* NaCl solution of his protein.

Rising boundary mobility values were found to be higher than those calculated from the descending boundary, and the difference between these two values was found to depend upon the protein concentration, as previously noted by HALL[10] for his thymus and thyroid nucleoprotein preparations.

When the heterogeneous protein complex of 1 *M* NaCl Chromosin is precipitated and redissolved in distilled water, the nucleoprotein now migrates as a relatively homogeneous material with a mobility much higher than that which any of the components in the 1 *M* NaCl solution had possessed. In fact, the mobility value of $-12.2 \cdot 10^{-5}$ in barbital buffer p_H 8.50, ionic strength 0.02, is in close agreement with the value of $-11.8 \cdot 10^{-5}$ obtained for distilled water nucleoprotein preparations in barbital buffer p_H 8.43, ionic strength 0.02.

The distilled water nucleoprotein solution when precipitated and dissolved in 1 *M* NaCl in turn yields a heterogeneous mixture with complex electrophoretic pattern very similar to that obtained for 1 *M* NaCl Chromosin and with components of mobilities much the same as those of the 1 *M* NaCl Chromosin.

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SUMMARY

Desoxyribosenucleoprotein has been prepared from bovine spleen by water extraction after exhaustive extraction of spleen pulp with 0.14 *M* NaCl. Repeated reprecipitation in 0.14 *M* NaCl and clarification of the water solutions by centrifugation at 30 000 g has been used to purify the protein.

Ultraviolet absorption, electrophoretic and solubility studies have been done to partially characterize the preparation, and its stability throughout the p_H range from p_H 2–12 and on prolonged standing has been studied. The preparation has been compared with Chromosin obtained by 1 *M* NaCl extraction by the method of MIRSKY AND POLLISTER.

It is suggested that the water preparations here studied are essentially the same as the Genoprotein T from calf thymus of STERN and coworkers.

Light scattering, ultracentrifuge and further analytical chemical studies are planned to further characterize this material, and similar preparations from other tissues are being studied.

RÉSUMÉ

Nous avons préparé de la desoxyribose nucléoprotéine par extraction à l'eau d'un tissu de rate de boeuf, préalablement épuisé par 0.14 *M* NaCl. Cette protéine a été purifiée par reprécipitation répétée avec une solution de NaCl 0.14 *M* et par clarification des solutions aqueuses dans la centrifugeuse à 30 000 g.

La préparation ainsi obtenue a été charactérisée partiellement par l'étude de son absorption dans l'ultraviolet et de sa solubilité et par électrophorèse; sa stabilité a été étudiée dans tout le domaine de p_H 2–12 et pendant un repos prolongé. Enfin nous l'avons comparée à la chromosine, obtenue par extraction à l'aide d'une solution de NaCl 1 *M* d'après la méthode de MIRSKY ET POLLISTER.

Nous voudrions suggérer l'idée que les préparations aqueuses étudiées soient essentiellement identiques à la Génoprotéine T isolée par STERN et coll. à partir du thymus de veau.

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Afin de charactériser davantage le matériel étudié nous allons nous servir de dispersion de la lumière, d'ultracentrifugation et d'autres méthodes analytiques; en plus, des préparations semblables obtenues à partir d'autres tissus sont à l'étude.

ZUSAMMENFASSUNG

Desoxyribosenukleoprotein wurde aus Ochsenmilz durch Extraktion mit Wasser eines, mit 0.14 *M* NaCl erschöpfend extrahierten Milzgewebes hergestellt. Zur Reinigung des Proteins wurde wiederholtes Umfällen mit 0.14 *M* NaCl und Klären der wässrigen Lösung durch Zentrifugieren bei 30 000 g angewendet.

Das Präparat wurde durch Ultraviolett-Absorption, durch Elektrophorese und durch Untersuchung der Löslichkeit teilweise charakterisiert und seine Beständigkeit bei p_H 2-12 und beim langen Stehen untersucht. Es wurde mit Chromosin, das nach MIRSKY UND POLLISTER durch Extraktion mit 1 *M* NaCl hergestellt war, verglichen.

Es wird angenommen, dass die hier untersuchten Präparate im Wesentlichen mit dem von STERN *et al.* aus Thymusdrüse vom Kalb isolierten Genoprotein T identisch sind.

Lichtstreuung, Ultrazentrifugieren und andere analytische Methoden sind zur weiteren Charakterisierung des Materials vorgesehen und ähnliche Präparate aus anderen Geweben werden untersucht.

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