SOME PHYSICO-CHEMICAL PROPERTIES OF THYMO-NUCLEOPROTEIN PREPARED ACCORDING TO MIRSKY AND POLLISTER

by

GÖSTA FRICK

Institutes of Biochemistry and Physical Chemistry, University of Uppsala, Uppsala (Sweden)

The first methods for the preparation of nucleoproteins were rather drastic. A method more likely to give well-defined and not extensively degraded or decomposed proteins was introduced by HUISKAMP[1] and modified by CARTER AND HALL[2]. In this procedure the freshly pulped calf-thymus is extracted with water at 5° C for twenty-four to thirty-six hours, and the protein precipitated by the addition of sufficient salt to give 1% NaCl.

MIRSKY AND POLLISTER[3, 4, 5] have taken advantage of the fact that nucleoproteins will dissolve if the salt-percentage is raised to about 5%.

The protein prepared in this way will also dissolve in pure water, but according to MIRSKY AND POLLISTER its structure seems to change, as, if it is once dissolved in pure water and then redissolved in 1 M NaCl, the viscosity and the streaming birefringence have decreased when compared to the original solution in the same salt concentration. As a very small addition of salt will cause precipitation of the nucleoprotein from its solution in pure water, it is for most purposes much better to work with 1 M salt solution. This will also yield a higher concentration of nucleoprotein.

Preparation: Calf thymus is collected immediately after the animal has been killed. The fresh tissue is frozen with solid carbon dioxide and 200 g are ground in a mill together with additional solid carbon dioxide. 400 ml 1 M NaCl are then added to the powder of mixed protein and solid carbon dioxide. The solid carbon dioxide is allowed to evaporate in the cold storage room during the following night. The salt solution and the protein are now in the form of gel. This gel is then added to 2400 ml distilled water and the protein precipitates. The precipitate is thoroughly stirred to allow most of the blood to go into solution and is then collected and dissolved in 1 M NaCl. This new solution is centrifuged at a moderate speed in medium sized tubes, and the insoluble parts form an easily removable cake on the top of the solution. By the addition of six parts of distilled water a new threadlike precipitate is obtained. This is collected by twirling it around a glass rod and merely lifting it up. The precipitate is redissolved and the very viscous rather opalescent solution, is centrifuged at about 9500 r.p.m. for two hours. (The medium diameter of the centrifuge is 13 cm). This procedure is repeated until a total of five or six precipitations and redissolvings have been performed. All preparations are made in the cold storage room. Ample stirring is essential. In some cases the 1 M NaCl. solution is buffered with a phosphate of 0.0125 M NaH_2PO_4 plus 0.0125 M Na_2HPO_4, ionic strength 0.05. (This buffer is referred to as "phosphate buffer" on the following pages). The p_H of the nucleoprotein-solutions will be very stable between 6.2 and 6.3. When no buffer is added the p_H has a great tendency to drop to about 5.6.

The nucleoprotein prepared in this manner gave a white powder, when freeze-dried. For analysis it was dried at 105° C for 12 hours. The amount of phosphorus in the nucleoprotein-preparations was 3.7–3.8% and the nitrogen amounted to about 14.8%. (Preparations which had only been freeze-dried gave 3.4–3.5% P and 14.8% N). These values proved to be fairly constant after five precipitations and redissolvings. They

References p. 116.

were also found to be constant if, when clearing the solutions of opalescence, the speed of the centrifuge was changed between the limits 27000 and 9500 r.p.m. (In both cases centrifugation was carried out for one hour). This is an interesting fact to be considered in the discussion of the degree of dispersity of the protein.

The analytical values are, however, a little too low, because of the adsorption of NaCl on the protein precipitate. If this factor is taken into account the values will be 3.8–3.9% P and 15.1% N. The sodium was determined as sodium sulphate and then weighed.

The nucleoprotein was checked for tryptophane with glyoxyl and copper sulphate plus sulphuric acid. The check was absolutely negative.

FEULGEN AND DISCHE reactions were positive.

All these facts agree very well with those given by MIRSKY AND POLLISTER.

ULTRACENTRIFUGATION

When the nucleoprotein, dissolved in 0.02 M NaCl, was centrifuged in the Svedberg ultracentrifuge, it showed a high degree of polydispersity. In 1 M NaCl the protein gave highly viscous solutions and then naturally the sedimentation constant was dependent on the concentration of the protein; giving one very sharp peak in the centrifugation diagram.

Only a few runs were made, because no striking differences from the values given by CARTER[6] were discovered. It must be stressed that due to the high viscosity no conclusions concerning the number of components can be drawn from the fact that only one peak was to be seen.

Two runs were made in the centrifuge at 40000 r.p.m. The sedimentation constants found were 12.9 for 0.62% nucleoprotein and 14.2 for 0.34% nucleoprotein. In both cases the solvent was 1 M NaCl and phosphate buffers (ionic strength 0.05) with p_H 6.3.

ELECTROPHORESIS

When the nucleoprotein dissolved in neutral phosphate buffers (ionic strength 0.02) was investigated in the Tiselius' electrophoresis apparatus with the Svensson-Philpot optical system only one component was seen. This was negatively charged.

For one and the same preparation the mobility changes greatly with p_H and concentration. Different preparations also give great differences in mobility. To give the order of magnitude it may be mentioned that the mobility was found to be $13.4 \cdot 10^{-5}$ cm^2/volt sec at p_H 6.24 for 0.27% nucleoprotein.

The investigation of nucleoprotein in 1 M NaCl in the electrophoresis apparatus was found to involve difficulties both in the performance of the experiments and their interpretation.

The compensation in the apparatus before starting each run must be done very slowly. If not, the boundary area will be extremely curved, because, due to the viscosity the middle of the area will be moved faster than the sides. The boundaries will then be very unstable.

Even if compensation is done with great care it is very difficult to avoid this effect on the descending side and in the following table only the values for the ascending side, moving towards the cathode, should be taken into consideration.

References p. 116.

These values will give an average mobility of $7.9 \cdot 10^{-5}$ cm²/volt sec of the main component. (See Table I). The voltage was in all runs, 50 V and the current about 43 mA. The runs were observed for about 18 hours.

TABLE I

ELECTROPHORESIS RUNS OF NUCLEOPROTEIN IN 1 M NaCl PLUS BUFFER AT 0.5 C. (BELOW PH 8 PHOSPHATE BUFFER, OVER PH 8 GLYCOCOLL-NaOH BUFFER. IONIC STRENGTH OF BUFFERS 0.1)

Prep. no.	Precipitated times	pH		% nucleoprotein	mobility · 10^5 cm²/volt sec	
		solution	buffer		+	—
17	4	5.1	5.1	0.35	8.0	6.6
17	4	6.3	6.3	0.35	7.8	6.5
17	4	6.3	6.3	0.18	7.8	7.4
18	4	6.3	6.3	0.65	8.1; 6.5	7.8
17	5	6.3	6.3	0.36	8.0; 7.0; 6.4	6.9
18	5	6.3	6.3	0.50	7.9; 6.5	7.1
17	4	6.6	6.7	0.35	7.9	6.7
18	4	9.6	8.9	0.65	7.8	8.0
18	4	10.3	9.6	0.65	9.5	(7.9)

The average value for the mobility does not take into consideration the value for the solution with the highest p_H as this solution showed a very high opalescence and as the nucleoprotein solutions in this p_H region change their qualities. (See below).

The electrophoresis diagrams gave in all cases at least two and sometimes three peaks. The others were moving more slowly than the main peak, but were in some cases difficult to observe.

From the best experiment, that with preparation no. 17, precipitated 5 times (see Table I), it was found that the fastest component contributed to the area under the curve in the diagram with 50%, the slower with 16, and the slowest with 34%. In some cases there was also seen a very small peak in the diagram slowly moving backwards towards the anode.

Another difficulty was that some material diffused out of the cellophane bag during the dialysis. In these experiments, 10–13% of the nitrogen content went out in the buffer when dialysis was performed for 36 hours with stirring.

To make a more exact estimation of the extent of this loss 20 ml of a thymo-nucleoprotein in 1 M NaCl and phosphate buffer with ionic strength 0.05 (p_H 6.3) were dialysed in a cellophane bag of the type used in the other experiments, against 40 ml of the same buffer and 1 M NaCl. The bag was rotated at a good speed. At the beginning the protein solution contains 1.09 mg N per ml. After 26 hours 13.8% of the nitrogen had passed out through the wall of the bag into the buffer, and after 98 hours, 17.0%. Samples for nitrogen determination were taken both outside and inside the bag. The dialysate was investigated for nucleic acid by the ultra-violet absorption method[7], but it was found that all the nucleic acid had stayed in the bag.

The specific volume of the nucleoprotein in the dialysed solutions was 0.65.

Phosphate buffers were used for p_H lower than 8. For higher p_H glycocoll-NaOH buffers were used.

While this work was being done van WINKLE AND FRANCE[8] published an investigation with the electrophoresis apparatus and the ultracentrifuge of a nucleoprotein prepared

References p. 116.

according to MIRSKY AND POLLISTER from rabbit liver. The mobilities found were of the same order of magnitude as those found for my preparations from calf thymus.

PRECIPITATION CURVES

An investigation was made of the solubility of nucleoprotein in NaCl-solutions of different molarities. The object of this investigation was to determine the form of the precipitation curve and see if under the usual conditions there were any changes in the percentage of nucleic acid in the precipitates.

As there is a marked solubility minimum at 0.14 M NaCl two separate series of determinations were made, one on each side of this minimum.

1. *Distilled water to 0.14 M NaCl.* A thoroughly washed protein precipitate was dissolved in distilled water. The solution was centrifuged for three hours at 9500 r.p.m. and contained 0.350 mg N/ml (Table II). 2 ml were taken from it, and 20 ml of accurately

TABLE II

THE SOLUBILITY OF NUCLEOPROTEIN IN 0.00–0.14 M NaCl AT 3.8–4.2° C

M NaCl	Nitrogen mg/ml	Maximum extinction (260 mμ)	$\frac{\text{Extinction}}{\text{N in mg/ml}}$	PH
0.000	0.350	2.072	59	6.01
0.018	0.0276	1.758	64	5.61
0.036	0.0092	0.653	70	5.70
0.055	0.0080	0.520	65	5.70
0.073	0.0062	0.411	66	5.75
0.082	0.0060	0.392	65	5.70
0.091	0.0056	0.337	60	5.58
0.100	0.0048	0.341	71	5.58
0.109	0.0056	0.428	76	5.50
0.118	0.0048	0.331	69	5.62
0.141	0.0036	0.265	74	5.57

prepared salt solutions of different concentrations were added. Precipitates resembling small pieces of cotton wool immediately appeared in all mixtures, except that to which 0.020 M NaCl was added. It, however, showed opalescence. The mixtures were allowed to stand for three hours and the precipitate was then centrifuged down at 9500 r.p.m. The temperature during the whole procedure was between 3.8 and 4.2° C.

All solutions, together with the original one, were analysed for nitrogen by the Kjeldahl method. As nucleic acid shows a very specific absorption of light in the wavelength region of 260 mμ[7], the absorption curves were determined for all solutions between 400 and 230 mμ in the Beckman spectrophotometer. The irrelevant absorption was very small, the absorption at 400 mμ being less than one per cent of the maximum absorption, and in the following table (No. 2) no correction has been made for it. The absorption measurements were made against the solvent.

The table values for N were obtained from the analysis of 5 ml of solution. As the values for the absorption of the original solution were somewhat high, they were measured after dilution at half the original protein concentration. The readings were then multiplied by two. The same method is used when necessary in all the following investigations. It was proved by separate experiments that BEER's law holds for these

References p. 116.

preparations up to the highest extinction values which can possibly be determined directly and accurately with the apparatus, *i.e.*, an extinction of about 2 (see below).

From the quotient for the maximum absorption and the amount of nitrogen per ml, which is nearly constant over the whole curve, one can see that the nucleic acid is precipitated in parallel with the protein part. When judging the table values it must be understood that quotients of the nitrogen poor solutions are naturally somewhat uncertain.

2. *1 M to 0.14 M NaCl.* The original solution was centrifuged for 1½ hours at 3500 r.p.m. after being dissolved for the last time. (Earlier in the preparation the usual higher speed was used). To five ml of this nucleoprotein solution with 1 M NaCl and phosphate buffer (ionic strength 0.05) were quickly added different amounts of the same phosphate buffer. The mixtures were thoroughly shaken for a few minutes and then left for 5–6 hours. They were then centrifuged for one hour at 3500 r.p.m. All work was done in the cold storage room. The values measured are found in Table III.

TABLE III

THE SOLUBILITY OF NUCLEOPROTEIN IN 1.00–0.14 M NaCl PLUS PHOSPHATE BUFFER. (IONIC STRENGTH OF BUFFER 0.05). FIRST CURVE IN FIG. 1

Solution no.	M NaCl	Nitrogen mg/ml	λ_{max}	Dilution for the absorption measurement	Observed maximum extinction	Theoretical extinction (corr. for dilution)	Extinction / N in mg/ml
1	0.139	0.0036	265	1/1	0.093	0.093	—
2	0.139	0.0026	267	1/1	0.062	0.062	—
3	0.194	0.0024	265	1/1	0.042	0.042	18
4	0.292	0.0052	265	1/1	0.091	0.091	18
5	0.398	0.0732	260	2/7	0.714	0.250	34
6	0.422	0.1050	260	2/10	1.095	5.475	52
7	0.480	0.337	260	2/25	1.614	20.175	60
8	0.524	0.400	260	1/25	0.934	23.350	58
9	0.544	0.406	260	3/50	1.451	24.183	60
10	0.590	0.517	260	3/50	1.836	30.600	59
11	0.682	0.558	260	1/25	1.325	33.125	59
12	0.770	0.617	260	2/50	1.430	35.750	58
13	0.872	0.703	258	1/50	0.817	40.850	58
14	0.970	0.786	260	2/50	1.894	47.350	60

TABLE III (*continued*)

Solution no.	Temperature	pH	Type of precipitation (before centrifugation)
1	4.0–4.3	6.64	Immediate flocculant precipitation, clear liquid
2	4.0–4.3	6.64	,, ,, ,, ,, ,,
3	4.0–4.3	6.59	,, ,, ,, ,, ,,
4	4.0–4.3	6.51	,, ,, ,, ,, ,,
5	4.0–4.3	6.45	,, ,, ,, ,, ,,
6	4.0–4.3	6.49	Good precipitation, liquid somewhat opalescent
7	4.0–4.3	6.37	,, ,, ,, more ,,
8	1.2–1.6	6.35	,, ,, ,, ,, ,,
9	4.0–4.3	6.32	Slight precipitation
10	4.0–4.3	6.30	Very little precipitation
11	1.2–1.6	6.28	Opalescence
12	4.0–4.3	6.28	,,
13	1.2–1.6	6.34	,,
14	4.0–4.3	6.27	,,

References p. 116.

It is seen from the values for the temperature in the table that the precipitations were made in two different series.

It may also be stressed that in the cases were the maximum absorption did not occur at 260 mμ, the difference between the maximum value and the reading at 260 mμ was very small; for solution no. 13, well within the limits of error.

Since the protein precipitate used for preparing the original solution cannot be dried completely, the salt molarity of the original solution will be somewhat diminished. For this reason, the values of the salt concentrations have an error of ± 0.01 M, but the relative values between the different solutions are correct to the second decimal.

A second curve was made in approximately the same manner as the first one (see Table IV). The difference is that another preparation was made and that the precipita-

TABLE IV

THE SOLUBILITY OF NUCLEOPROTEIN IN 1.00–0.14 M NaCl PLUS PHOSPHATE BUFFER AT 1.8° C. pH 6.60–6.25. IONIC STRENGTH OF BUFFER 0.05. SECOND CURVE IN FIG. 1

M NaCl	Nitrogen mg/ml	Extinction (corr. for dilution)	Extinction / N in mg/ml
0.243	0.005	0.054	—
0.340	0.017	0.229	—
0.399	0.057	1.680	29
0.423	0.130	3.930	30
0.468	0.200	10.85	54
0.483	0.205	11.12	54
0.506	0.231	12.75	55
0.534	0.257	14.22	55
0.563	0.278	15.44	56
0.584	0.321	17.20	54
0.683	0.380	21.40	56
0.722	0.400	22.30	56
0.795	0.452	25.20	56
0.825	0.481	25.80	54
0.892	0.509	27.86	55
0.970	0.578	31.68	55

tions were allowed to stand for 18 hours before centrifugation for one hour at 9500 r.p.m. The temperature was 1.8° C and p_H between 6.60 and 6.25, with decreasing values for increasing salt concentrations.

For all samples in both series the absorption at 400 mμ was less than 1.5% of the maximum absorption and also about the same for all solutions. No correction for the irrelevant absorption has therefore been made.

From the values it can be seen that the nucleic acid precipitates in parallel with the protein. It can also be observed that both curves in Fig. 1 show typical sudden changes. The nucleoprotein precipitates from different parts of these curves will be investigated later to determine if possible whether the form of the curves is due to a different type of nucleoproteins, to which the electrophoresis investigations hint, or to changes in the character of the highly viscous solution.

It can however be said with certainty that the precipitation by dilution from 1 M to 0.14 M NaCl, made in the preparation of nucleoprotein, approaches 100%. Less than 0.2% of the nitrogen content and extinction coefficient of the original solution remain in

References p. 116.

the solution which contains physiological saline solution. From the two experiments with 0.14 M NaCl, one centrifuged and the other decanted, it can be seen that this makes very little difference.

To control the degree of precipitation 14 samples were taken from the same nucleoprotein solution in 1 M NaCl, half of which were precipitated as above. That is to say the solutions were merely allowed to stand in the coldroom for a number of hours after dilution to different NaCl concentrations and the precipitates then centrifuged down.

The remaining seven samples were diluted to the same NaCl concentrations as the others and then shaken for twenty hours in an icewater bath.

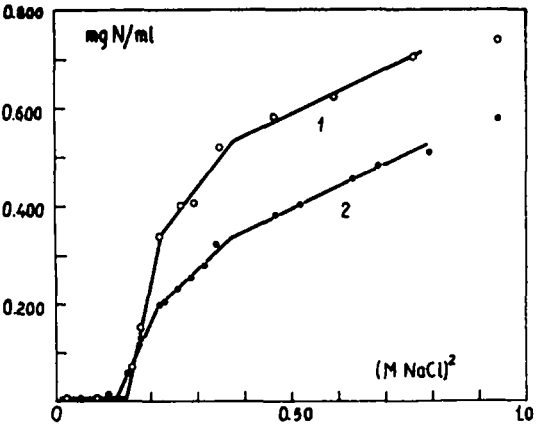

Fig. 1. Amount of nitrogen in mg/ml plotted against the square of the molarity of NaCl in two series, where a solution of nucleoprotein in 1 M NaCl plus phosphate buffer (ionic strength 0.05) was diluted to different NaCl concentrations. The two solutions have been prepared in somewhat different ways (See also Tables III and IV).

The precipitates in all samples were centrifuged down at 9500 r.p.m. under the same conditions.

Their nitrogen content was determined. The differences in nitrogen found between the two differently treated types of solutions were very small, and were well within the limits of error.

VISCOSITY

Measurements of viscosity have been made earlier by among others, CARTER[9] and CARTER AND HALL[3] in preparations made according to HUISKAMP and with dilute phosphate buffer as a solvent, with or without the addition of NaCl. The protein content was varied and the temperature was kept either at 0.7 or 25° C.

VON EULER AND FISHER[10] have prepared cell nuclei by a method according to DOUNCE, dissolved them in 1 M NaCl and measured viscosity with variations in p_H and protein concentration.

GREENSTEIN AND JENRETTE[11] have prepared nucleoprotein from calf thymus and cow liver. They precipitated the protein at p_H 4.2 in an 0.8 M salt solution and washed it with KCl-acetate buffer. The protein was dissolved in "fairly strong" NaOH, to which was afterwards added guanidine — HCl or urea in different concentrations. This addition resulted in a great decrease in the very high viscosity.

GREENSTEIN AND JENRETTE[12] have also shown, that the viscosities of solutions of pure nucleic acid are very sensitive to salt additions and especially to the guanidinium-ion. Urea also has a strong effect.

The purpose of the following viscosity measurements was to determine whether or not the effect found when salt is added to nucleic acid solutions also exists for additions of salt to solutions of mildly prepared nucleoprotein.

References p. 116.

I. Measurements on Solutions with a p_H of about 6.3

This investigation was carried out partly on protein, which had already been dissolved in 1 M NaCl, to which had been added solutions of NaCl, NaI, guanidine-HCl, guanidine-HNO_3 and urea, and partly on protein which had been prepared and precipitated in the usual manner and then directly dissolved in solutions of NaI, and guanidine-HCl. Viscosity measurements on these solutions were made with different protein concentrations. In urea practically no nucleoprotein dissolved.

The measurements were carried out in an Ostwald viscosimeter, which was placed in a well insulated thermostat filled with ice water.

The different measurements, obtained after the addition of different salts are given in Table V. "Relative viscosity" in the table is obtained directly by dividing the time for the outflow of the nucleoprotein solution by the time for the solvent in each case.

TABLE V

VISCOSITY CHANGES AT 0° C IN NUCLEOPROTEIN SOLUTION CONTAINING 0.165 mg N/ml DISSOLVED IN 1 M NaCl PLUS PHOSPHATE BUFFER (IONIC STRENGTH 0.05) WITH ADDITION OF DIFFERENT SALTS

Added salt solution Final conc.	Rel. visc. of protein-solution and solvent + added salt	pH
2 M urea	2.98	6.3
2 M NaCl	3.03	6.2
Saturated gu-HNO_3	3.03	5.8
About 1.8 M gu-HCl	3.13	5.9
About 1.8 M gu-HCl	3.14	6.0
2 M gu-HCl	3.12	6.2
2 M NaI	3.05	6.2
No salt added	2.81	6.3

From the table it is quite clear that there is no change in the viscosity by the addition of guanidinium-ion, urea or NaI to nucleoprotein solutions in 1 M NaCl slightly buffered at p_H 6.3. This is in striking contrast to the large decrease in viscosity obtained by the addition of these salts to nucleic acid solutions.

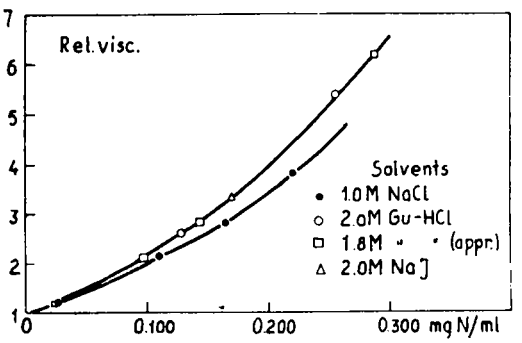

Fig. 2. Relative viscosity at 0° C for nucleoprotein in different salt solutions with p_H between 6.2 and 6.3

The result of directly dissolving nucleoprotein freshly precipitated in three different salt solutions will best be understood from the curve in Fig. 2.

The nucleoprotein itself is from the same preparation as that in the first experiment above.

It can be stated that any decrease in the viscosity of nucleoprotein solutions, when changing from NaCl to guanidine-HCl or NaI as the solvent is not observed.

In two preparations the possible change of viscosity with time was investigated (see Table VIa and b). The solutions were kept at 0° C the whole time. Time is measured from the start of the last dissolving.

References p. 116.

When stirring, the viscosity, as seen from the table changed a little in the second decimal. The slight decrease probably depended on the fact that water condensed in the cold solution when it was allowed to stand exposed to the air for a while. No thixotropic effect seems, however, to exist.

TABLE VIa

INFLUENCE OF TIME ON VISCOSITY AT 0° C OF NUCLEOPROTEIN (PREPARATION Th 13) IN 1 M NaCl PLUS PHOSPHATE BUFFER (IONIC STRENGTH 0.05). THE PROTEIN SOLUTION STORED AT 0° C

Time in days	Rel. visc.	
1	2.81	
3	2.83	pH 6.3–6.2
8	2.89	0.165 mg N/ml
9	2.91	

TABLE VIb

INFLUENCE OF TIME ON VISCOSITY AT 0° C OF NUCLEOPROTEIN (PREPARATION Th 12) IN 1 M NaCl PLUS PHOSPHATE BUFFER (IONIC STRENGTH 0.05). THE PROTEIN SOLUTION STORED AT 0° C

Time in days	Rel. visc.	
9	2.88	
11	2.93	
19	3.06	
30	3.27	pH 6.3–6.2
33	3.27	0.24 mg N/ml
Stirring for 100 min	3.22	
,, ,, 180 min	3.20	

For the most part, one can say that the viscosity of these nucleoprotein solutions remains constant for several weeks if the solutions are correctly stored. Depolymerization does not seem to take place. It must however, be stressed that after a longer time the solutions seem to undergo essential changes, even with the viscosity unchanged, as the tendency to precipitate on dilution is diminished.

II. Measurements on Solutions of p_H 6.3 and higher

It was found that fresh nucleoprotein dissolved in both 0.05 M NaOH or in 1 M NaCl gave high viscosities. Relative viscosity was about 2.9 in both cases. If salt was added to the solution of protein in base, the viscosity decreased very rapidly down to about 1.1. The same viscosity value was obtained if NaOH was added to a solution of nucleoprotein in 1 M NaCl. The decrease, however, is somewhat slower in the latter case.

After these first experiments a series of measurements of additions of NaOH to nucleoprotein in 1 M NaCl were made. Small samples of the protein solution were taken, and solvent and 0.100 M NaOH were added to each sample, giving the same protein concentration, the same 1 M NaCl, but a different amount of NaOH.

The solutions were kept for about 24 hours in the refrigerator after the addition of NaOH. (Except the one marked with an X in the curve Fig. 3, which was kept at room temperature).

References p. 116.

The increase in viscosity, which preceded the final decrease, is remarkable. The curve in this region is dotted to show that the values both for p_H and viscosity are rather unstable. The time for outflow in the viscosimeter decreased by as much as 10 seconds from one reading to another. The p_H before and after three readings usually differed by one or two tenths. The other parts of the curve gave reproducible and stable values.

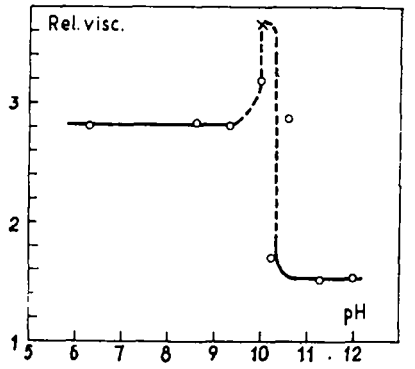

Fig. 3. Relative viscosity at 0° C of nucleoprotein in 1 M NaCl at different p_H. Different amounts of 0.100 M NaOH have been added 24 hours before the measurements

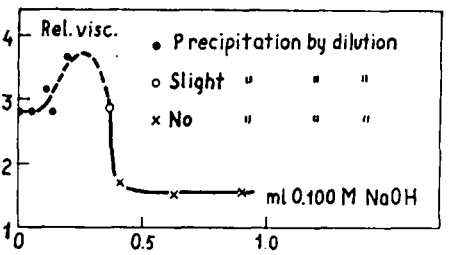

Fig. 4. Relative viscosity at 0° C of a nucleoprotein (same as in Fig. 3) in 1 M NaCl after adding different amounts of 0.100 M NaOH. The measurements were made 24 hours after the addition of the base

The definite change in viscosity seems to come after an addition of $4 \cdot 10^{-5}$ mol NaOH to 10.7 mg nucleoprotein, calculated on basis of a N-content of 15.1%.

It has also been indicated on the curve (Fig. 4) that the nucleoprotein solutions lose their ability to precipitate on dilution at the same time that their viscosities tend to become dependent on salt concentration. Tests of precipitability were made by adding 5 ml of distilled water to 1 ml of each solution. The precipitates were centrifuged down at 9500 r.p.m. for half an hour. 4 ml of the solutions were investigated for N according to KJELDAHL (see Table VII).

TABLE VII

AMOUNT OF NITROGEN LEFT IN THE SOLUTION AFTER PRECIPITATION BY DILUTION AT DIFFERENT PH VALUES

mg N/4 ml	ml 0.100 M NaOH added to the original samples
0.015	0.00
0.011	0.05
0.018	0.11
0.010	0.14
0.010	0.20
0.084	0.37
0.095	0.42
0.109	0.63
0.119	0.90

THE ULTRAVIOLET ABSORPTION

I. Nucleic Acid Content from Percentage P and Light Absorption

As has been already stated the nucleoprotein solutions gave the typical curve for

References p. 116.

ultra-violet absorption which was given by CASPERSSON[7] and others. This curve, with the maximum about 258–260 mμ, was of the same type for dried preparations dissolved in dilute NaOH and for fresh nucleoprotein dissolved in 1 M NaCl.

The fact that the nucleoprotein followed BEER's law was proved with a preparation first freeze-dried and then dried in vacuum over phosphorus pentoxide at 90° C for 72 hours and then dissolved in 0.100 M NaOH. The absorption in ultra-violet light at 260 mμ was then measured for four different concentrations of nucleoprotein: 0.0059 mg N/ml gave an absorption of 0.460, 0.0118 mg N/ml gave 0.940, 0.0148 mg N/ml gave 1.180 and 0.0178 mg N/ml gave 1.415. For all four measurements this means a quotient of 80 between extinction and mg N/ml.

This value gives a percentage of nucleic acid of about 44%, if compared with absorption measurements made by B. DRAKE (*unpubl.*) on pure nucleic acid, (the same preparation as used by BJÖRNESJÖ AND THEORELL[13]). The P-content of the nucleoprotein preparations described in this paper was 3.8–3.9% which gives a nucleic acid content of only 38–39%, if calculated on the basis of the value from LEVENE's formula — 9.91% P for thymus nucleic acid. It is, however, assumed nowadays that this formula gives too high a phosphorus percentage.

The nucleic acid used as comparison gives 7.6% P as tetrasodium salt. The highest percentages yet found in tetrasodium nucleate preparations according to HAMMERSTEN are 8.8 and 8.4 given by GREENSTEIN[14]. These values give a nucleic acid content, calculated from the phosphorus content, closer to that given by estimation from absorption curves. GULLAND, JORDAN, AND THRELFALL[15] have, however, with another preparative method obtained tetrasodium nucleate with a phosphorus content quite near to the value calculated from LEVENE's formula. It thus seems clear that all the phosphorus in the nucleoprotein preparations used here comes from the nucleic acid.

II. The Influence of p_H on the Absorption Curve

The calculation above is valid for nucleoprotein dried in the way above mentioned and then dissolved in dilute NaOH. If the absorption is measured on fresh preparations in 1 M NaCl, there will be a lower quotient between the maximum extinction at 260 mμ and the nitrogen content measured in mg N/ml.

In the three solubility curves quotients about 59 and 55 are found for protein in 1 M NaCl at the beginning, and between 65–76 for protein originally dissolved in distilled water. In the first case the lower values belong to the curve where the precipitate was centrifuged down at the highest speed. In the second case the first and lower value is the most reliable, as the N-determinations become more uncertain for solutions near 0.14 M NaCl, which give higher quotients.

Some control experiments were carried out. It was first found that if from the same fresh precipitate one half was redissolved in 1 M NaCl and phosphate buffer (p_H 6.3; ionic strength 0.05) and the other half dried as above and then dissolved in dilute NaOH, the quotient between extinction at 260 mμ and N/ml was for the first solution only 59 or 72% of the same quotient for the second solution, which gave the value 82.

In Fig. 5, curve number 1, a typical curve is seen, for the light absorption of nucleoprotein in 1 M NaCl at a p_H of about 6.3, obtained with the Beckman apparatus and measured against the solvent. The extinction per mg N/ml is 56.

It was now found that if diluted NaOH was added to such a nucleoprotein solution the quotient between extinction and mg N/ml rose to 74. To compare the two absorption

References p. 116.

curves the solution of nucleoprotein giving curve number 1 was diluted with 1 part diluted NaOH to 3 parts of solution and curve number 2 in Fig. 5 was obtained. The NaCl content is kept constant at 1 M.

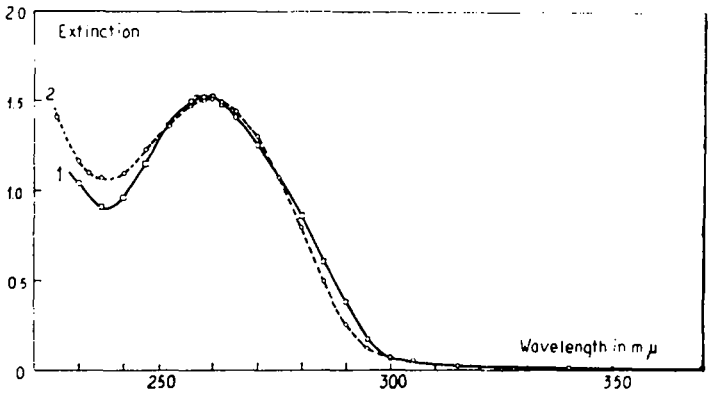

Fig. 5. Light absorption for nucleoprotein in 1 M NaCl (0.0271 mg N/ml) is shown in curve no. 1. The dotted line, curve no. 2, gives the values if 1 vol. part of 0.10 M NaOH + 1 M NaCl is added to 3 parts of the first solution

To avoid any effects of the added substances, the dilution is done parallel in the two BECKMAN cells.

With this more gentle treatment the light absorption of neutral nucleoprotein is only 75% of the basic one.

It can be concluded from what has been said above, that the absorption increases at 260 mμ if the nucleoprotein is treated with NaOH. If the nucleoprotein is treated more roughly, that is to say, dried and then redissolved in NaOH, the absorption increases still further. HOLIDAY[16], HEYROTH AND LOOFBOUROW[17] have shown that some purines and pyrimidines increase their ultra-violet absorption at higher p_H values. Concerning the nucleoprotein it may also be pointed out that the change in absorption occurs at the same p_H at which the viscosity falls from the addition of salts.

I wish to thank my teacher, Professor A. TISELIUS, who introduced me to this field of work, for all his kind advice and helpful discussions. I also wish to thank Professor T. SVEDBERG for allowing me to perform this work at the Institute of Physical Chemistry in Uppsala.

SUMMARY

Calf thymus nucleoprotein has been prepared according to MIRSKY AND POLLISTER giving a content of 3.8–3.9% phosphorus and 15.1% nitrogen.

Ultracentrifugation and electrophoresis have been carried out on the nucleoprotein dissolved both in 1 M NaCl and in distilled water. The nucleoprotein in 1 M NaCl and phosphate buffer shows three components, the main one having a mobility of $7.9 \cdot 10^{-5}$ cm²/volt sec.

When nucleoprotein dissolved in 1 M NaCl was precipitated through dilution, the nucleic acid precipitated in parallel with the protein. From 1 M down to about 0.35 M NaCl the precipitation curve is composed of three parts, giving three approximatively straight lines if the nucleoprotein is plotted against the square of the salt molarity. Between 0.30 and 0.05 M NaCl practically all the nucleoprotein is precipitated. The nucleoprotein dissolves in distilled water.

From the measurements of light absorption at 260 mμ it was found that about 44% of the nucleoprotein is nucleic acid. This agrees with the percentage of phosphorus, showing that all phosphorus comes from the acid.

References p. 116.

The nucleoprotein has a high viscosity both in 1 M NaCl and in dilute NaOH. But if NaOH is added to the former type of solution the viscosity drops at a p_H about 10.5. The viscosity also drops if salts are added to the solution of nucleoprotein in NaOH.

No decrease in viscosity was obtained, when guanidinium-ion or urea was added to the solution of nucleoprotein in 1 M NaCl.

The viscosity does not decrease with time if the nucleoprotein solution is stored in the cold.

The maximal extinction of light at 260 mμ increases if the nucleoprotein is treated with NaOH. The position of the extinction maximum does not change.

RÉSUMÉ

La nucléoprotéine du thymus de veau a été préparée d'après la méthode de MIRSKY ET POLLISTER. La substance contient 3.8 à 3.9 % de phosphore, et 15.1 % d'azote. La nucléoprotéine dissoute soit dans NaCl 1 M soit dans de l'eau distillée, a été soumise à l'ultracentrifugation et à l'électrophorèse. Dans NaCl 1 M tamponné par des phosphates, la nucléoprotéine est formée de trois constituants dont le principal a une mobilité de $7.9 \cdot 10^{-5}$ cm²/volt sec.

Lorsque la nucléoprotéine dissoute dans NaCl 1 M est précipitée par dilution, l'acide nucléique précipite parallèlement à la protéine. Pour des concentrations de NaCl allant de 1 M à environ 0.35 M, la courbe de précipitation comporte trois parties, s'exprimant approximativement par des lignes droites si la teneur en nucléoprotéine est exprimée en fonction du carré de la molarité du sel. Lorsque la concentration en NaCl est entre 0.30 et 0.05 M, pratiquement toute la nucléoprotéine est précipitée. La nucléoprotéine se dissout dans l'eau distillée.

La mesure de l'absorption lumineuse à 260 mμ montre que 44 % de la nucléoprotéine consiste en acide nucléique. Ceci correspond à la teneur en phosphore, ce qui montre que tout le phosphore appartient à l'acide nucléique.

La nucléoprotéine possède une viscosité élevée, aussi bien dans NaCl 1 M que dans la soude diluée. Mais si l'on ajoute de la soude à la solution dans NaCl, la viscosité diminue à un p_H voisin de 10.5. La viscosité diminue également si on ajoute des sels à la solution de nucléoprotéine dans NaOH.

La viscosité ne diminue pas lorsque l'on ajoute l'ion guanidinium ou de l'urée à la solution de nucléoprotéine dans NaCl 1 M.

La viscosité ne diminue pas avec le temps lorsque la solution de nucléoprotéine est conservée au froid.

L'extinction maximum de la lumière à 260 mμ s'accroît si la nucléoprotéine est traitée par NaOH, mais la position du maximum d'extinction reste la même.

ZUSAMMENFASSUNG

Kalbsthymusnukleoprotein wurde nach MIRSKY UND POLLISTER mit einem Phosphorgehalt von 3.8–3.9 % und einem Stickstoffgehalt van 15.1 % bereitet.

Mit Lösungen des Nukleoproteins, sowohl in 1 M NaCl als in destilliertem Wasser wurden Ultrazentrifugierungs- und Elektrophoresemessungen ausgeführt. In 1 M NaCl und Phosphatpuffer zeigt das Nukleoprotein drei Komponenten, von denen die Hauptkomponente eine Beweglichkeit von $7.9 \cdot 10^{-5}$ cm²/Volt sec. hat.

Bei Fällung von Nukleoprotein, das in 1 M NaCl gelöst war, durch Verdünnen schlug die Nukleinsäure parallel mit dem Eiweiss nieder. Von 1 M ab bis zu ungefähr 0.35 M NaCl ist die Präzipitationskurve aus drei Teilen zusammengesetzt, die drei angenähert gerade Linien ergeben, wenn der Nukleoproteingehalt gegen das Quadrat der Salzmolarität aufgetragen wird. Zwischen 0.30 und 0.05 M NaCl wird praktisch das gesamte Nukleoprotein gefällt. Das Nukleoprotein löst sich in destilliertem Wasser.

Aus Messungen der Lichtabsorption bei 260 μm wurde festgestellt, dass ungefähr 44 % des Nukleoproteins Nukleinsäure ist. Dieses Ergebnis stimmt mit dem Phosphorgehalt überein. Es zeigt sich also, dass der gesamte Phosphor aus der Nukleinsäure stammt.

Das Nukleoprotein hat sowohl in 1 M NaCl wie auch in verdünnter NaOH eine hohe Viskosität. Wenn jedoch zu ersterer Lösung NaOH zugefügt wird, fällt der Viskositätswert bei einem p_H von ungefähr 10.5. Die Viskosität wird auch erniedrigt, wenn zu der Lösung des Nukleoproteins in NaOH Salze hinzugefügt werden.

Bei Zugabe von Guanidinium-ionen oder Harnstoff zu der Nukleoproteinlösung in 1 M NaCl wurde keine Viskositätsverringerung erhalten.

Die Viskosität nimmt im Laufe der Zeit nicht ab, wenn die Nukleoproteinlösung kalt aufgehoben wird.

Die maximale Lichtextinktion bei 260 mμ nimmt bei Behandeln des Nukleoproteins mit NaOH zu. Die Lage des Extinktionsmaximums ändert sich dabei nicht.

References p. 116.

REFERENCES

1 W. HUISKAMP, *Z. Physiol. Chem.*, 32 (1901) 145.
2 R. O. CARTER AND J. L. HALL, *J. Am. Chem. Soc.*, 62 (1940) 1194.
3 A. E. MIRSKY AND A. W. POLLISTER, *Proc. Natl. Acad. Sci., U.S.*, 28 (1942) 334.
4 A. E. MIRSKY AND A. W. POLLISTER, *Biol. Symposia*, 10 (1943) 247.
5 A. E. MIRSKY AND A. W. POLLISTER, *Trans. N.Y. Acad. Sci.*, 5 (1943) 190.
6 R. O. CARTER, *J. Am. Chem. Soc.*, 63 (1941) 1960.
7 T. CASPERSSON, *Scand. Arch. Physiol.*, 73 Suppl. 8 (1936).
8 Q. V. WINKLE AND W. G. FRANCE, *J. Phys. Colloid Chem.*, 52 (1948) 207.
9 R. O. CARTER, *Dissert. Univ. of Wisconsin* (1939) 88.
10 H. V. EULER AND I. FISHER, *Arkiv Kemi, Mineral. Geol.*, 22 A (1946) No. 4.
11 J. P. GREENSTEIN AND W. V. JENRETTE, *J. Nat. Canc. Inst.*, 1 (1940/41) 91.
12 J. P. GREENSTEIN AND W. V. JENRETTE, *Cold Spring Harbor Symposia Quant. Biol.*, 9 (1941) 236.
13 K. B. BJÖRNESJÖ AND T. TEORELL, *Arkiv Kemi, Mineral. Geol.*, 19 A (1945) No. 34.
14 J. P. GREENSTEIN, *J. Nat. Canc. Inst.*, 7 (1946) 10.
15 J. M. GULLAND, D. O. JORDAN, AND C. J. THRELFALL, *J. Chem. Soc.* (1947) 1129.
16 E. R. HOLIDAY, *Biochem. J.*, 24 (1930) 619.
17 F. F. HEYROTH AND J. R. LOOFBOUROW, *J. Am. Chem. Soc.*, 56 (1934) 1728.

Received September 3rd, 1948