THYMO-NUCLEOPROTEINS IN NaCl SOLUTIONS OF DIFFERENT STRENGTHS

by

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In an earlier article[1] it was shown that when thymo-nucleoprotein, prepared according to MIRSKY AND POLLISTER[2,3] and dissolved in 1 *M* NaCl, was precipitated by decreasing the salt concentration, the precipitation curve was composed of three parts (see Fig. 1).

It was also reported that two, or sometimes three, peaks were observed in the electrophoresis diagrams, when the nucleoprotein was examined in 1 *M* NaCl plus phosphate buffer solutions. Three peaks were also observed by WINKLE AND FRANCE[4] in their electrophoresis runs.

The question now is, whether the different parts of the precipitation curve and the different peaks in the electrophoresis diagram, correspond to different components of the original nucleoprotein or whether this multiplicity is an effect of the changes in the salt concentration of the solvent.

Electrophoresis

In order to decide between the above alternatives, precipitations were made by diluting to different salt concentrations, the precipitates then being redissolved in 1 *M* NaCl and investigated in the Tiselius electrophoresis apparatus. The original nucleoprotein was dissolved in 1 *M* NaCl buffered with phosphate at p_H 6.3 (ionic strength of buffer 0.05). A detailed description of the preparation of the nucleoprotein is given in the earlier article[1]. The dilution was made with buffer solution only and carried out in a cold storage room.

As the precipitate used to make the original solution cannot be dried completely, the molarity of the 1.0 *M* NaCl solution will be somewhat diminished. The values for the NaCl concentrations have an error of 0.01 *M*. To obtain the molarity the sodium was determined as sodium sulphate and weighed. The relative values between the different salt molarities within the same series will, however, be correct to the second decimal.

In those cases where the salt concentrations were diminished from 1.00 *M* to 0.46, 0.53 and 0.60 *M* respectively, the precipitate was collected by centrifuging for 60 minutes at 9500 r.p.m. (diameter of centrifuge 13 cm) in the cold storage room, in the same manner as when the precipitation curve itself was determined. The precipitates were redissolved in 1 *M* NaCl and phosphate buffer in order to make a comparison with the

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earlier electrophoresis runs possible. Before the runs the solutions were dialysed for 24 hours against 1 *M* NaCl and phosphate buffer (p_H 6.3, ionic strength 0.05).

From two of the centrifuge tubes, those containing 0.46 and 0.60 *M* NaCl, the supernatant fluid was collected and directly dialysed against 1 *M* NaCl and phosphate buffer (marked* in Table I).

TABLE I

ELECTROPHORESIS RUNS ON NUCLEOPROTEIN PRECIPITATED BY DILUTION FROM 1.00 *M* NaCl TO DIFFERENT SALT CONCENTRATIONS AND REDISSOLVED IN 1.00 *M* NaCl, AT WHICH CONCENTRATIONS THE RUNS WERE CARRIED OUT

(For the detailed treatment of the two runs marked with*, see text). All solutions contained phosphate buffer (ionic strength 0.05, p_H 6.3)

Preparation	Precipitation Diluting from	to	Nitrogen in mg/ml	Mobility (after resolution in 1 *M* NaCl + phosphate buffer) $\cdot 10^5$ cm²/volt sec
22	1.00 *M*	0.46 *M*	0.076	8.3
22	*0.46 *M*	(0.14) *M*	0.079	8.3
22	1.00 *M*	0.53 *M*	0.077	8.6
22	1.00 *M*	0.46 *M*	0.249	8.3
23	1.00 *M*	0.60 *M*	0.472	8.2
23	*0.60 *M*	(0.14) *M*	0.256	8.1

p_H 6.3

As seen from Table I the electrophoretic mobilities turned out to be nearly the same in all runs. The difference in value is only in the first decimal place, whereas the values of the mobility of the two other peaks, previously observed (see earlier article[1]), had values which were at least one whole unit less.

As very little material was used it is not possible to draw any conclusions from the fact that in some runs only one component was observed. In three runs, however, with a higher content of nucleoprotein, two peaks were observed in the apparatus, one of which was considerably smaller than the other. The main peak was followed for 14–15 hours in all runs, but it was not possible to observe the smaller one for more than 6 hours.

The voltage used was 50 V, giving a current of about 43 mA.

Thus it seems impossible to distinguish different nucleoproteins from the different parts of the precipitation curve with the electrophoresis apparatus. It also seems unlikely that those parts are due to different nucleoproteins since all the precipitates, as shown in an earlier article[1], give the same nitrogen/nucleic acid quotient and also, as shown above, manifest the same electrical behaviour when compared under the same circumstances.

Viscosity measurements

Two series of precipitations were made in the same manner as above (Fig. 1). The precipitates were centrifuged down and the viscosities of the supernatant fluids were determined with an OSTWALD viscosimeter in a well insulated ice bath. As before, the centrifuge was run at 9500 r.p.m., for 60 min in the first series, for 90 min in the second

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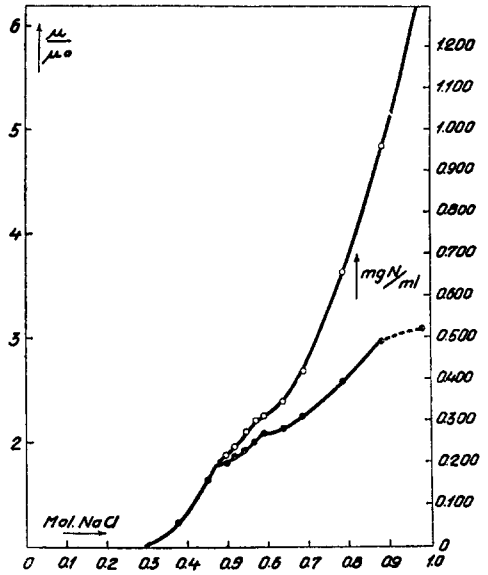

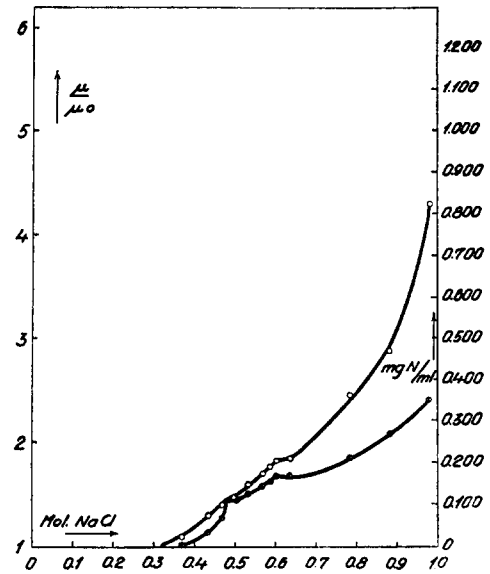

Figs. 1 and 2. Relative viscosity at 0° C (unfilled circles) and nitrogen content (crossed circles) plotted against the NaCl content for a series of solutions which were prepared from a nucleoprotein dissolved in 1 *M* NaCl and phosphate buffer (ionic strength 0.05 pH 6.3) by dilution with the buffer solution in the cold. The precipitated part of the protein was centrifuged down and the determinations carried out on the supernatant

series. The solution at 1 *M* NaCl in the first series was somewhat opalescent, whereas the same solution in the second series was quite clear. The nitrogen content was determined according to KJELDAHL. All procedures were carried out in a cold storage room.

The results of these determinations are seen in Figs. 1 and 2, where relative viscosity and nitrogen content are plotted against the content of NaCl to which the different protein solutions have been diluted. If instead of the relative viscosity μ/μ° (where μ° is the viscosity of the solvent), the expression $\frac{\mu/\mu^\circ - 1}{C}$ (where C is the concentration given in grams per 100 ml solution) is calculated and plotted as before against the salt molarity, the curves shown in Fig. 3 are obtained. These curves show that the characteristic form of the viscosity curve is dependent on the salt concentration, showing in both cases a decrease, quite steep

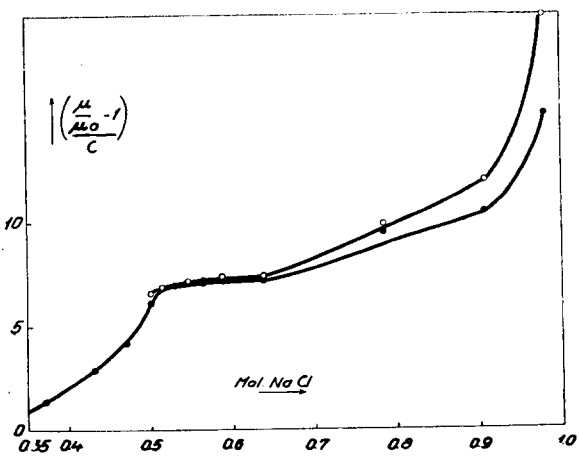

Fig. 3. The two series of viscosity measurements on nucleoprotein solutions of different NaCl content at 0° C, pH 6.3 given as relative viscosity in Figs. 1 and 2 are here given as specific viscosity divided by the concentration. The unfilled circles correspond to the series in Fig. 1, the filled circles to the series in Fig. 2. The protein concentration is given in grams protein per 100 ml solution and calculated from the nitrogen determinations

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in the beginning, between 1.00 and 0.65 *M* NaCl. The values then remain nearly constant till about 0.51 *M* NaCl and then decrease sharply. If instead of plotting against salt concentration, the $\frac{\mu/\mu^\circ - 1}{C}$ values against the concentration of protein are plotted, the significant changes in the two new curves would not coincide, as is easily concluded from the three figures shown here.

In order to investigate in more detail the different parts of the precipitation curve, one preparation of nucleoprotein was precipitated in two steps, one for each of the two main parts. The first fraction, precipitating on dilution from 1.0 *M* to 0.60 *M* NaCl and the second, from 0.60 *M* to 0.14 *M*, were collected separately. The two samples of nucleoprotein were then redissolved in 1 *M* NaCl and the precipitation curve for both solutions determined by dilutions, as described above. For each sample in both series viscosity and nitrogen content were determined. If these values were plotted against the salt concentration, typical curves, similar to those shown in Figs. 1 and 2, were obtained for both nucleoprotein precipitates.

The large increase in the sedimentation constant when diminishing the salt concentration, which is reported below, appeared also for both samples.

These results show, that the specific changes in the precipitation curve most probably depend on the change in salt concentration and its influence on the structure of the dissolved nucleoprotein.

Intrinsic viscosity

Finally, viscosities were determined for some preparations of nucleoprotein in different salt concentrations and with diminishing protein content.

As was to be expected, when dealing with solutions of such very high viscosities

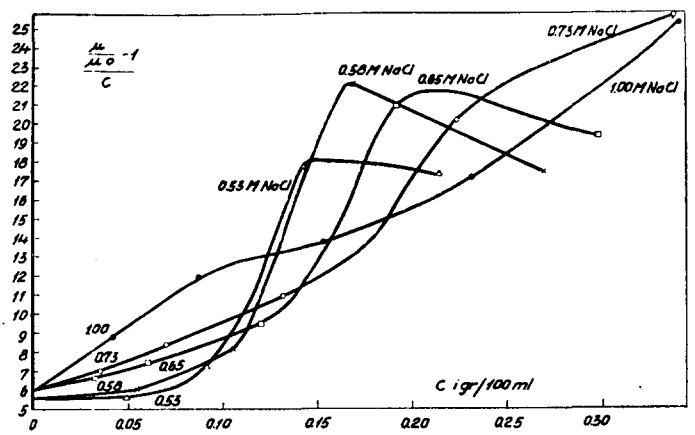

Fig. 4. Specific viscosity divided by the concentration of a nucleoprotein preparation in solutions of different NaCl content and plotted against the protein concentration. The concentration is calculated from nitrogen determinations. Each protein solution was obtained from the solution containing 1 *M* NaCl and phosphate buffer (ionic strength 0.05, pH 6.3) by diluting with the buffer solution in the cold. The precipitated part of the protein was centrifuged down and the different supernatant protein solutions then diluted with solutions containing phosphate buffer and NaCl of the new molarity. The first points in the curves correspond to the original solutions and all curves show a rise in viscosity after the first dilution. The curves are drawn through zero concentration. This can only be done, however, with any certainty if more points are determined in the range of very low protein concentration where the curves can be looked upon as straight lines

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it was necessary to use very low concentrations of protein in order to make it possible to extrapolate to zero concentration (Fig. 4). This is not possible using values higher than 0.1%. The difficulty then is to get enough concentration points with sufficient accuracy. These protein concentrations were determined in two ways. Firstly, in two complete series (Fig. 5), from the nitrogen content, determined according to KJELDAHL (N = 15.1% of the nucleoprotein) and seccndly, in two series (Fig. 6) from the ultra-violet absorption as measured at 260 mμ in the Beckman spectrophotometer. It has been shown[1] that the extinction/nitrogen content quotient is constant over the whole range of salt concentrations used here. The results of these measurements are given in Figs. 5 and 6. The discussion of these results follows below.

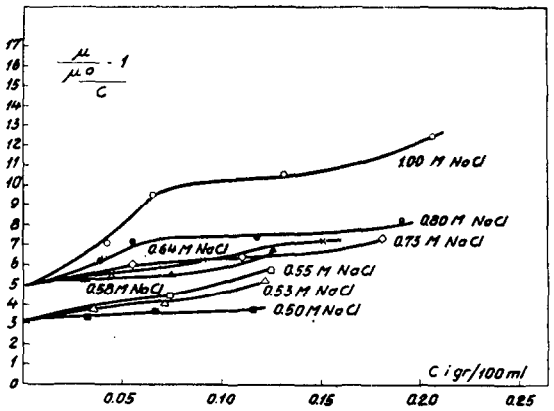

Fig. 5. Specific viscosity at 0° C, pH 6.3, divided by the concentration of protein and plotted against the protein concentration for a series of nucleoprotein solutions prepared as described under Fig. 4. Only the determinations at low concentration are shown here

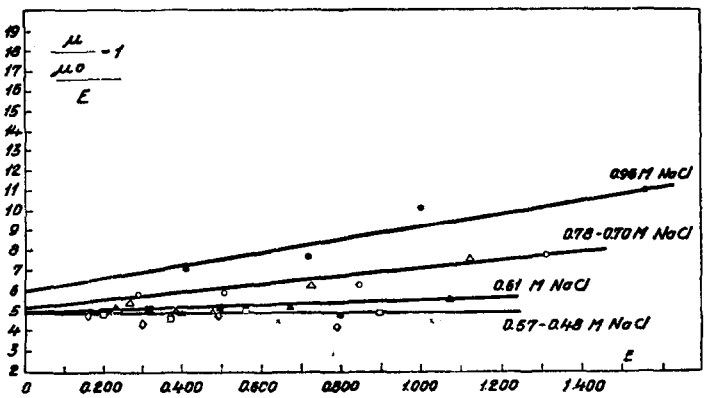

Fig. 6. Specific viscosity at 0° C, pH 6.3, divided by the extinction at 260 mμ for a series of nucleoprotein concentrations and plotted against the extinction. The solutions were prepared as described under Fig. 4. Only the determinations at low protein concentrations are shown here.

Filled circles correspond to 0.96 *M* NaCl
Unfilled triangles correspond to 0.78 *M* NaCl
Unfilled circles correspond to 0.70 *M* NaCl
Filled triangles correspond to 0.61 *M* NaCl
Crosses correspond to 0.57 *M* NaCl
Unfilled squares correspond to 0.53 *M* NaCl
Diamonds correspond to 0.48 *M* NaCl

Ultracentrifugation

Some of the solutions used for viscosity measurements were also examined in the SVEDBERG ultracentrifuge. They were run at a speed of 40,000 r.p.m. giving a single sharp peak in the diagram. From Fig. 7 it can be seen that the sedimentation constant increases very rapidly when the salt concentration is decreased. That this is not due to the usual effect following the dilution of the protein follows from the curves in Fig. 8,

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where the lowest curve shows the increase in the sedimentation constant if the salt content is kept at 1 *M* NaCl and the protein content decreased. Values given by CARTER[5] for different protein concentrations in a salt molarity of 0.85 would fall above this curve and nearly parallel to it, giving the value $S_{20} = 24.7$ for 0.10% protein.

For one solution (0.65 *M* NaCl and phosphate buffer p_H 6.3, ionic strength 0.05, 0.19 g nucleoprotein in 100 ml) the diffusion constant was also determined and found to be $0.25 \cdot 10^{-7}$ cm²/sec, the corresponding sedimentation constant being 37.9 *S*. These values would indicate a molecular weight of at least 11,500,000.

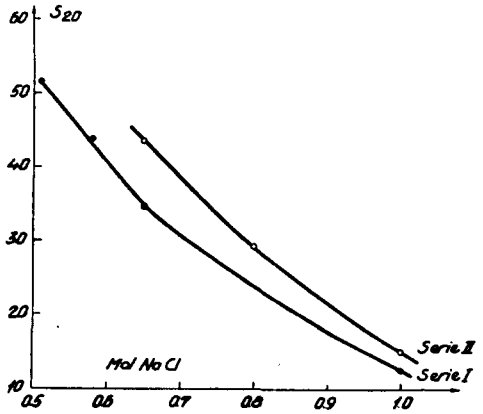

Fig. 7. Variation of the sedimentation constants with NaCl content for two nucleoprotein preparations. For the protein concentrations see Fig. 8. The nucleoprotein solutions of different salt strength were obtained by dilution from protein solutions of 1 *M* NaCl. The precipitated part of the protein was centrifuged down and the determinations carried out on the supernatant

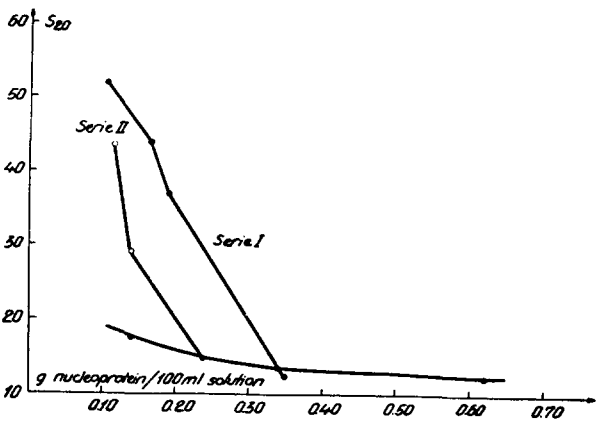

Fig. 8. Sedimentation constants for nucleoprotein plotted against the protein concentration. Curves I and II (filled and unfilled circles) are for the same solutions as those shown in Fig. 7, where the salt content is given. These solutions thus differ both in protein and NaCl content. The third curve is determined for nucleoprotein in 1 *M* NaCl and the solutions differ only in protein concentration. All solutions contain phosphate buffer, ionic strength 0.05, p_H 6.3

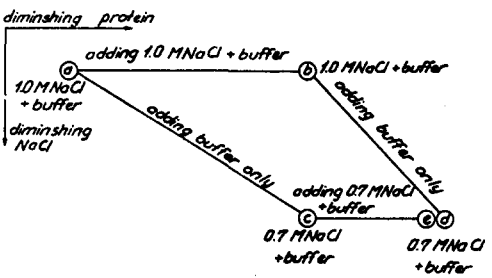

Fig. 9. Dilution diagram for the solutions in Table II. (a) is the original solution of nucleoprotein in 1.0 *M* NaCl and phosphate buffer (ionic strength 0.05, p_H 6.3; called buffer in the diagram). When the buffer solution only was added some of the protein precipitated, the supernatant giving solution (c) or (d)

To obtain sedimentation and diffusion constants of the some preparation of nucleoprotein at different concentrations both of protein and salt, three series of experiments were run using solutions from three separately prepared nucleoproteins.

The method of dilution is most easily understood from Fig. 9. In this Figure (a) is the original solution (1.0 *M* NaCl + phosphate buffer, p_H 6.3, ionic strength 0.05 + the recorded protein concentration, Table II). Moving clockwise from (a), solution

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(b) is obtained by dilution with 1.0 *M* NaCl and buffer, thus diminishing the protein concentration only. Solution (b) is then diluted with buffer to 0.7 *M* NaCl to give solution (d). When the NaCl concentration is decreased some of the protein precipitates as usual and is centrifuged down, solution (d) being the supernatant liquid.

TABLE II

SEDIMENTATION AND DIFFUSION CONSTANTS FOR THREE THYMO-NUCLEOPROTEIN PREPARATIONS AT DIFFERENT NaCl AND PROTEIN CONCENTRATIONS

The method of dilution from the original solution (a) is shown in Fig. 9

Solution	S_{20} corr.	$\frac{S_{20}(\times)}{S_{20}(a)}$	Content NaCl	Diff. const. D_A	Conc. mg N/ml
a	17.7	1.0	1.0 *M*	—	0.444
b	22.7	1.3	1.0 *M*	—	0.258
c	34.0	1.9	0.7 *M*	—	0.283
d	39.8	2.3	0.7 *M*	—	0.192
e	36.5	2.1	0.7 *M*	—	0.212
a_1	(17.1) *16.1	1.0	1.0 *M*	$0.19 \cdot 10^{-7}$	0.318
b_1	*19.1	1.2	1.0 *M*	$0.11 \cdot 10^{-7}$	0.197
c_1	*26.4	1.7	0.7 *M*	$0.10 \cdot 10^{-7}$	0.231
d_1	*30.4	1.9	0.7 *M*	$0.14 \cdot 10^{-7}$	0.144
e_1	*30.2	1.9	0.7 *M*	—	0.143
a_2	11.9	1.0	1.0 *M*	$0.16 \cdot 10^{-7}$	0.372
b_2	15.4	1.3	1.0 *M*	$0.18 \cdot 10^{-7}$	0.425
c_2	20.5	1.7	0.7 *M*	$0.14 \cdot 10^{-7}$	0.419
d_2	27.3	2.3	0.7 *M*	$0.17 \cdot 10^{-7}$	0.282

* Also dialysed for centrifuge

The first step moving anticlockwise from (a) also includes decreasing the salt molarity to 0.7 *M* with buffer only, some protein precipitating and solution (c) being the supernatant liquid. Solution (c) is brought to a protein concentration near that of (d) by dilution with 0.7 *M* NaCl and buffer. This last solution is called (e).

The solutions (c) and (d) were centrifuged clear from the resulting precipitates at 10,000 r.p.m. in 60 minutes. The solutions (a) and (b) were run in the same way in the centrifuge so that the same treatment was obtained for all solutions except (e).

The nucleoprotein solutions were investigated in the ultracentrifuge at 40,000 r.p.m. (see Table II). The peak sedimented normally, *i.e.*, the velocity was constant throughout the whole run, thus giving no indication of gel formation whatsoever.

The diffusion constants were determined for series 2 and 3. After dialysis, the runs were made against pure solvent and in all cases, except two, at 20° C. They were followed for 4 days. A cell, according to CLAESSON[7], was used in four of those experiments (a_1, b_1, c_1, d_1), and a glass cell in two (a_2, d_2)[8].

In the two remaining cases b_2 and c_2, the glass cell of a TISELIUS electrophoresis apparatus was used. The temperature was +1° C and the diffusion was followed for 14 days. These last two runs were by far the best in quality (For results see Table II). The nitrogen content was determined by the KJELDAHL method.

The diffusion diagrams were in all cases asymmetrical. The curve for solution b_2 is shown in Fig. 10.

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The molecular weight has not been calculated, for it must be stressed that in such a case as this, only a mean value would result.

In the calculations, the value 0.65 for the specific volume was used in all runs above. This value was determined by the use of a pycnometer at 0° C.

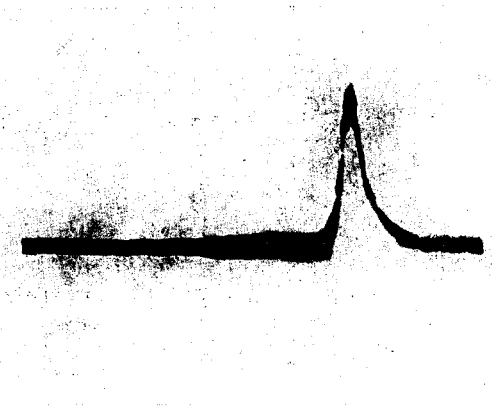

Fig. 10. Diffusion curve for solution b_2 (see Table II) of nucleoprotein in 1.0 *M* NaCl and phosphate buffer (ionic strength 0.05, p_H 6.3) against the solvent 8 days after the start of the experiment. The diffusion was carried out at + 1° C in an electrophoresis cell. The curve shows a very marked asymmetry. The right-hand part of the curve represents the nucleoprotein solution.

DISCUSSION

If the results of the centrifugation and diffusion measurements are compared with the measurements of the intrinsic viscosity, the following picture of the behaviour of the nucleoprotein in different NaCl concentrations seems the most likely.

When diluted between 1.0 *M* and 0.6 *M* NaCl some nucleoprotein precipitates and the remaining parts show a strong association. The gradient of the specific viscosity curves divided by the concentration plotted against the concentration diminishes and the intrinsic viscosity decreases somewhat indicating a slight curling up of the molecule.

At about 0.6 *M* NaCl there is a more marked drop in intrinsic viscosity (see Figs. 4–6). Thus the molecule curls up and the first discontinuity in the precipitation curve (see Figs. 1 and 2) appears. The precipitation is now more rapid and the precipitate is of a more cotton-like type. The diminishing gradient for the specific viscosity curves indicates some curling up of the molecule in this part of the precipitation as well. Unfortunately, the concentration is too low to admit investigation in the ultracentrifuge or by diffusion.

Below a NaCl content of 0.48 *M* the solubility of the nucleoprotein drops very fast and no measurements of the shape of the molecule have been possible. Essentially, 0.48 *M* NaCl may be taken as the limiting boundary for the solubility of the nucleoprotein. The nucleoprotein is not to be looked upon as a homogeneous substance and this may account for the fact that the solubility does not drop absolutely sharply.

This above-mentioned sensitivity of the nucleoprotein to changes in salt concentration may also account for the observations of more than one peak in the electrophoresis diagram.

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It should be observed that the nucleoprotein solutions of different salt strength investigated here were obtained by dilution from standard solutions of 1 *M* NaCl and were, after the dilution, solutions of a critical concentration in equilibrium with a precipitate.

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SUMMARY

Thymo-nucleoprotein, prepared according to MIRSKY AND POLLISTER, has been shown to change its sedimentation greatly with changes in the salt concentration of the solvent.

The separate discontinuous parts of the precipitation curve obtained when decreasing the salt concentration to different extents from 1.0 *M*, of a nucleoprotein/NaCl solution, have been related to different shapes of the protein molecule.

It has also been suggested that the multiplicity of peaks in the electrophoresis diagrams may have arisen from effects due to salt concentrations.

RÉSUMÉ

Nous avons montré que la sédimentation de thymo-nucléoprotéine préparée selon MIRSKY ET POLLISTER varie considérablement lorsque la concentration des sels dissous dans le solvent varie.

Un rapport a été établi entre les tronçons discontinus de la courbe de précipitation, obtenus lorsque l'on diminue à divers degrés la concentration en sels d'une solution de nucléoprotéine/NaCl 1.0 *M*, et différentes formes de la molécule de protéine.

Nous avons suggéré l'idée que les divers maxima des diagrammes électrophorétiques pourraient être dus à des effets de concentration de sels.

ZUSAMMENFASSUNG

Wir haben gezeigt, dass die Sedimentation von nach MIRSKY UND POLLISTER hergestelltem Thymo-Nukleoprotein stark variiert, wenn sich die Salzkonzentration des Lösungsmittels ändert.

Die einzelnen diskontinuierlichen Stücke der Präzipitationskurve, welche man erhält, wenn man die Salzkonzentration einer ursprünglich 1.0 *M* Nukleoprotein/NaCl-Lösung in verschiedenem Masse herabsetzt, wurde mit verschiedenen Formen der Protein-Molekel in Zusammenhang gebracht.

Es wurde auch darauf hingewiesen, dass die Vielfältigkeit der Maxima, welche in den Elektrophorese-Diagrammen beobachtet worden war, auf Einflüsse der Salzkonzentration zurückzuführen sein könnte.

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