EFFECT OF LYOPHILIZATION ON THE DEGREE OF POLYMERIZATION AND THE IMMUNOLOGICAL PROPERTIES OF DEOXYRIBONUCLEOPROTEINS

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Considerable interest is being taken in the problems of the antigenic and serological properties of deoxyribonucleoproteins (DNP) and deoxyribonucleic acids (DNA). The effects on malignant tumors of antisera prepared from the DNP of human tumor tissues have been described in a number of papers, both for the whole organism [1, 14], and for tissue cultures [11]. It has been shown that antibodies to DNP were formed only when animals were immunized with freshly excised tissues. Formation of antibodies to DNP did not take place when autolyzed material taken at autopsy was used [2]. It was shown that high-polymeric DNA preparations possessed antigenic and serological activities, but that these were lost following depolymerization [7, 9, 15]. J. Colter [11] obtained cytotoxic antisera by immunizing animals with lyophilized DNP preparations. V. Hasekova et al. [13] were, however, unable to detect any antigenic or serological activity of DNP or DNA; this finding could, in our opinion, be ascribed to their use of lyophilized material for their immunization experiments. We could find no references in the literature to the results of comparative studies of the serology of DNP and DNA preparations before and after their lyophilization.

Since the immunological properties of DNP and DNA preparations depend on their physicochemical state, the present research was devoted to the study of the effect of lyophilization on the degree of polymerization and the immunological properties of DNP.

EXPERIMENTAL METHODS AND RESULTS

DNP and DNA were prepared by a slight modification of Mirsky and Pollister's method [3] from freshly frozen calf thymus. The DNA preparations were reprecipitated 5-6 times in 6 volumes of distilled water, with subsequent dissolution and filtration through 3-4 layers of muslin. Nucleoproteins of low protein content were prepared by deproteinizing the initial DNP according to Sevag [16], involving treatment of the DNP solutions with 3 : 1 chloroform–n-butanol mixture, and separating the resulting emulsion on the centrifuge. The deproteinized products were then dialyzed against 1 M NaCl solution for 5-6 days.

All the operations were conducted at 2-4°C. Portions of the resulting preparations were stored in salt solution at low temperature, while the remainder was lyophilized in the Dry Preparations Department (Head: E. K. Dolinov) of the N. F. Gamaleya Institute of Epidemiology and Microbiology, AMN SSSR, under the following conditions: freezing at −40°, drying at room temperature, at pressures of 0.5-0.2 mm, for 42 hr; final water content 0.3-0.6%. Some preparations were also lyophilized in the Institute of Biological and Medical Chemistry, AMN SSSR, under the following conditions: freezing at −73°, drying at room temperature for 4-5 hr.

The lyophilized preparations were taken for experiment after reconstitution to their original volume, by adding distilled water. The DNP and DNA preparations were analyzed for their nitrogen content (Conway's modification of the Kjeldahl method) and their phosphorus content (Spirin's spectrophotometric method [4]). The molecular weight of the DNP and DNA was determined by Spitkovskii's viscosimetric method [6]. The characteristic viscosity of DNP was measured in 1 M NaCl solution, and of DNA in 0.2 M NaCl solution, using an Ostwald viscosimeter (length of the capillary tube 10 cm, diameter 0.6 mm) at 25° ± 0.01°. The calculation of the molecular weights was based on the assumption that the fall in characteristic viscosity of the lyophilized preparations was due basically to depolymerization, and only to a much smaller degree to changes in molecular configuration.

TABLE 1. Data for the Molecular Weight of DNP and DNA Preparations

Serial No.	Calf thymus preparation	N/p	Native			Lyophilized			Percentage of lowering of characteristic viscosity
			characteristic viscosity	molecular weight (million)		characteristic viscosity	molecular weight (million)		
				DNP	DNA of DNP		DNP	DNA of DNP	
1	DNP	3.50	26.0	12.7	5.8	22.0	10.8	4.9	15.4
2	DNP	3.12	27.2	11.9	6.0	18.0	7.8	4.0	33.8
3	DNP, initial$_1$	3.20	26.0	11.6	5.8	15.5	6.9	3.4	40.4
4	DNP-8 (DNA)	1.87	38.5	–	4.3	20.0	–	2.2	48.1
5	DNP, initial$_2$	3.16	25.5	11.3	5.65	17.8	7.7	3.9	30.2
6	DNP-5 (DNA)	2.12	29.0	8.7	6.4	11.6	3.4	2.6	60.0
7	DNP-8 (DNA)	1.73	42.0	–	4.7	15.4	–	1.7	63.4
8	DNP-10 (DNA)	1.63	44.0	–	4.9	12.7	–	1.4	71.2

Note: The figures shown for preparations Nos. 4, 6, 7, and 8 refer to the number of deproteinizations performed

The data of Table 1 illustrate the effect of lyophilization on the molecular weight of DNP and DNA, for which the N/p ratio varied from 3.5 to 1.63. It is evident from these data that the molecular weights of the initial DNP and DNA preparations were fairly high. The molecular weights found after lyophilization of both DNP and DNA were appreciably lower.

The fall in characteristic viscosity of DNP varied (with the exception of preparation No. 1) by from 30.2 to 40.4%, and of DNA from 48.1 to 71.2%, depending on the individual properties of the preparations, of which the most important was the extent to which they had been freed of protein. The greatest fall in characteristic viscosity was found for preparations of DNA Nos. 5-8, the degree of polymerization of the molecule falling with the number of deproteinization operations (see Table 1).

It may thus be concluded that the molecular weights of DNP and DNA fall appreciably as a result of lyophilization.

The redissolution of some DNP preparations took place with difficulty, sometimes requiring a few days for completion. There was, however, little change in the solubility of DNA preparations following lyophilization. The changes in the molecular weight and solubility of DNP and DNA preparations lyophilized under the above-specified conditions were of about the same magnitude, notwithstanding differences in the drying procedure.

Having ascertained the effect of lyophilization of DNP on its molecular weight, we proceeded to examine the antigenic and serological properties of the preparations. We were not concerned with establishing the presence or absence of immunological activity of DNA preparations having a N/p of about 2 or less. For the examination of the serological activity of DNP before and after lyophilization we used a quantitative complement fixation reaction (CFR) to a 50% titer with DNP bound on adsorbents.

The antisera were prepared in the following way. Rabbits were given four intramuscular or subcutaneous injections, at daily intervals, of 2-2.5 ml of DNP solution. Blood was taken from an ear vein on the 8th day after the last immunizing injection. One group of rabbits was given native DNP preparations Nos. 1 and 3, while a second group received the same preparations after they had been lyophilized (see Table 1). The antisera were heated at 56° for 30 min, and were then stored at 2-4°.

For the preparation of test antigens we immersed weighed 12 mg portions of Whatman No. 1 filter paper for 20 min in solutions of native and lyophilized DNP. The papers, with adsorbed DNP, were then washed for 25 min in four changes of 0.14 M NaCl solution. We used DNP preparations Nos. 1, 2, 3, and 5 (Table 1) for the preparation of the test antigens.

TABLE 2. Results of Serological Examination of DNP Preparations

Antigen (DNP)	Normal rabbit serum		Native DNP antiserum No. 51		Lyophilized DNP antiserum No. 103		Control antigen	
	complement units							
	free	bound	free	bound	free	bound	free	bound
Native	13.3	0.5	10.3	2.3	11.5	2.3	13.8	–
Lyophilized	12.9	0.9	10.7	1.9	11.6	2.2	13.8	–
Control of serum	13.9	–	12.6	–	13.8	–		
Control of complement	13.8	–	13.8	–	13.8	–		

Note: Control tests of the sera and of the complement were performed in the presence of a weighed portion of filter paper, since it has been shown that a certain amount of complement may undergo fixation on the paper.

In all, we performed 16 quantitative CFR experiments, the reactions between the various antigens and sera being always conducted at the same dosage levels. The sera were taken at identical dilution. The reactions were conducted in veronal-medinal buffer of pH 7.4-7.7, and Ca^{++} and Mg^{++} ions were added [12].

The data of Table 2 illustrate a typical experiment, comparing the serological properties of a DNP preparation before and after lyophilization.

The results show that both native and lyophilized calf thymus DNP preparations possess serological activity, giving equal fixation of complement with both native and lyophilized DNP antisera. Native DNP reacts in cross-reactions with antisera to both native and lyophilized DNP, binding about the same number of complement units in each case. Similarly, lyophilized DNP reacts in the same way with antisera to both native and lyophilized DNP.

It follows that the antigenic and serological properties of DNP were unaffected by lyophilization. The reason for this finding may be that the initial DNP preparations were of high molecular weight, and that it was still high enough after lyophilization for immunological activity to be retained.

The conclusion may hence be drawn that preparations of DNP intended for immunological studies may be lyophilized only on condition that the molecular weight is carefully checked. Especial care has to be taken in lyophilizing DNP preparations, in view of their extreme sensitivity to this procedure. It is conceivable that the negative findings reported by V. Hasekova et al. [13] were due to the loss of antigenic and serological properties during the lyophilization process.

In conclusion we thank D. M. Spitkovskii, of the Institute of Experimental Biology, AMN SSSR, for valuable consultations regarding the physicochemical operations described in this paper.

SUMMARY

A method of viscosimetry was used to compare the molecular weight of the crude and lyophilized desoxynucleoproteids (DNP) and DNA preparations at various stages of their purification from protein. As demonstrated, lyophilization leads to a marked reduction of polymerity of the preparations, especially in the DNA lyophilized preparations. However, the immunological activity of the DNP preparations remained unchanged after lyophilization. The study of the DNP by means of the quantitative complement fixation reaction has shown that crude and lyophilized DNP preparations had an equal initial antigenic and serological activity which could be explained by their sufficiently high polymerity.

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