FRACTIONATION OF DESOXYRIBONUCLEOPROTEINS ISOLATED FROM THE LIVER

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Investigations during recent years have shown that desoxyribonucleic acid (DNA), isolated from a single biological source, consists of a group of closely connected polymers, differing essentially in their structure. Several authors [2, 4, 5, 6] have succeeded in separating DNA into several fractions with different proportions of nitrogenous bases. The methods of fractionation which have so far been used may be subdivided into the following groups:

1) extraction of denatured desoxyribonucleoprotein (DNP) with sodium chloride solution and paying attention to the concentration gradient [4, 6] or the time gradient [9];

2) fractionation of the isolated DNA by treatment with ion-exchange reagents [2], with polypeptides [12] or with proteins [3];

3) fractionation of denatured DNP by replacement of the protein by powerful anionic polymers – heparin and dextran sulfate [7];

4) fractionation by means of paper electrophoresis [11].

It is mainly the DNP from microorganisms that has been subjected to fractionation by the above methods. Less work has been done on the fractionation of DNP isolated from animal tissues; fractionation here of the DNP obtained from the liver by extraction with saline has not been undertaken at all.

The aim of the present work was to carry out fractionation of DNP obtained from the liver. For our attempt we utilized the simplest methods of saline fractionation with NaCl solution of constant or varying concentration, in the cold and at different temperatures. We suggested this last modification of saline fractionation, using different temperatures, and worked out the details in this particular investigation in view of the idea that the stability of the bond between protein and nucleic acid varies with the temperature.

EXPERIMENTAL METHOD

We obtained the DNP from the ground-up liver of cattle and rabbits by the method of Mirsky and Pollister [10]. The liver was taken without delay after the animals had been slaughtered and was kept frozen at −20°. The product was precipitated 3 times from 6 volumes of distilled water until the N/P ratio was 3.6-3.7. The DNA content of the preparations was estimated as phosphorus (by the method of Fiske and Subbarow) and as nitrogen (by Conway's method).

The solution of DNP in 1 M NaCl was precipitated before fractionation in 6 volumes of distilled water. To the gel was added an equal volume of NaCl solution of concentration required by the conditions of the experiment,

and the preparation was kept in the refrigerator for 30 minutes, being stirred slowly all the time. After 30 minutes a mixture of chloroform and butyl alcohol (3:1) was added, in a volume equal to one half that of the gel solution, and the whole was mixed for 2 minutes in the cold by means of a high-speed mixer. The emulsion thus obtained was centrifuged in the cold at 3000 rpm for 10 minutes.

TABLE 1

Fractionation of DNP from Ox Liver by NaCl Solutions of Different Concentration at 5°

Material examined	Concentration of NaCl	Content of		N/P	a/t	g/c	$\frac{a+g}{t+c}$	$\frac{a+t}{g+c}$
		P per ml (in γ)	N per ml (in γ)					
Whole nucleoprotein	—	132	488.4	3.7	0.95	1.11	1.02	1.28
	1. Group of experiments							
1st fraction	0.5 M	54.6	220.8	4.04	0.98	1.11	1.03	1.31
2nd fraction	0.9 M	60.2	125.3	2.08	0.94	1.13	1.02	1.33
3rd fraction	1.3 M	13.0	47.7	3.66	1.03	1.07	1.05	1.24
	2. Group of experiments							
1st fraction	0.35 M	10.3	35.8	3.45	0.87	1.17	0.99	1.34
2nd fraction	0.5 M	138.0	256.0	1.85	0.96	1.15	1.05	1.05
3rd fraction	0.7 M	44.5	161.0	3.61	1.06	1.06	1.08	1.15
4th fraction	0.9 M	10.7	35.7	3.33	1.99	1.07	1.06	0.82

Symbols used in this and later tables: a — adenine, t — thymine, g — guanine, c — cytosine.

The supernatant fluid was the first fraction. The protein gel formed with part of the unfractionated nucleoprotein was treated by the same method, but when different temperatures were used the last 30-minute extraction took place at 20° (for the second fraction) and at 37° (for the third fraction). During fractionation with NaCl solution of constant concentration, the same 0.5 M solution was used for each successive fractionation; during fractionation with NaCl solutions of different concentration, this was successively increased. The concentration of DNA, protein, purines and pyrimidines in the fractions thus obtained was estimated by paper chromatography, using A. S. Spirin's [1] modification of Wyatt's method [13].

Each fractionation was usually repeated twice. In the chromatographic analysis, 2 parallel samples of hydrolyzate were taken from each fraction, and 2 chromatograms were taken of each sample (the mean values are shown in the tables).

EXPERIMENTAL RESULTS

In the course of our research we at first believed that fractionation would be most successful when using NaCl solutions of different concentration. In the first group of experiments, fractionation was therefore carried out by the use of NaCl solutions in concentrations of 0.5, 0.7 and 1.3 M, and in the second group — of 0.35, 0.7 and 0.9 M at 5°. The results of these experiments are shown in Table 1.

As may be seen from Table 1, in the first group of experiments 3 DNA fractions were obtained with an insignificant difference in the composition of their nitrogenous bases, and in the second group 4 fractions were obtained, of which the second and third differed only slightly.

No form of sequence was observed in the changes in the specificity factor of the nitrogenous bases ($\frac{a+t}{g+c}$).

Thus, from our point of view, this fractionation did not give satisfactory results. We therefore carried out experiments using solutions of constant concentration for the fractionation. Under these circumstances we obtained (see Table 2, 1st method) 3 fractions with an insignificant difference between the second and third fractions,

i. e. this method also, according to our view, fails to give sufficiently clear fractionation of the DNA. We thereupon carried out experiments in which fractionation of DNP from ox liver was done at different temperatures and with a constant concentration of NaCl. In this case clear differences were found between all three fractions, with a successive fall in the specificity factor from the first to the third (see Table 2, 2nd method).

TABLE 2

Fractionation of DNP from Ox Liver with 0.5 M NaCl Solution at Constant and Differing Temperatures

Material examined	Temperature	Content of P per ml (in γ)	Content of N per ml (in γ)	N/P	a/t	g/c	$\frac{a+g}{t+c}$	$\frac{a+t}{g+c}$
Whole nucleoprotein	—	168.0	605.0	3.6	0.97	1.08	1.02	1.25
		1st method						
1st fraction	5°	23.6	98.9	4.20	1.07	0.94	1.02	1.32
2nd fraction	5°	37.1	98.9	2.66	1.02	1.09	1.05	0.99
3rd fraction	5°	12.0	59.6	4.96	1.04	1.08	1.06	1.06
		2nd method						
1st fraction	5°	23.5	86.1	3.66	0.95	1.05	1.00	1.35
2nd fraction	20°	29.2	92.7	3.17	0.93	1.10	1.00	1.25
3rd fraction	37°	12.0	66.2	5.52	0.95	1.06	1.03	0.74

TABLE 3

Fractionation of DNP from Rabbit Liver with 0.5 M NaCl Solution at Different Temperatures

Material examined	Content of P per ml (in γ)	Content of N per ml (in γ)	N/P	a/t	g/c	$\frac{a+g}{t+c}$	$\frac{a+t}{g+c}$	Temperature
Whole nucleoprotein	86.0	320.0	3.72	0.98	1.08	1.02	1.19	—
1st fraction	41.5	140.0	3.37	0.96	1.03	0.99	1.20	5°
2nd fraction	13.3	35.0	2.63	1.04	1.05	1.05	0.99	20°
3rd fraction	20.8	70.0	3.36	1.10	1.08	1.09	0.85	37°

In order to test the reproducibility of this method for the liver DNP of another species of animal, we carried out fractionation experiments on DNP isolated from the liver of the rabbit. Table 3 shows that in this case too, the 3 fractions obtained had a different specificity factor, its value falling progressively from the first to the third fraction.

From a comparison of the methods of fractionation with 0.5 M NaCl at constant and different temperatures (see Table 2), it can be seen that the first fraction from the first method and the first fraction from the second method, obtained under practically the same conditions and at the same temperature, resembled each other very closely in their specificity factor, as was to be expected. Subsequent changes in the temperature, however, led to the production of fractions quite different in their composition from those obtained by the first method. This suggested that the method of fractionation which we were using was effective.

A control determination of the viscosity showed the high degree of polymerization of the test preparations and their fractions. The ratio of purines to pyrimidines in all the fractions was close to unity, i. e. was in agreement with Chargaff's rule with all the methods of fractionation.

When the results were compared with those reported in the literature, some lack of correspondence was observed with the results of saline fractionation described by a number of workers [4-6], for in their research an increase was observed in the coefficient of specificity from the first fractions to the last. Our method of fractiona-

tion differed, however, from the methods used by the authors cited, chiefly by the nature of the initial preparation. We thought that for fractionation it was necessary to select nucleoprotein preparations of sufficient stability, for only these would have the characteristic composition of the particular tissue. Accordingly, by triple precipitation we freed the initial nucleoprotein from weakly combined and possibly contaminating fractions, and obtained in every case preparations which were soluble in 1 M NaCl and with N/P = 3.6-3.7, i. e. in the proportion characteristic of purified nucleoprotein. According to the literature the initial nucleoproteins usually selected for fractionation were water-soluble preparations with a higher N/P ratio.

It must also be pointed out that the preliminary dissolving of the initial preparation in 1 M NaCl in our own investigation could possibly increase the lability of the bonds between the protein and nucleic acid with an increased content of adenine and thymine. It is interesting to point out that, according to Kent [7], during the fractionation of nucleoprotein dissolved in 1 M NaCl with heparin and dextran sulfate, the same sequence was observed in the changes in the specificity factor through the fractions as in the present work.

We therefore have demonstrated the possibility of saline fractionation of the DNP obtained from the liver of cattle and rabbits; we have suggested a new method of fractionation of DNP at different temperatures; and we have obtained fractions of nucleic acid which differed clearly from each other in the factor of specificity of composition of nitrogenous bases, which decreased from the first fraction to the last.

SUMMARY

The possibility of the salt fractionation of desoxyribonucleoproteins (DNP) obtained from the liver of cattle and rabbits was demonstrated. A new method of fractionation of DNP at various temperatures was suggested. The authors obtained three fractions of nucleic acid which have a pronounced difference from each other by the specific content of the nitrous bases which decrease from the first fraction to the next.

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