Nucleic Acids

I. Nucleic Acids of Hog Spleen

First Paper

By

Kiyosi Ogawa

(小川 清)

(From the Medico-chemical Institute, Tohoku University,
Sendai; Director: Prof. H. Masamune)

(Received for publication, December 22, 1951)

It is generally not easy to prepare PNA and DNA from an organ without any contamination of each other. And even of recent investigators of spleen DNA, Petermann & Lamb¹⁾ and Chargaff *et al.*,²⁾ the former group could not separate it entirely pure in this respect although a fine-devised method was applied. As to the spleen PNA none appears to have isolated it.

In the present work, isolation of DNA in the highly associated state was not intended so that slight alkalization or acidification of its solutions was dared to simplify the procedure. Hence only low-viscous fractions of DNA were yielded. All PNA was carried down at pH 9.4 together with a part of DNA by baryta and from the deposit, dried for several days, could the former be separated from the latter by repeated extractions with 10% NaCl. The preparations purified were either DNA or PNA and not mixtures of those two kinds of nucleic acid, but one of the fractions of DNA, whose amount allowed electrophoretic examination, proved inhomogeneous. The crop of PNA was less than 10% of that of DNA in any run.

To particularly mention, this account introduces a new expedient method of preparing pure pentose nucleic acid. Despite of their admirable means Kerr and Seraidarian³⁾ was not successful in obtaining pancreas-PNA free from DNA. Besides it is worthy to note that neither glucidmin nor carbohydrate had come into saline (10%) extracts of the tissue.

EXPERIMENTAL

Preparation Procedure

A typical example will be given.

The preparation was carried out during winter in a cold room ($<5^{\circ}$

C). Hog spleens quickly brought from a slaughter house were freed at first from adipose tissue and then from blood by perfusion of physiological saline through a. lienalis. 14 fresh spleens thus treated and weighing 1,450 g. were masticated and shaken with three volumes of 10% NaCl for two days. Percolated and filtered. The filtrate—**Fluid I**—was an extremely viscid, red-tinted and transparent fluid of pH 6.8. The tissue residue was further subjected twice to a similar extraction. The final residue (87 g.) contained a trace of phosphorus but no purine ($\text{AgNO}_3\text{-NH}_3$ test).

The successive extracts were acidified each, when filtered, by addition of 10% HCl carefully to a pH of 1.4, whereby maximum precipitation occurred. The following description concerns the first extract. The precipitate (acid-insoluble part) was separated from the mother fluid—**Fluid II**—by quick centrifugation and shaken with an equal volume of 10% NaCl, adjusting the pH of the mixture to 7.0 with 0.1 *N* NaOH, for 6 hours. The centrifugate was manipulated twice more in a like manner, extending over 12 and 24 hours respectively, when no more purine remained in the residue. The solutions were dialyzed together against running water for two days and distilled at low temperature to about 20 cc. and the saturated Ba(OH)_2 was added in drops to pH 9.4. The abundant deposit centrifuged (The mother fluid will be referred to as **Fluid III**) was removed from the excess baryta by stirring up with portions of a dilute HCl of pH 1.8, until a nearly neutralized washing was given, further washed with alcohol and ether and dried in a vacuum-desiccator over CaCl_2 for 5 days. 1,430 mg. of a white powder were given. The corresponding products from the second and third extracts amounted only to 24 and 5 mg. respectively. All of the products at this stage were biuret-negative, but Dische (for DNA)-, Bial (for PNA)- and Molisch-positive.

They were combined and kneaded with 10% NaCl (0.5 cc. per 100 mg.), adding 0.1 *N* HCl to maintain the pH at 7.0, and centrifuged. On the undissolved was repeated the extraction again and again as long as the residue showed the positive Molisch reaction (The supernatant combined—**Fluid IV**—were set aside for obtaining PNA. See below). Next, the operation was resumed, replacing 10% NaCl with 3 *M* NaCl. Almost 20 repetitions were here requisite to reduce purine to practically nil. The second solutions (in 3 *M* NaCl) were dialyzed for three days against running water and condensed (by distillation in vacuo to about 15 cc.) and 3 volumes of alcohol added. And the precipitate was reprecipitated from water with alcohol twice. To change the Ba,Na-salt into free acid, it was washed with a mixture of 99 volumes of methanol and 1 volume of 16% HCl three times. It was further washed with alcohol and ether

and after drying, the white powder obtained was taken up in a smallest possible quantity of water by the aid of NaOH (The pH was not raised above 8.0), followed by precipitation with two volumes of alcohol and thorough washing with alcohol. Finally a trace of the insoluble part was eliminated by redissolution of the substance in water (5 cc.), centrifugalization and precipitation of the supernatant with 1.5 volumes of alcohol⁴¹. The deposit was washed with methanol and ether. The Na-salt given—**DNA I** (a white powder)—amounted to 225 mg. It formed a low-viscous neutral solution in water and was also soluble in 97% glacial acetic acid. Molisch negative, Bial faintly positive, Dische distinctly positive. Purine and pyrimidine were detected (Ag-test).

Fluid IV that contained a minute quantity of DNA besides PNA was dialyzed for two days, distilled in vacuo to about 10 cc. and precipitated with 3 volumes of alcohol. The precipitate (crude Ba₂Na-salt of PNA) was treated, with the aim of obtaining free acid, like as the corresponding salt of DNA above, then reprecipitated twice from a little water (A very small insoluble part that consisted of DNA was discarded) with methanol and washed with methanol and ether. 18 mg. of a white powder were yielded—**PNA**. It gave a slightly acid and slightly viscous solution in water, but did not dissolve in 97% glacial acetic acid. In 1 *N* HCl it dissolved into a milky solution (Centrifugation separated no sediment). Bial and Molisch distinctly positive but Dische negative. Presence of purine and pyrimidine was demonstrated.

On acidification of Fluid III (the supernatant fluid of the baryta-precipitate of DNA and PNA when the first tissue extract was fractionated) with HCl, there happened a voluminous precipitate at pH 1.4, which was centrifuged, washed with alcohol and ether. 865 mg. was the product. Those from the second and third extracts weighed 66 and 20 mg. They were strongly biuret-positive and faintly Molisch-positive. A trace of phosphorus was detected but no purine. Osaki-Turumi tests (the direct as well as the indirect) were negative and an acid hydrolysate scarcely gave Elson-Morgan reaction. Neither Neuberg-Saneyoshi nor Goldschmiedt was shown. All of the three acid mother fluids were dialyzed, evaporated and precipitated with alcohol. The deposits were washed and dried as above. Yields, 465, 45 and 36 mg. respectively. These were entirely devoid not only of purine but also of phosphorus. Biuret remarkably positive but Molisch negative.

Fluid II (the mother fluid of the acid precipitate from the first tissue extract) was evaporated at low temperature to about 15 cc. and as much 20% CuCl₂ was added as to produce maximum precipitation (The pH was lowered to 4.2). The centrifugate (The supernatant will be referred to as **Fluid V**) was washed with alcohol and ether. 390 mg. was the substance given (Cu-salts), which was biuret-positive and faintly Molisch-positive. From the mother fluids after acidification of the second and third tissue extracts were given 45 and 9 mg. respectively.

The products were united, well washed with a dilute HCl of pH

1.4 to perfectly liberate from Cu (The precipitate from the washings by alcohol was biuret-positive and Molisch-negative but contained no purine) and then agitated with portions of 3 *M* NaCl. The extracts here were dialyzed together, condensed and after precipitation at pH 9.4 with baryta as above, quickly centrifuged. The centrifugate that was biuret-negative was exhausted, employing 2~3 cc. portions of 3 *M* NaCl and when no more purine was detected in the residual, the solutions were combined and worked as when the sodium salt of DNA above (DNA I) was prepared from the corresponding solutions. The yield of the product—**DNA II**—was 13 mg. This showed resembling solubilities and chemical qualitative properties as DNA I. The centrifuged supernatant fluid of the baryta-precipitate was dialyzed and 5 volumes of alcohol were added. Some precipitate occurring showed the positive biuret reaction but negative Molisch and $\text{AgNO}_3\text{-NH}_3$ (for purine) test.

Fluid V (the mother fluid of the CuCl_2 -precipitate in the run started with the first tissue extract) was dialyzed and after distilling in vacuo to about 10 cc., precipitated with 3 volumes of alcohol, and freed from Cu with changes of alcohol containing a small quantity of 10% HCl. The undissolved was washed with alcohol and ether and dried. 34 mg. was the substance obtained. In the second and third runs 19 and 8 mg. were the crops. Purine and phosphorus absent; Molisch faintly positive; biuret distinctly positive. Osaki-Turumi reactions: the direct negative, the indirect faintly positive. Neuberg-Saneyoshi and Goldschmiedt, negative. An acid hydrolysate gave only faint Elson-Morgan reaction.

TABLE I
Composition of Nucleic Acids Separated from Hog Spleen

	DNA I* (sodium salt)	DNA II* (sodium salt)	PNA
N† (%)	10.3	10.4	11.0
P‡ (%)	5.9	5.8	6.4
N/P	1.7	1.8	1.7
Ash (§)	21.7§	20.0§	4.2//

* Ba was not detected. † Micro Kjeldahl. ‡ A modification of Plimmer method.⁵⁾

§ Pregl's micro method. // Pregl's micro method without the use of H_2SO_4 .

Fig. 1. An electrophoretic pattern of a 1% solution of DNA I (See the text) in phosphate buffer, pH 7.8, $I=0.29$; 20°C ; mobility of the faster moving boundary $17.0\text{ cm}^2\text{-sec}^{-1}\text{-volt}^{-1}\cdot 10^5$. Descending limb to the right.

Composition and Homogeneity of the Nucleic Acids

Composition. See Table I.

Electrophoresis of a solution at pH 7.8 of DNA I demonstrated two components (Fig. 1).

Through the Grant Committee of the Science Council was given a grant from the Education Department, which enabled us to carry out this work.

H. M.

References and Notes

- 1) Petermann & Lambs, J. Biol. Chem., 1948, **176**, 685.
 - 2) Chargaff, Vischer, Doniger, Green and Misani, *ibid.*, 1949, **177**, 405.
 - 3) Kerr and Seraidarian, *ibid.*, 1949, **180**, 1203.
 - 4) It was often necessary to add NaCl for perfect precipitation.
 - 5) Haneda, Sinokawa and Yasuoka, Tohoku J. Exp. Med., 1944, **46**, 263.
-