Nucleic Acids

X. Chemical Comparison between DNAs of Human Placenta and of Human Spleen

Third Report: Schiff Reaction of the DNAs during the Course of Acid Hydrolysis

By

Mutuo Sakamoto

(阪本 睦男)

From the Medico-chemical Institute, Tohoku University, Sendai;

Director: Prof. H. Masamune

(Received for publication, July 10, 1956)

In the Second Report,¹⁾ it was suggested that pyrimidinenucleates of the DNAs from the two sources are of different structures despite of similar compositions. If then, the purines of the placental DNA might be cleft off by acids more rapidly or more slowly than those of the splenic DNA, because hydrolysis constant of glycosides depends upon the structure of aglycon as well as upon that of the sugar moiety even among α - or β -glycosides. In the present case, pyrimidinenucleate represents the sugar moiety. To follow up the course of the hydrolysis, the writer adapted Schiff reaction as a measure of free aldehyde group corresponding to deoxyribose whose glycosidic link is cleft.²⁾

Solutions of DNAs in water or in 10% NaCl become Schiff-positive by mere dialysis, and Stacey³⁾⁻⁵⁾ ascribed it to fission of labile glycosidic phosphate links which partake in polymerization of DNA, being based on the fact that inorganic phosphorus appears in the dialysate simultaneously. However, the writer found with a quantitative-analytical method which will be described below that the amount of deoxyribose residues reacting with Schiff reagent in a dialyzed DNA (Experimented on Prep. 4 of the splenic DNA in the First Report) only reaches 3-4% of the maximum value when the DNA is hydrolyzed with a mild acid to set free all the purines. Judging from the very weak union between purines and the remaining part of DNAs as has been revealed in preparation of a pyrimidinenucleate (See the Second Report¹⁾), it seems more plausible to suppose that DNAs turn Schiff-positive in dialysis owing to detachment of a tiny part of purines by the faint acidity of water. Although purines are not detected in the outer fluid, such traces of purines as are liberated in that way may be retained in the inner fluid through interaction between

them and the negatively charged residual part of the DNA, and the phosphorus coming out into the dialysate is possibly a contamination of the DNA preparation. Apart from this, even if the opinion of Stacey hold true, deoxyribose glycosidically bound with phosphoric acid can occupy only a small percentage of the total deoxyribose and is neglected in the argument below.

EXPERIMENTS AND COMMENTS

Quantitative Schiff Reaction

In 1928, Widström⁶⁾ scrutinized the conditions under which Schiff reaction can be used for estimation of DNAs. Recently, however, Overend⁷⁾ found that the extinction of the color developed does not parallel the amount of deoxysugars, and so the reaction remains unutilized as a quantitative method. Fortunately, the writer proved that the extinctions follow Lambert-Beer law, if reaction mixtures are stood in an ice-chest until they show constant extinctions. The devised method which is a modification of Widström's is as follows:—

Schiff reagent. A 0.4% fuchsine (Fuchsin kleine Kristalle E. Merck) solution is bubbled with SO₂ gas until it turns straw-yellow, and then aerated to remove the irritative smell. It is preserved in a stoppered brown bottle at below 5°C. This reagent should show a pH of 4 or more when diluted twenty times with water.

The technique. An accurate quantity from 0.8 to 8 mg. of a partial degradation product of a DNA is dissolved in 100 cc. of water, and 4 cc. of the solution is pipetted into a stoppered (ground joint) test tube, added to with 0.2 cc. of the Schiff reagent and stood at 26°C for 30 minutes at first and afterwards at 8–10°C for 80 minutes. Thereupon the extinction at the wave-length of 550 mμ is measured. The control must be always carried out in parallel employing a definite degradation product of a DNA, f. i., a sodium pyrimidinenucleate specimen, because the extinction varies with the SO₂-content of the reagent.

Fig. 1 shows an example on a sodium pyrimidinenucleate revealing that the extinction is a straight line function of the amount of the material analyzed.

Schiff Reaction of the DNAs after Various Durations of Acid Treatment

1) 40 mg. each of the DNAs in the First Report⁸⁾ (Preps. 4 (spleen DNA) and 4' (placenta DNA)) were dissolved in 50 cc. of distilled water, made up to 100 cc. with 0.02 N HCl (pH of the solutions ca. 2.1) and stood in an incubator at 37°C. 2 cc. portions were pipetted out at in-

Fig. 1. Showing the correlation of extinction (Cell 1 cm. thick) in Schiff reaction with amount of substance on Prep. 6 from the splenic DNA in the Second Report.

TABLE I

Relation of Incubation Time with Schiff Reaction,
when 40 mg% Solutions of the Splenic and Placental
DNAs in 0.01 N HCl Are Stood at 37°C

Extinctions were measured in a 1 cm. thick cell, after neutralizing and diluting the solutions to 4 mg%.

Incubating time (hrs.)	Human spleen DNA	Human placenta DNA
	Extinction at λ of 550 $m\mu$	
1	0.094	0.135
2	0.264	0.396
4	0.439	0.530
6	0.550	0.561
8	0.545	0.558
10	0.552	0.560
12	0.558	0.552
14	0.550	0.560
16	0.554	0.560
24	0.530	0.550
28	0.530	0.545
36	0.516	0.548
48	0.514	0.539

Fig. 2. Showing the increase of Schiff-positive residue with time when 40 mg% solutions in 0.01 *N* HCl of the splenic (●) and placental (○) DNAs were incubated at 37°C (Curves plotted from the figures in Table I).

tervals from the solutions, neutralized and after addition of water to 20 cc., examined as for the Schiff reaction. The solutions gave the maximum reaction after 6 hours from the commencement of incubation, and thereafter the reaction showed no noticeable alteration of strength within 16 hours. The maximum reactions of the solutions equaled each other. But, as shown in Table I and Fig. 2, the placental DNA gave rise to much stronger reaction than the splenic DNA in the period before the sixth hour, that is, purines were split off with a larger hydrolysis constant from the placental DNA than from the splenic. At the 24th hour of hydrolysis, the diphenylamine reaction of the solutions diluted to 40 mg% of the placental and splenic DNAs gave extinctions of 0.791 and 0.806 (at λ of 595 $m\mu$) respectively, deoxyribose reactive to this reagent of the two solutions amounting nearly equal.

2) Two preparations each of the DNAs (Preps. 4, 5, 3' and 5' in the First Report) were taken, and 20 mg. portions of them were separately dissolved in 40 cc. of water. The solutions were adjusted with 0.1 *N* HCl to pH 4.0 a weaker acidity than in 1) and placed in a thermostat at 25°C for 20 hours. Then, they were neutralized with 0.1 *N* NaOH and made up to 50 cc. with water and their Schiff reaction was examined. The hydrolysates of the placental-DNA preparations gave larger $E_{\max}$ values than the splenic-DNA preparations, notwithstanding that the diphenylamine reactions of them were rather of strengths in reversed

TABLE II

Schiff and Diphenylamine Reactions of the DNAs Stood
as 50 mg% Solutions of pH 4.0 for 20 Hours at 25°C

Extinctions in Schiff and diphenylamine reactions were measured in a 1 cm. thick cell after dilution of the neutralized solutions to 4 and 40 mg% respectively. The figures in parentheses are extinctions for the unhydrolyzed materials.

Source of DNA		Human spleen		Human placenta	
No. of preparation*		4	5	3'	4'
Extinction	Schiff reaction $\lambda_{\max}$ 550 m μ	0.542	0.540	0.625	0.671
	Diphenylamine reaction $\lambda_{\max}$ 595 m μ	0.760 (0.764)	0.758 (0.765)	0.700 (0.715)	0.769 (0.766)

* Preparations in the First Report.⁸⁾

relation (Table II).

SUMMARY

1. Schiff reaction has been made up into a quantitative-analytical method for determination of deoxyribose with free carbonyl in degraded DNAs.

2. Occurrence of the Schiff-positive residue was followed up during mild acid hydrolyses of DNAs from human placenta and spleen.

3. The Schiff-positive residue was produced more quickly from the placental DNA than from the splenic DNA, in other words, purines in the placental DNA are less stably linked than those in the splenic DNA. After reaching the maximum, the intensities of the reaction of the DNAs were nearly the same within 16 hours and then decreased gradually. The maximum values equaled each other.

The expenses of this work were defrayed by a grant from the Ministry of Health and Welfare, which is gratefully acknowledged. H. Masamune.

References

- 1) Sakamoto, Tohoku J. Exp. Med., this volume, p. 281.
- 2) Tamm & Chargaff, J. Biol. Chem., 1953, **203**, 689.
- 3) Li, Overend & Stacey, Nature, 1949, **163**, 538.
- 4) Lee & Peacocke, J. Chem. Soc., 1950, 3361.
- 5) Overend, Stacey & Webb, *ibid.*, 1951, 2450.
- 6) Widström, Bioch. Z., 1928, **199**, 298.
- 7) Overend, J. Chem. Soc., 1950, 2769.
- 8) Sakamoto, Tohoku J. Exp. Med., this volume, p. 269.