b-Nucleic Acid, and the Initial Step of Deoxyribonucleic Acid-Degradation by Deoxyribonuclease I

In 1935, FEULGEN observed that the solution of DNA prepared according to his own method was strongly liquefied by the action of pancreatin without the liberation of purines and phosphates, and the reaction product, which was obtained in high yield, yet maintained nearly the same acid-precipitability as that of the parent DNA. He called this substance β-nucleic acid, and the enzyme concerned nucleogelase.

For some time we have also studied the nucleogelase reaction and obtained the following results. To 0.4 ml of 1% solution of Feulgen's DNA in M/10 acetate buffer (pH 6.0) was added 0.1 ml of enzyme solutions of various activities by extracting NBC's pancreatin with water, and the viscosity change of the reaction mixture was observed. The relative viscosity which was at first ca. 4, dropped to the final value of ca. 1.2 after various time passages, according to the concentrations of nucleogelase. An aliquot of the reaction mixture was poured into 30 vol of cold 0.25 N HCl and the resultant precipitate was dissolved in M/15 Na2HPO4; then an aliquot of this solution was subjected to paper electrophoresis (200 V, 4 mA, the current flow lasting 2 h, the electrolyte solution used being M/50 acetate buffer of pH 5.0). After the finish the paper was observed with mineralight Model SL 2537.

The original DNA of FEULGEN did not move from the starting line, while β-acid showed a circumscribed sharp spot of the original DNA detectable by paper electrophoresis diminished rapidly, while that of β-acid increased quickly. When crystalline DNase 1 was used instead of pancreatin without Mg++, the same electrophoretic pattern as above was observed, suggesting that FEULGEN's nucleogelase might be the same enzyme with DNase 1. Therefore, more detailed comparisons of the two enzymes were carried out as follows. A solution of DNase 1 and the nucleogelase solution prepared as described above were fractionated with ammonium sulphate, and every fraction of both enzymes was compared in DNA-liquefying activity. As indicated in Figure 1 (a), the enzyme activity in both cases was found to be concentrated on the fraction between 57 and 71% ammonium sulphate saturation. As the next step, the b-acid-decomposing activity of nucleogelase was compared with that of DNase.

To 1% solution of FEULGEN's DNA dissolved in M/10 acetate buffer of pH 6.0 was added each of the following solutions containing, in liquefying activity order, DNase I (10 μ/ml) > pancreatin extract > DNase I (5 μ/ml) respectively. After incubation, an aliquot was taken from each reaction mixture and poured into 30 vol of 0.25 N HCl and centrifuged. The supernatant solution was investigated for its light absorbency at 260 μm in order to estimate the degradation of b-acid. As indicated in Figure 1 (b), no difference was seen between the b-acid decomposing activity of nucleogelase and that of DNase 1. Furthermore, the pH-activation curve of liquefying activity (production of b-acid) and that of b-acid decomposing were compared between these two enzymes. For this purpose, DNA or β-nucleic acid was dissolved to 1% in phosphate buffer of various pH's as indicated in Figure 1 (c), and to this solution was added nucleogelase solution.

2. Abbreviations: DNA, deoxyribonucleic acid; DNase I, deoxyribonuclease I; NBC, Nutritional Biochemicals Corporation, Cleveland, Ohio; OD, optical density; P, phosphate.
(the fraction precipitated by 57–71% ammonium sulphate saturation) or DNAse 1 solution. As the viscosity-lowering activity varied with the pH changes, it was expressed by the percentage of difference of the relative viscosities between 1% original DNA and 1% purified b-acid solution at various pH's (Figure 1(c-M)).

As indicated in Figure 1(c-M) the pH-optimum of the viscosity-lowering activity was 7 in both cases and that of the b-acid decomposing activity (Figure 1(c-L)) existed at 6.0–6.5 in both cases.

Next, we pursued the process of DNA degradation by DNase 1 by determining the acid-soluble P, the acid-precipitable P, the residual DNA and the produced b-acid. To 1% DNA solution in M/10 acetate buffer (pH 6.0) was added 1/4 vol of DNase 1 solution (final concentration of crystalline DNase was 2 γ/ml). At various intervals of incubation an aliquot was taken, and the acid-soluble and precipitable P were estimated by Allen's method. The acid-precipitable part was subjected to paper electrophoresis as described above, and the resultant spots of DNA and b-acid were eluted by heating with M/15 Na2HPO4 solution and used for measuring their light absorbency at 260 mp. As demonstrated in Figure 2, the original DNA decreased rapidly coincidentally with the

prompt lowering of viscosity and disappeared after 2 h, while b-acid increased rapidly and then decreased very slowly. Concurrently with this phenomenon the acid-soluble P increased very slowly and the acid-precipitable P decreased at the same rate. Anyhow, it is due to the fact that the production of b-acid is rapid, whereas the degradation of b-acid is very slow, that the main product in both cases of pancreatin and DNase 1 is b-acid. When Mg++ was added to the reaction system the pattern of the DNA-degradation changed completely, the accumulation of b-acid being scarcely observed. This fact seemed to indicate that the effect of Mg ++ on DNase 1 exists in a uniform shape than the parent DNA. Molecular sizes (end to end distance) were 600 Å and 3000 Å respectively. From these facts it can be conceived that FEULGEN'S DNA is degraded by the action of nucleogelase or DNase 1 into about 1/100 of the original DNA. Contrary to their relatively large molecular weights, their intrinsic viscosities were rather small, especially in the case of FEULGEN'S DNA, that is, 0.26 and 2.5 respectively. Hermanns has found likewise a small intrinsic viscosity for a heat-denatured DNA derived from a high-molecular one having a large intrinsic viscosity. In order to know whether nucleic acid samples concerned in this study were double-stranded or single-stranded, they were dissolved in a solution containing 0.15 M NaCl and 0.015 M sodium citrate, and the light absorbency at 260μm of the solutions at various temperatures (T) were measured to see the relation of the increase in the ratio of OD250/OD260 to the temperature; and furthermore the increase in OD260μm by the action of formaldehyde upon DNA were investigated.

was dissolved in 1% sodium acetate, filtered and again precipitated with alcohol; 320 mg of b-nucleic acid were obtained. The acid was shown to contain no more original DNA and could not be distinguished from b-acid prepared by degrading DNA with the extract of pancreatin, judging from the electrophoretic pattern and other tests.

In order to investigate the molecular weights and molecular sizes of b-acid and Feulgen's DNA, b-acid prepared from Feulgen's DNA by treating with pancreatin and Feulgen's DNA, after they were purified by Sevag's method, were subjected to ultracentrifugation, diffusion and light-scattering: b-acid So, $3.5 \times 10^{-13}$ D, $0.2 \times 10^{-12}$; DNA, So, $28.8 \times 10^{-13}$ D, $0.2 \times 10^{-12}$. From these constants were calculated molecular weights for b-acid and FEULGEN'S DNA to be $6 \times 10^4$ and $700 \times 10^4$ respectively. Based on the ultracentrifugal and diffusion patterns, it could be imagined that b-acid had a more uniform shape than the parent DNA. Molecular sizes (end to end distance) were 600 Å and 3000 Å respectively. From these facts it can be conceived that FEULGEN'S DNA is degraded by the action of nucleogelase or DNase 1 into about 1/100 of the original DNA. Contrary to their relatively large molecular weights, their intrinsic viscosities were rather small, especially in the case of FEULGEN'S DNA, that is, 0.26 and 2.5 respectively. Hermanns has found likewise a small intrinsic viscosity for a heat-denatured DNA derived from a high-molecular one having a large intrinsic viscosity. In order to know whether nucleic acid samples concerned in this study were double-stranded or single-stranded, they were dissolved in a solution containing 0.15 M NaCl and 0.015 M sodium citrate, and the light absorbency at 260μm of the solutions at various temperatures (T) were measured to see the relation of the increase in the ratio of OD250/OD260 to the temperature; and furthermore the increase in OD260μm by the action of formaldehyde upon DNA were investigated.

500 mg of FEULGEN'S DNA were incubated with 250 γ of crystalline DNase 1 at 37°C under almost the same conditions as mentioned above, and precipitated by the addition of hydrochloric acid. The precipitate obtained