26. A Study of Deoxyribonucleoprotein Fibers by a Modified Freeze-Replica Method

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The present study was undertaken in an aim to elucidate the fine structure of deoxyribonucleoprotein fibers under their hydrated state. A method favourable for such a study was developed by improving a spray-freezing technique which was released from the formation of any artifact produced by fixatives or cryoprotective agents following the suggestion of Backmann and Schmitt (1971).¹⁾

Material and methods. Deoxyribonucleoprotein (DNP) was extracted from calf thymus following the method of Zubay and Doty (1959)⁶⁾ with some modifications. Final dialysis was carried out against 0.7 mM phosphate buffer solution (pH 6.8) for 48 h at 4°C. After the dialysis, the solution was centrifuged at $70,000\times g$ for 30 min. The supernatant was used as material for study. The concentration of DNP in the solution was about 0.5 mg per ml, and the chemical composition was 1.00:1.30:0.20:0.12 for DNA:histone:non-histone protein:RNA.

For spray-freezing of the DNP solution, a special specimen holder was designed. The holder was made with a 3 mm thick brass plate (5×10 mm) with a hollow, 2 mm in diameter and 0.3 mm in depth, in the center of the plate. The surface of the hollow was scratched with a needle in order to well-attach the specimen to it.

The DNP solution was transferred to an atomizer, and sprayed directly onto the liquid-nitrogen-precooled holder. The frozen specimen consisted of a number of small droplets, approximately 10 μm in diameter. The spraying was carried out several times to form an about 1 mm high frozen mound on the hollow of the holder. The excess of the frozen specimen around the mound was carefully scraped in liquid nitrogen with the aid of a forceps. The holder was placed into a JEOL freeze-etching apparatus. Freeze-fracturing at 1×10^{-5} Torr was achieved by a cold pin at -100°C . The specimen was deeply etched by rewarming the cold stage upto -90°C at a rate of 4°C per min. Then, the deep-etched surface was shadowed with platinum-

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palladium (8:2) and backed with carbon. The replica of the DNP specimen, thus prepared, was examined under a JEM 100C electron microscope at an accelerating voltage of 100 kV, and photographed at a magnification of 50,000 times.

Results and discussion. The DNP solution contained fibrous elements, preferring to use a term of DNP fibers.²⁾ The DNP fibers appeared to be formed by a coiling of fine fibrils (Fig. 1). Without any purposeful selection, 100 determinations were made on the diameters of the DNP fibers and fibrils which were enlarged 400,000 times. The DNP fibers ranged from 110 Å to 260 Å in diameter with a mean

Fig. 1. Freeze-replica of deoxyribonucleoprotein (DNP) fibers. Bar represents 1,000 Å.

diameter of 183 Å. The fine fibrils measured between 20 Å and 32 Å with a mean value of 23 Å.

The configuration of the DNP fibers is essentially similar to that of the DNP fibers observed under a hydrated state with the scanning electron microscope equipped with cryo-units.^{3),4)} The DNP fibers demonstrated by the present method seem to correspond to the 'elementary chromatin fiber' with the diameter of 200–300 Å which was observed in preparations of newt erythrocytes by the method of air-water interface spreading.⁵⁾ The fine fibrils are presumed to be DNA fibers based on the diameter measurement.

Although a final conclusion must be left to future studies, it seems very probable that the DNA fiber winds around the protein core, resulting in the formation of the spiral structure of the DNP fiber.

Summary. Deoxyribonucleoprotein (DNP) is extracted from calf thymus. The DNP solution is sprayed onto a specially designed specimen holder which is precooled with liquid nitrogen. The frozen specimen is deeply etched by rewarming upto -90°C at 1×10^{-5} Torr, and subsequently shadowed with platinum-palladium. The freeze-replica of the DNP is examined under an electron microscope. The DNP fibers with a mean diameter of 183 Å are formed by a coiling of fine fibrils, 23 Å in mean diameter.

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