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The presence of the 30 nm filament structure of chromatins in intact chicken erythrocytes observed by ^{31}P NMR

Shozoh Nishimoto, Hideo Akutsu* and Yoshimasa Kyogoku

Institute for Protein Research, Osaka University, Suita, Osaka 565 and **Department of Physical Chemistry, Faculty of Engineering, Yokohama National University, Tokiwadai, Hodogaya-ku, Yokohama 240, Japan*

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A ^{31}P NMR spectrum of chicken erythrocyte chromatins in vivo was obtained. The spectrum was characterized by examining the spectra of isolated nuclei and nondigested chromatins under various conditions. The results have shown that most chromatins in chicken erythrocyte nuclei assume the 30 nm filament structure as a second step of condensation of DNA in vivo.

^{31}P-NMR; Chromatin; Chromatin structure; Solid-state NMR; (Chicken erythrocyte nucleus)

1. INTRODUCTION

Eukaryotic chromosomes are known to have an organized and compact structure. An electron microscopic study of nuclease-digested chromatins (soluble chromatins) showed that they undergo a compaction, from 10 to 30 nm filaments, depending on the salt concentration [1]. Finch and Klug [1] proposed a solenoid model for the 30 nm filament. An important question is whether the 30 nm filament really exists in in vivo chromatins. Low angle X-ray diffraction of intact chicken erythrocytes or sea urchin sperm gave broad 40 nm reflections, which were ascribed to side-to-side packing of chromatin fibers [2–4]. This suggests that the 30 nm filament structure does exist in in vivo chromatins. Although these results are very important, the presence of the 30 nm filament in vivo is not yet well established. It should also be clarified whether it is the general structure in an intact cell. We have investigated the higher order structure of in vivo chromatins from the view point of the dynamic state of DNA by the use of ^{31}P solid-state NMR in this study. A pioneering work on the soluble chromatins was reported by DiVerdi et al. [5].

Correspondence address: S. Nishimoto, Institute for Protein Research, Osaka University, Suita, Osaka 565, Japan

2. MATERIALS AND METHODS

The erythrocytes were obtained from chicken blood diluted with an approximately equal volume of SSC buffer (150 mM NaCl, 15 mM sodium citrate), followed by centrifugation at 4000 × *g* for 15 min. The pelleted cells were used for the NMR measurements. The erythrocyte nuclei were prepared according to Olins et al. [6]. The pellet was dialyzed against STM buffer (10 mM NaCl, 10 mM Tris, 3.0 mM $MgCl_2$, pH 7.4) containing 30% sucrose and used for NMR measurements. The nondigested chromatins were obtained as follows. Nuclear envelopes were ruptured by gently suspending chicken erythrocyte nuclei in a large volume of TE buffer (1 mM Tris, 0.2 mM EDTA, pH 7.4). Then, 1 M NaCl was added dropwise and gently up to 100 mM final concentration. The aggregated chromatins were pelleted by centrifugation at 4000 × *g* for 15 min and then dialyzed against TE buffer containing 30% sucrose and 0.1–3 mM $MgCl_2$. A sample in a dialysis tubing immersed in the buffer containing 30% sucrose was used for the NMR

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measurements. The ^{31}P NMR spectra were obtained at 40.3 MHz with a Jeol FX-100 NMR spectrometer equipped with a solid-state NMR system. A 1.0 ms contact time and 3.0 s relaxation delay were used for the cross-polarization pulse sequence, and a 45°C pulse and 3.0 s relaxation delay were used for the single pulse measurements. An exponential window function with a 100 Hz broadening factor was used.

3. RESULTS AND DISCUSSION

Presented in fig.1A is the ^{31}P NMR spectrum of intact chicken erythrocytes at 4°C measured by the conventional single pulse method. The spectrum represents the whole phosphorus-containing molecules in an erythrocyte. The sharp and symmetrical peak at the center would comprise overlapping signals originating from soluble molecules in the cytoplasm such as inorganic phosphate. Since an exponential window function with a 100 Hz broadening factor was used, the signals are not resolved. The broader components can be attributed to insoluble molecules such as phospholipids in the membranes and DNA in the nucleus. The contents of such molecules per cell are 1.72 pg DNA (76.8% of DNA, RNA and phospholipids in phosphorus base), 0.19 pg RNA (8.5%) and 0.75 pg phospholipids (14.7%) [7]. In order to obtain information on the DNA, a cross-polarization pulse sequence was used. For this method, the polarization of proton spins is transferred to the phosphorus spin system through the thermal contact between these spin systems [8]. Since the polarization transfer is mediated by the dipolar interactions between ^{1}H and ^{31}P, only the molecules undergoing slow motions can be observed. The efficiency of the cross-polarization was maximum with about 1 ms thermal contact for DNA but with 10 ms thermal contact for the phospholipid bilayer in the liquid-crystalline state [9]. Therefore, we should be able to observe DNA with minimum contribution from the phospholipid membranes when the cross-polarization spectrum is measured with a 1 ms contact time.

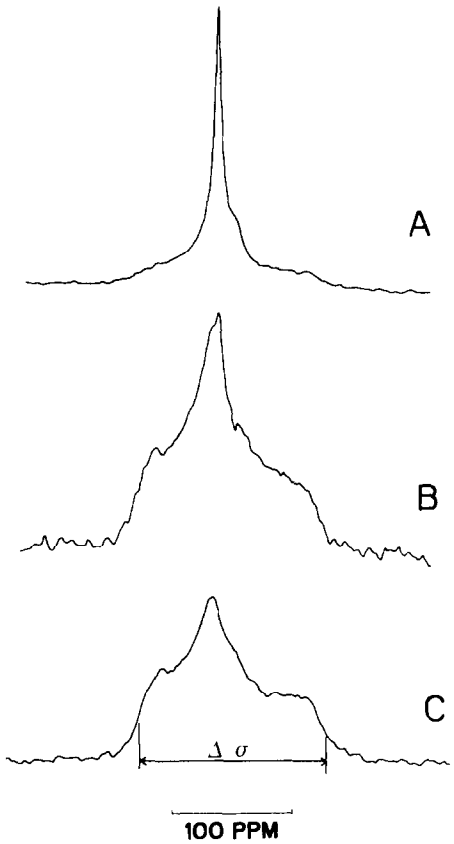

Fig.1. ^{31}P NMR spectra of intact chicken erythrocytes and isolated nuclei at 4°C. (A) Chicken erythrocytes, measured by the single pulse method; (B) chicken erythrocytes, measured by the cross-polarization method; and (C) isolated nuclei, measured by the single pulse method. 10000, 8000 and 1000 transients were accumulated for (A), (B) and (C), respectively. The 90° pulse width was 11 and 6.6 μs for the erythrocytes and isolated nuclei, respectively.

Fig.1B was obtained under such conditions. The spectrum is a typical asymmetric powder pattern which is characteristic of rigid phosphorus. The chemical shift anisotropy ($\sigma_{11} - \sigma_{33}$), which is indicated at the bottom of fig.1, can be used as a measure of the rigidity of phosphorus. The chemical shift anisotropy in fig.1B was about -156 ppm. This value corresponds to the broadest width in fig.1A. Since the 'axially symmetric' powder pattern typical of the phospholipid bilayers does not appear at all in fig.1B, it can be said that the contribution from the membranes is actually eliminated as expected and the spectrum in fig.1B represents in vivo chromatins.

To confirm the assignments of the spectrum, isolated chicken erythrocyte nuclei were examined by means of the conventional single pulse method. The spectrum of the isolated nuclei in the presence of 3.0 mM $MgCl_2$ and 30% sucrose is presented in

fig.1C. 30% sucrose was included for stabilization and condensation of the nuclei. All phosphorus spins in the isolated nuclei contribute to the spectrum. Phosphorus is located mainly in DNA (91.2% of DNA, RNA and phospholipids in phosphorus base), RNA (7.0%) and phospholipid (1.8%) [7]. Since the content of phospholipids is negligibly small, one can say that the spectrum in fig.1C mainly represents phosphorus spins located in the DNA and RNA. The spectrum also reveals a typical asymmetric powder pattern with the chemical shift anisotropy of -155 ppm. Although the spectral contribution of RNA cannot be identified, the major feature of the spectrum must be determined by DNA. The well-characterized asymmetric powder pattern suggests that most DNA in the nucleus takes on a similar dynamic state. The chemical shift anisotropy was compared between intact chicken erythrocytes and isolated nuclei at different temperatures. The temperature dependence was found to be identical for both (not shown). Now one can conclude that the spectrum in fig.1B represents in vivo chromatins and that the dynamic properties of DNA of chicken erythrocyte chromatins are identical in in vivo nuclei and isolated ones. Since the chemical shift anisotropy ($\sigma_{11}-\sigma_{33}$) of solid DNA was reported to be about -194 ppm [10], that of the chromatin DNA is partially averaged by motions. Nevertheless, the value of -156 ppm in fig.1 means that the motions of DNA are highly restricted in in vivo chromatins.

The dynamics of the DNA were further investigated through the use of nondigested chromatins. In order to keep the chromatins as intact as possible, the nuclear envelope was just ruptured by dispersing the nuclei in the TE buffer. The chromatins prepared in this way are referred to as nondigested chromatins hereafter. A ^{31}P-NMR spectrum of the nondigested chromatins in TE buffer with 3.0 mM $MgCl_2$ was measured by the single pulse method. Surprisingly, the spectrum obtained was quite similar to that of the isolated nuclei (not shown). The residual chemical shift anisotropy was also identical for both. This means that the rigid structure observed in the nuclei also occurred in the nondigested chromatins in the presence of 3.0 mM $MgCl_2$. To characterize the rigid structure represented by this residual chemical shift anisotropy, that of the nondigested chromatins was examined for various Mg^{2+} concentrations. As shown in the upper panel of fig.2, the residual chemical shift anisotropy changed sharply in the range 0.1 - 0.4 mM $MgCl_2$, with a midpoint at about 0.2 mM, showing that magnesium ions induce a structural change of chromatins. The Mg^{2+} concentration range which induces the structural

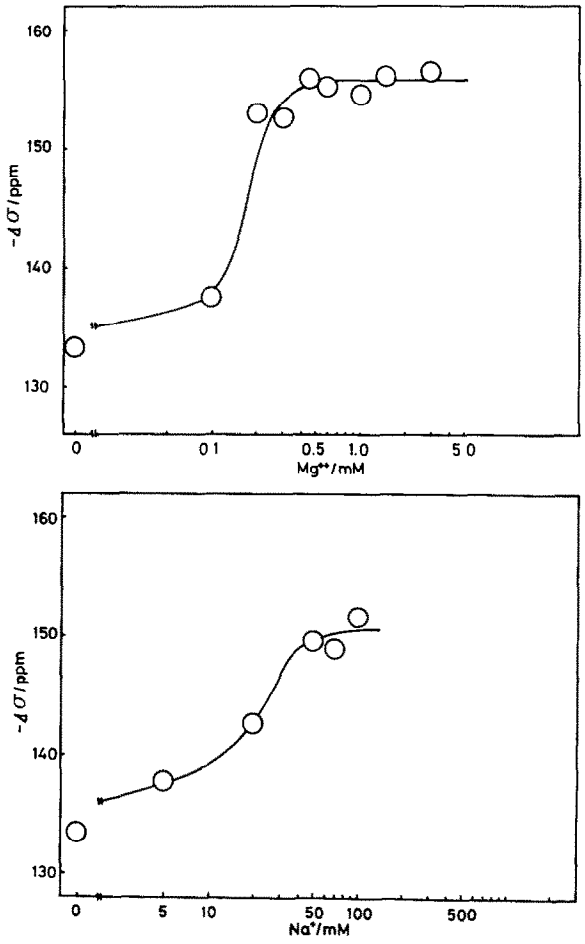

Fig.2. Residual phosphorus chemical shift anisotropy ($\Delta\sigma=\sigma_{11}-\sigma_{33}$) of the nondigested chromatins of chicken erythrocyte at various Mg^{2+} and Na^+ concentrations. In the case of the upper panel, the pellet obtained on centrifugation at 4000 × *g* for 15 min was dialyzed first against TE buffer and then against TE buffer containing various concentrations of $MgCl_2$. In the case of the lower panel, the pellet was dialyzed against TE buffer containing various concentrations of NaCl. All spectra were obtained by the cross-polarization method. The residual chemical shift anisotropy was identical for the spectra obtained by single pulse and cross-polarization methods.

change strictly corresponds to that which induces a structural transition from the 10 filament to the 30 nm filament in soluble chromatins [11–14]. Since it was reported that Na^+ also induces the same structural transition at a much higher concentration, the Na^+ concentration dependence of the residual chemical shift anisotropy was examined, and the results are shown in the lower panel of fig.2. The chemical shift anisotropy changed in the range 5 – 40 mM NaCl. This range also corresponds to the NaCl concentration range which induces the structural transition from the 10 nm filament to the 30 nm filament in soluble chromatins [11–14]. These facts indicate that the structural change observed in the soluble chromatins also occurs in the non-digested chromatins. Since the spectra obtained by means of the single pulse and cross-polarization methods are similar, it can be concluded that the structure of the 30 nm filament is the general structure in the second step of condensation of DNA in chicken erythrocyte chromatins.

There is a difference in the residual chemical shift anisotropies of the nondigested chromatins in the presence of Mg^{2+} and Na^+. They are -156 and -150 ppm, respectively, suggesting that the Mg^{2+}-induced structure is dynamically different to some extent from that induced by Na^+. This fact shows that the residual chemical shift anisotropy is sensitive enough to characterize the nature of the higher order structure of the chromatins. The residual chemical shift anisotropy of chromatins in isolated nuclei and in intact erythrocytes was identical with that of the nondigested chromatins in the presence of 3.0 mM $MgCl_2$. Its temperature dependence was also identical for all three kinds of chromatins. Thus, we can conclude that the chromatins in the isolated nuclei and intact erythrocytes assume the Mg^{2+}-induced 30 nm filament structure as their general second-step higher order structure.

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