Electron Spin Resonance and Cell Division in Silkworm Egg

Shizuo FUJIWARA, Hiroshi TADANO*1 and Makoto NAKAJIMA*2

Department of Chemistry, Faculty of Science, The University of Tokyo, Bunkyoku, Tokyo

(Received June 29, 1970)

Several biological systems have so far been investigated by electron spin resonance (ESR).[1-3] The sources of ESR signals were generally identified and the results of investigation by ESR have presented direct information on the kinetic and chemical behaviors of the sample. ESR can also be used for looking at the free radicals of unknown identity, as this technique can be used as a means of seeing inside the living material without causing any distortion or damage.

Information hitherto given on the commercial silkworm[4] aroused our interest to investigate silkworm eggs by ESR. Studies were carried out on the eggs of six varieties of *Bombyx mori*, including two Japanese (KONJIKI and AOJYUKU), three Chinese (KANSEN, HIKO and SEKKO) and one European (ASCOLI). All showed ESR signal of the same g value, 2.004 ± 0.001, but of different intensity.

All measurements were carried out at room temperature. For the measurements, twenty eggs of each kind were loaded in a glass capillary of about 1 mm I.D. without sealing and placed in the field of ESR spectrometer. All eggs had previously been about one year in diapause. The g-value and signal intensity were measured with two reference substances, *i.e.*, the diphenylpicrylhydrazil and the manganous ions of a known number of spins in magnesium oxide. The g-value, of all six varieties did not change with time, either before incubation, or after the eggs were activated by treatment with hydrochloric acid.

In order to locate the radicals, ESR measurements were carried out on egg fractions of nucleus, mitochondria, and microsome, which were separated from each other according to standard procedures. The shell did not show any signal, and mitochondrial and microsomal fractions showed weak signals. We suspect that these signals are due to contamination with nuclear material. A very strong signal was observed with nucleus. The records of ESR signals are shown in Fig. 1.

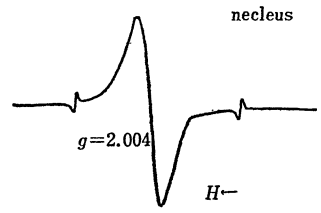

Fig. 1. ESR signal (central peak) in the nucleus of silkworm egg. Satellites in both sides of the central peak refer to the hyperfine signals of Mn^{2+} (in MgO), which has been added to the sample as the reference substance.

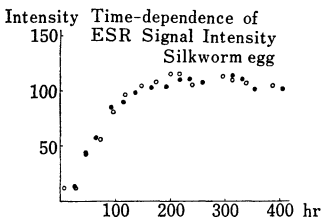

Fig. 2. Time-dependence of ESR signal intensity of silkworm egg.

Figure 2 shows the time dependence of the ESR signal in freshly laid eggs of HIKO. The origin of the time scale in Fig. 2 refers to the time of *OVIPOSITION*. Two sets of measurements were carried out: one was started 3 hr after *OVIPOSITION* (white circles in Fig. 2); the other after 12 hr (black circles).

As shown in Fig. 2, the signal intensity increases linearly during the first 180 hr and then plateaus. The intensity scale in Fig. 2 is arbitrary; after the 180th hour, the number of electron spins in one egg is about 1.4×10^{14}.

If we assume that an egg originally has n spins, and that they increase linearly until equilibrium is achieved, then there is approximately a 60-fold increase during this time.

Further effort has been made in order to locate the spins, *i.e.*, the nuclear material has been separated into DNA and protein by extracting nucleus with 1% Na_2CO_3 or 0.8% NaCl solution. Although a spurious signal is observed in the DNA part, spins are mainly located in that of protein. The principal problem is the source of the ESR signal in these eggs. Whatever the free radicals be, they increase systematically during the early hours of incubation and then stabilize in an equilibrium. Additional studies are being made.

*1 On leave from Rikkyo University, Tokyo.

*2 Tokyo Nōkō University, Fuchū, Tokyo.

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