SHORT COMMUNICATION

Protein Damages in Irradiated Deoxyribonucleoprotein

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When the solid-state deoxyribonucleoprotein (DNP) was irradiated by gamma rays, Lücke-
Huhle et al.1) found that single strand breaks of DNA were produced more frequently in the
DNP than in pure DNA. Ormerod2) and Kuwabara and Yoshii3) reported that the ESR spectra
of DNP after irradiation showed radicals to be located preferentially on the DNA moiety, and
the results were explained by the electron transfer from the protein moiety to the DNA moiety.
From studies on the luminescence decay curve of DNP when irradiated with electron pulses,
Llilicrap and Fielden4) also found that the excitation energy formed in the protein moiety was
transferred to the DNA moiety.

When the DNP solution was irradiated by gamma rays, Weiss and Wheeler5) and Hayashi
et al.6) found that the template activity of DNP for RNA synthesis was considerably enhanced
by irradiation (10-20 krad), whereas Hagen et al.7) found that the template activity of purified
DNA was markedly reduced. Using an irradiated DNP template, Mee et al.8) also reported
that changes in the base composition of the synthesized RNA were found at doses of 10-20
krad.

These reports suggest that DNP is not affected by irradiation in the same way
as DNA alone. A variety of radiobiological studies suggests that radiation causes
DNA side damage in irradiated DNP; however, as far as we know, no report on the
protein side damage in irradiated DNP has appeared. This report reveals protein
damage in DNP due to irradiation: firstly, radical formations were found on the
protein side in solid-state DNP; secondly, physico-chemical changes of the protein
in DNP solutions affecting the template activity were found.

DNP was extracted from calf thymus by the method of Zubay and Doty.9) The
protein to DNP ratio in the extracted DNP was about 1.45 : 1.0 by weight. DNA
was extracted from calf thymus by the method of Kay et al.10) The protein content
of the DNA preparation was less than 1%. Histone was isolated from calf thymus
DNP by acid extraction.11) No DNA content was detected in the histone preparation.

Gamma-irradiated dry state calf thymus DNP, DNA and histone were investiga-
gated using the method of electron spin resonance (ESR). Irradiation and observation
were carried out at room temperature. The ESR spectrum of DNP came to resemble

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closely that of the DNA. The presence of an octet hyperfine structure, which represents a thymine radical, was also markedly clear (Fig. 1, a, b). Moreover, it was found that resonance at \( g = 2.0012 \), indicated by an arrow in the DNP spectrum, represented the absorbance caused by free radicals on the histone moiety.\(^9\) The ESR spectrum of pure histone is shown in Fig. 1, c. The spectrum consists of a characteristic doublet with an additional signal in the wings. Holroyd’s experiment\(^{13}\) on hydrogen-bombarded histone leads us to assign the central doublet spectrum to the polypeptide \( \alpha \)-carbon radical. The satellite resonance indicated by the arrows seems to be attributed to the radicals created in the aromatic rings of the histone. This is confirmed by Liming and Gordy’s work,\(^{14}\) where, when dry state polyamino acids, namely poly-tyrosine, poly-phenylalanine and poly-tryptophan were used, weak lines were observed on either side of the central region of the spectrum, and these were assigned to radicals in the aromatic ringed groups.

The importance of these radicals is clear when one notes the research of Charlesby,\(^{14}\) where it was shown that intramolecular cross-linkings in the protein were easily formed by ionizing radiation (where a \( G \) value of about 1 was found in most cases). Thus, when DNP is exposed to ionizing radiation and radicals are formed in the protein moiety, \( \alpha \)-carbon radicals on the polypeptide main chain and radicals in the aromatic ringed groups may interact either between themselves or

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Fig. 1. First derivative of the ESR spectra of (a) DNP, (b) DNA and (c) histone. Irradiation and observation at room temperature. Dose 1 M rad. The resonance at \( g = 2.0012 \) in (a) and the satellite resonance in (c) are indicated by arrows.
with each other, resulting in the formation of intramolecular cross-linkings.

The enhancement of the template activity by gamma irradiation, as mentioned above, has been interpreted as the removal of protein from DNP, which is inferred from physico-chemical studies done on the subject. On the other hand, since only small amounts of protein were released from DNP with irradiation of a high dose (170 krad), it was suggested that it would be difficult to account for the enhancement of the template activity on the basis of the removal of proteins by irradiation. These conflicting arguments may be resolved as a result of the experiment where the protein molecules were dissociated on irradiation and then re-associated with other histone molecules or with DNP molecules. Therefore, the authors have attempted to test whether or not the protein molecules would become dissociated on irradiation and would become re-associated. Sodium chloride was added to the irradiated DNP solution to a final concentration of 0.15 M. After centrifugation, the amount of protein in the supernatant, which gave the amount of dissociated protein, was analysed. The amount of dissociated protein is shown in Table I, (a). Only small amounts of protein, 2.65%, were dissociated from DNP even at a dose of 160 krad. This result corresponds to results obtained by Robinson et al., if any re-association did occur, it probably resulted in aggregation. The DNP-salt precipitates were extracted with 5 M urea-0.15 M NaCl. The urea was employed to disperse any protein aggregate. After centrifugation, the amount of aggregated protein in the supernatant, which gave the amount of aggregated protein after irradiation, was analysed. No significant difference in the amount of aggregated protein was found between the irradiated and the unirradiated DNP (Table 1, (b)). It is clear that re-association did not occur between the dissociated proteins and the DNP. Thus, these results indicate that rather than the dissociation of protein from DNP, an irradiation weakens the bond between the proteins and DNA, exposing DNA to serve as a template.

Table 1
The amounts of the dissociated proteins from DNP by irradiation and the aggregated proteins were analyzed by the method of Itzhaki (see text). The percentage of the dissociated proteins from DNP by irradiation is indicated in parentheses. The concentration of DNP was 991 µ/ml. The protein to DNA ratio in the DNP preparation was 1.45 : 1.

<table>
<thead>
<tr>
<th>Doses (krad)</th>
<th>Amounts of dissociated protein (a) µg/ml (%)</th>
<th>Amounts of aggregated protein (b) µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>26.5 (—)</td>
<td>38.0</td>
</tr>
<tr>
<td>13.4</td>
<td>23.5 (0.00)</td>
<td>38.7</td>
</tr>
<tr>
<td>26.9</td>
<td>28.1 (0.28)</td>
<td>34.9</td>
</tr>
<tr>
<td>41.5</td>
<td>33.6 (1.16)</td>
<td>37.0</td>
</tr>
<tr>
<td>72.9</td>
<td>35.7 (1.58)</td>
<td>31.2</td>
</tr>
<tr>
<td>162.0</td>
<td>41.9 (2.65)</td>
<td>—</td>
</tr>
</tbody>
</table>
To find the cause of the weakening of the bonds, the physico-chemical changes of histones were investigated by means of polyacrylamide gel electrophoresis (15% polyacrylamide gel contained 6.25 M urea, pH 3.2). Urea was served to avoid aggregation of histones. Histones were extracted from the irradiated DNP with 0.2 M H₂SO₄ and applied to the electrophoresis. As seen in Fig. 2, the height of main bands which represent five histone fractions decreased, whereas the height of the bands showing higher molecular weight materials than H₁ increased. The band showing lower molecular weight materials than H₃ did not appear. These results indicated that: 1. higher molecular weight materials than H₁ were not aggregates of histones but products of the intramolecular cross-linkings in the histones, since only small amounts of the aggregate of histones were present in 6.25 M urea; and 2. that no significant degradation of histones was observed at a dose of 40 krad. Thus, steric hindrance caused by the intramolecular cross-linkings in the histone prevented the protein from interacting again with DNA, and it may contribute to the weakened bond between proteins and DNA.

Fig. 2. Histones were extracted from DNP with 0.2 M H₂SO₄ and analyzed with polyacrylamide gel electrophoresis by the method of Fanyim and Chalkley. Electrophoresis patterns of histones were recorded by a gel scanner using the Joyce Loebel Scanner. — histones from unirradiated DNP, ...... histones from 40 krad irradiated DNP.
The results were summarized as follows.

1. Protein radicals were formed in the irradiated DNP.
2. An irradiation did not actually cause the dissociation of protein from DNP.
3. The amount of higher molecular weight materials than H1 increased markedly in the histones extracted from the irradiated DNP.

From this series of experiments, it may be concluded: (1) after the electrons were liberated from the protein moiety and transferred to the DNA moiety, electron holes remaining on the protein moiety contributed to the protein radical formation; (2) the intramolecular cross-linkings in protein due to radical-radical reactions caused a weakening of the bond between the proteins and DNA, which affected a change in the template activity of DNP due to gamma-irradiation; and (3) in addition, the amino acid radicals increased by OH radicals participated in intramolecular cross-linkings.

The cross-linkings of amino acid in proteins may inhibit the functional capabilities of the proteins which provide structural support in the complex chromatin of higher organisms.

REFERENCES


