High Energy Radiation-Induced Crosslinking of Histone Octamer Complexes

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Calf thymus histone octamer complexes were irradiated in the native state in N_2O-saturated dilute aqueous solution (0.5 g/l, pH 9, $[NaClO_4] = 1 - 4$ mol/l) with 50 or 100 ns pulses of 16 MeV electrons or ^{60}Co-γ-rays. Time resolved light scattering measurements and optical absorption measurements yielded the following: the octamers underwent a volume contraction due to intra-complex-crosslinking induced by the attack of OH-radicals. Crosslinking proceeded to a certain extent *via* 2,2′-biphenol coupling as inferred from product analyses.

Introduction

This paper describes radiation effects detected in *natural* histone octamer complexes that were extracted from calf thymus chromatin and irradiated in the isolated form by 16 MeV electron beam pulses or ^{60}Co-γ-rays. Octamer complexes consisting of two sets of the histones, H2A, H2B, H3 and H4, are major constituents of nucleosomes in which double stranded DNA tied up by histone H1 is wound around the octamer complexes [1–3].

Pulse radiolysis experiments using light scattering detection showed that the octamer complexes underwent a contraction as a consequence of intra-complex-crosslinking (intra-cc). Optical absorption detection yielded evidence for the transient existence of tyrosyl radicals that subsequently formed dityrosyl links (2,2′-biphenols). These results were affirmed by product analysis using gel electrophoresis and optical absorption and emission spectroscopy. The irradiation experiments were carried out in N_2O-saturated dilute aqueous solution (pH 9) containing $NaClO_4$ (1–4 M). Some typical results are presented below. In this connection experiments by Prütz *et al.* are noteworthy [4]. With a commercial histone product consisting of a mixture of calf thymus core histones, these authors observed 2,2′-biphenol coupling induced by the reaction of $\cdot N_3$ radicals. 2,2′-biphenol coupling subsequent to the attack by reactive free radicals ($\cdot OH$, $\cdot N_3$, Br_2^-) has been evidenced in various proteins [5–10]. It appears to be an important reaction during the irradiation of proteins in N_2O-saturated aqueous solution if solvated electrons are converted to OH-radicals ($e_{aq}^- + N_2O + H_2O \rightarrow \cdot OH + OH^- + N_2$).

It should be pointed out that up to now only non-complexed proteins were investigated. In these cases 2,2′-biphenol coupling led to larger molecules (dimers etc.) with the consequence of an increase in the molecular weight and therefore in the light scattering intensity (LSI) [7]. In the present case 2,2′-biphenol coupling occurs within the protein complexes causing their contraction. This leads also to an increase in the LSI, as will be outlined below.

Results and Discussion

Fig. 1 shows an oscilloscope trace depicting the increase of the light scattering intensity (LSI) after the pulse as a function of time. The first halflife of the LSI increase, $\tau_{1/2}$ (LSI) = 80 ± 20 ms, and the total extent of the LSI increase $\Delta U/(U_\infty - U_L) = 0.14 \pm 0.03$ were independent of the absorbed dose (100–400 Gy). These findings indicate that the observed effect is due to intra-cc. In the case of inter-complex-crosslinking (inter-cc), the extent of LSI increase is expected to increase with the absorbed dose because an increasing number of complexes should be aggregated with increasing absorbed dose. Intra-cc, on the other hand, is restricted to the isolated complexes, within which

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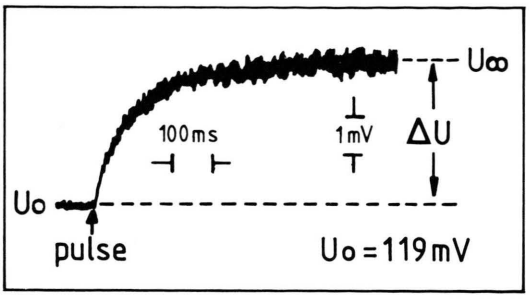


Fig. 1. The increase of the light scattering intensity of a N_2O-saturated aqueous histone-octamer solution (0.5 g/l) at pH 9 after irradiation with a 100 ns pulse of 16 MeV electrons. Absorbed dose: 100 Gy; [$NaClO_4$]: 1 mol/l; U (signal voltage) $\propto$ light scattering intensity. The subscripts 0 and 00 refer to times before and a long time after the pulse. L designates solvent. $\Delta U = U_{00} - U_0$.

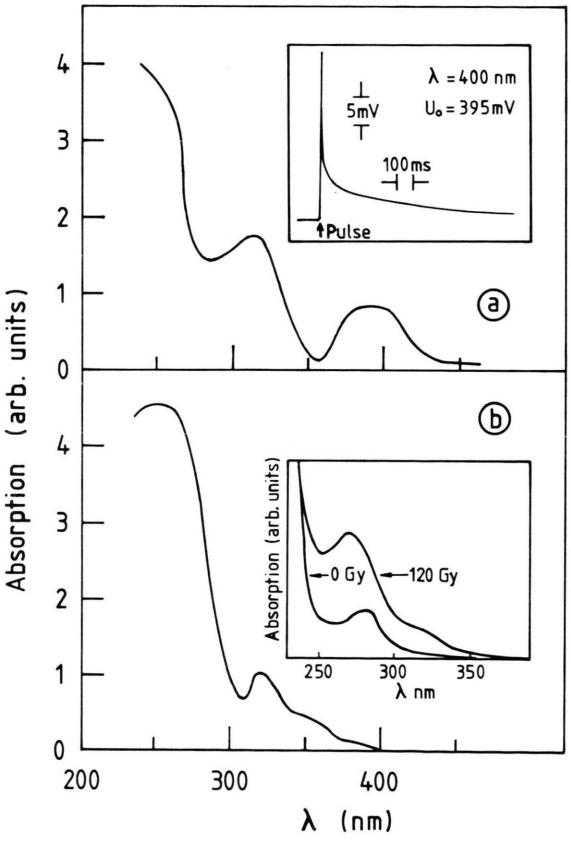


Fig. 2. Difference absorption spectra recorded after irradiation of a N_2O-saturated aqueous solution of histone-octamer complexes at pH 9 and [$NaClO_4$]: 1 mol/l. Absorbed dose per pulse: 115 Gy; pulse duration: 50 ns. (a) $(\text{absorpt.})_{t=0} - (\text{absorpt.})_{t=900\,\mu s}$. Insert: Oscilloscope trace depicting the change of the optical absorption at $\lambda = 400$ nm with time. (b) Permanent absorption, recorded 1.8 s after the pulse. Insert: Absorption spectra recorded before and after irradiation.

only a limited number of crosslinks can be formed with the consequence of limited possible maximum contraction of the complexes and therefore limited possible maximum extent of increase in LSI. That a contraction of the complexes is expected to cause an increase in the LSI follows from Eqn. (1) [11–13]:

$$\frac{Kc}{R_\vartheta} = \frac{1}{M_w} + \frac{16\pi^2\langle s^2\rangle}{3\lambda^2 M_w}\sin^2\vartheta/2 + 2A_2c\,. \qquad (1)$$

K = constant, R_ϑ = Rayleigh ratio $\propto$ LSI, M_w = weight average molecular weight, $\langle s^2\rangle$ = radius of gyration, λ = wavelength of analytical light, ϑ = scattering angle, A_2 = 2nd virial coefficient, c = polymer concentration.

Generally, a decrease in the radius of gyration is accompanied by a decrease in A_2. Thus, it is obvious from Eq. (1) that the LSI should increase provided $\bar{M}_w$ remains constant. In the present case this is true since, as far as light scattering is concerned, the octamer complexes can be considered as integral units regardless of the state of bonding. Hence, before irradiation the histone molecules in the octamer complexes are held together by physical forces whereas after irradiation the same histone molecules are chemically bonded to each other within the complex.

By time-resolved optical absorption measurements the transient difference absorption spectrum shown in Fig. 2 (a) with maxima around 320 nm and 400 nm was observed. Because OH radicals attack proteins not very specifically, the transient spectrum is composed of the spectra of various radicals which are produced during the pulse. It appears, however, that the spectra of cyclohexadienyl and of tyrosyl radicals, which are expected to be formed in the case of histones, can be distinguished in Fig. 2 (a) against the background of the absorption of other radicals. Based on relevant literature data [14–18], the maxima around 320 and 400 nm are ascribed to cyclohexadienyl type radicals and to tyrosyl radicals, respectively. These species presumably were formed upon reaction of OH radicals with phenylalanine and tyrosine moieties. The insert of Fig. 2 (a) shows an oscilloscope trace depicting the decay of the optical absorption at 400 nm formed during or a few μs after the pulse. The decay occurred according to two modes: a relatively fast mode with a halflife of about 75 μs and a slow mode with a halflife of 80 ± 20 ms corresponding to

the halflife of the LSI change. This finding indicates that, at least to a certain extent, intra-cc occurs *via* the combination of tyrosyl radicals. This assumption is affirmed by the permanent absorption spectrum, shown in Fig. 2 (b), that remains after the decay of the transient absorption. The difference spectrum possesses a maximum at about 320 nm indicating the formation of 2,2′-biphenol-groups and a strong absorption band between 250 and 300 nm indicating the formation of other products such as 3,4-dihydroxyphenylalanine moieties.

From fluorescence measurements on irradiated octamer solutions the characteristic emission of dityrosine ($\lambda_{max} = 410$ nm) was observed as shown in Fig. 3. Analogous results were obtained by Boguta and Dancewicz with various proteins (insulin, ribonuclease, papain, collagen) [8 b, c] that were subjected to γ-radiation. These authors ascribed the emission band with $\lambda_{max} = 410$ nm to dityrosine groups on the basis of the emission spectrum observed with dityrosine [8 a]. By SDS-polyacrylamide gel electrophoresis of irradiated histone octamer solutions, carried out after denaturation, chemically bonded histone dimers, tetramers and hexamers were evidenced. For these determinations a modified method based on the high pH SDS-polyacrylamide gel system of Hardison and Chalkley was used [19]. The gel concentration was 15%, the pH 8.8. Amidoblack (E. Merck) was used for staining.

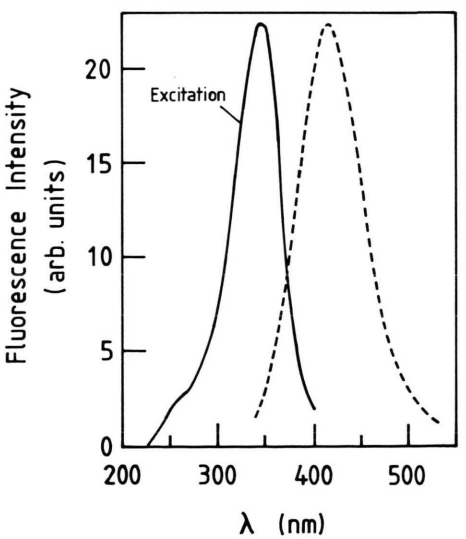
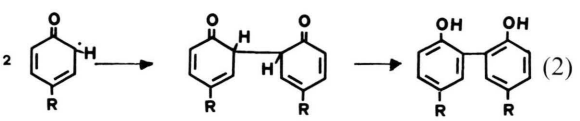


Fig. 3. Fluorescence spectra recorded with a histone octamer solution after irradiation with 16 MeV electrons. pH 9; $[NaClO_4] = 2$ mol/l; absorbed dose: 115 Gy. Full curve: excitation spectrum, $\lambda_{record} = 410$ nm. Dotted curve: Emission spectrum, $\lambda_{exc} = 325$ nm. The spectra were corrected for a small contribution, obtained with the unirradiated solution.

Summarizing the results described above it may be concluded that native histone complexes undergo intra-complex-crosslinking upon irradiation with high energy radiation in dilute aqueous solution containing $NaClO_4$ (1 mol/l). Evidence was obtained for 2,2′-biphenol bridges being formed between different histones within the complexes *via* the combination of dityrosyl radicals: Crosslinking



(2)

occurs with a halflife of ca. 80 ms as inferred from the change of the light scattering intensity caused by contraction of the complexes and the decay of the optical absorption at $\lambda = 400$ nm. Regarding the postulated predominance of intra-cc *vs.* inter-cc *via* the combination of tyrosyl radicals in native octamer complexes it is noteworthy to point out that according to the studies of Michalski-Scrive *et al.* [20] histone octamers possess no tyrosine groups exposed to the outer sphere of the complexes. This implies that inter-cc is expected to occur only after denaturation of the complex.

Preparation of Histone Octamer Complexes

The isolation of native histone octamer complexes was based on methods described in the literature [21–26]. Chromatin was obtained by extraction from calf thymus. For this purpose the thymus gland was homogenized in 0.14 M NaCl solution containing the disodium salt of ethylenediaminetetraacetic acid (2 Na-EDTA) at a concentration of 10 mM, pH 8.0. The cell nuclei, so obtained, were washed several times with the same saline solution by renewed homogenization followed by centrifugation to recover the insoluble chromatin. Nonhistone proteins and histone H1 were separated by extraction with 0.35 M and 0.65 M NaCl containing 10 mM 2 Na-EDTA, pH 8, respectively. The H1 depleted chromatin was dissolved in 1.98 M NaCl, 6.85 mM $Na_2B_4O_7$, pH 9. After stirring for 24 h, the DNA

was pelleted by ultracentrifugation for 16 h in a Beckman 60 Ti rotor at 55,000 UpM. All procedures were carried out at 4 °C. Octanol was present during homogenization to prevent surface denaturation, and 1 mM phenylmethanesulphenylfluoride (PMSF) (added as a 50 mM solution in iso-propanol) was present in all solutions to prevent protease activity. The histone-octamer solution was dialyzed against 1.98 M NaCl, 6.85 mM $Na_2B_4O_7$, pH 9, to remove PMSF and octanol. NaCl was substituted by $NaClO_4$ *via* dialysis against solutions containing appropriate perchlorate concentrations. Final solutions, thus obtained, were analyzed for DNA using the "diphenylamine method" of Burton [27] and for protein using the method of Lowry *et al.* [28]. In all preparations the DNA concentration was below the sensitivity limit of the method, *i.e.*, below 2 μg/ml. The histone concentrations were between 0.5 and 0.7 mg/ml.

Acknowledgement

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