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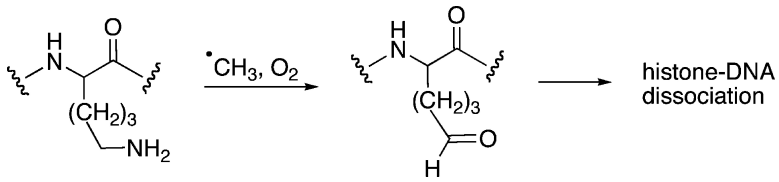
J. Am. Chem. Soc., **2007**, 129 (37), 11328-11329• DOI: 10.1021/ja073683s • Publication Date (Web): 28 August 2007



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Published on Web 08/28/2007

Dissociation of DNA-Histone Assemblies Resulting from Protein Side-Chain Functionalization

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Received May 22, 2007; E-mail: mohlerdl@jmu.edu

The modification of biomolecules by radical intermediates has profound implications in chemistry, biology, and medicine. Oxygen-centered radicals have been the most widely studied, primarily because of their participation in biomolecular damage by disease or radiation[1] but also as active species in therapeutic agents such as bleomycin.[2] With the discovery of the enediyne anticancer antibiotics, the study of the reactivity of carbon-centered radicals with DNA accelerated.[3,4] However, despite the hypothesis that the anticancer activity of the enediynes results from the radical-mediated oxidative cleavage of DNA, surprisingly few studies of organic radical reactivity have utilized DNA/histone assemblies to emulate the environment of DNA in eukaryotic cells. The importance of such investigations is suggested by the well-documented role of chromatin structure in gene regulation and by the known dependence of DNA structure and reactivity on nucleosome packing in chromatin.[5]

For example, the DNA cleaving patterns and intensities of calecheamicin γ_1^I and esperamicins A1 and C are modulated by chromatin structure, while the binding modes of the drugs are consistent with known models.[6] The enediyne-protein complexes kedarcidin, neocarzinostatin, and maduropeptin have been reported to cleave histones;[7] although there has been some controversy about whether the enediynes, the apoproteins, or protease contaminants are responsible for this behavior.[8]

In contrast, we now report that the action of photogenerated methyl radical on a DNA/histone assembly in air results in dissociation of the biomolecular complex, because of side-chain modifications including the conversion of lysine amines into aldehyde groups (in addition to some expected[9,10] DNA strand scission). This unanticipated outcome differs from that for oxygen-centered radicals, which give primarily DNA/protein crosslinking[11] and biomolecular cleavage or alkylation.[12]

The initial indication of this unusual reactivity occurred upon photolysis of $CpW(CO)_3CH_3$ (**1**, Scheme S1 in Supporting Information) in the presence of a DNA/histone H1 complex and analysis of the results by agarose gel electrophoresis (Figure 1). Compound **1** was chosen because of its well-precedented photochemical production of methyl radical, which was known to cleave DNA with no apparent interference from the metal fragment side product.[9] Thus, irradiation of **1** (12 mM, lane 6) led to dissociation of the biomolecular complex, as indicated by a return of the electrophoretic mobility of the plasmid to that of pure DNA (cf. lane 1). Both light and the organometallic species (lanes 4 and 5) were required for this effect, which was concentration dependent (lanes 6−8). The smearing apparent in lanes 6 and 7 occurs in part as a result of some cleavage of DNA, as demonstrated by the presence of form II DNA in similar samples treated with SDS after photolysis to cause DNA/H1 dissociation (Figure S1).

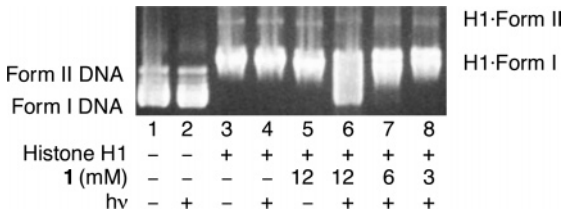

Figure 1. Dissociation of DNA/histone H1 complexes: pUC19 DNA (1 mM/bp) with or without H1 (0.23 mg/mL) in 10% DMSO/20 mM Tris buffer, pH 8. Irradiation for 30 min was accomplished with a 450 W mercury arc lamp through a Pyrex filter. The mobilities of free and H1-associated DNA are shown (left and right, respectively).

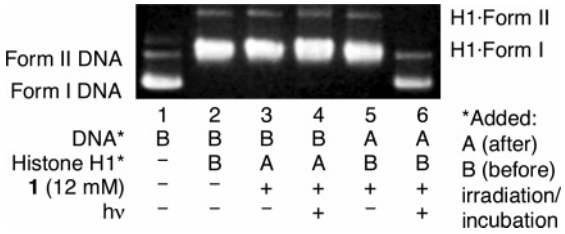

Figure 2. Effects on biomolecular dissociation of the addition of either DNA or H1 after irradiation: pUC19 DNA (1 mM/bp) and/or H1 (0.23 mg/mL) in 10% DMSO/20 mM Tris buffer, pH 8.

Further experiments demonstrated that dissociation of the biomolecular complex was inhibited by the radical trap DMPO (Figure S2, lane 9); and a potential side product of the photolysis of **1**, the $CpW(CO)_3$ metal-centered radical, was generated by the photolysis of $[CpW(CO)_3]_2$ (**2**)[13] but gave only intact DNA/H1 complex (Figure S3 lane 7). Additionally, the removal of O_2 by freeze/pump/thaw cycles prior to irradiation prevented dissociation of the DNA from the protein (Figure S3, lane 3), indicating that O_2 was required for this behavior.

Interestingly, the observed decomplexation results from the direct modification of H1 (Figure 2), as evident in lane 6, in which irradiation of a mixture of **1** and H1 yields protein that does not affect the mobility of subsequently added DNA. In contrast, irradiation of **1** and DNA prior to addition of H1 gives more DNA cleavage (lane 4); but the mobilities of both forms I and II DNA are still retarded by the protein. Lanes 3 and 5 show nonirradiated controls for each of these experiments.

Having established that the observed dissociation was caused by modification of the protein, we next sought to identify the alteration responsible. SDS-PAGE of the DNA/histone reaction mixture (lane 4, Figure S4a) revealed no major changes in H1 size as compared to a commercial sample (lane 1) or to control experiments (lanes 2 and 3), suggesting an intact protein backbone. Therefore, the possibility of side-chain modification was investigated by amino acid composition analysis, which showed a significant loss of the initial lysine content.[14] This result is intriguing in light of the dissociation experiments, because the positively

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10.1021/ja073683s CCC: $37.00

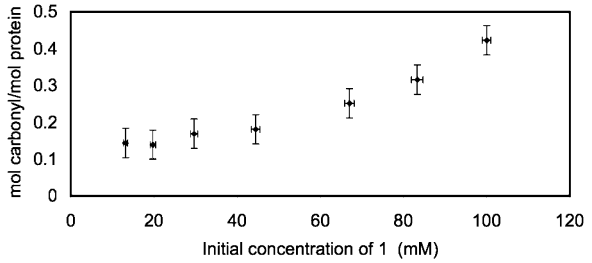
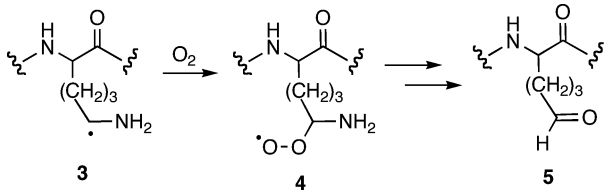


Figure 3. Extent of DNPH incorporation as a function of initial concentration of **1**. Various concentrations of **1** were photolyzed in the presence of H1 (5.1 mg/mL in 10% MeOH/20 mM Tris buffer, pH 8). A nonphotolyzed control containing **1** gave a value of 0.008 mol of carbonyl per mole of protein.

Scheme 1



charged ammonium groups on a fraction of lysine side chains are responsible for the attraction to the negatively charged plasmid DNA backbone in this nonspecific electrostatic supramolecular interaction. The change in charge and/or hydrophobicity of modified H1 was further indicated by a decrease in its electrophoretic mobility (lanes 8 and 9, Figure S4b) versus controls (lanes 5, 6, and 7) in acid/urea/Triton PAGE, which separates proteins by size, charge, and hydrophobicity.[15]

One mechanistic explanation consistent with these observations (Scheme 1) involves hydrogen atom abstraction from a lysine side-chain terminal carbon by methyl or methylperoxyl radical, followed by reaction of **3** with O_2 to give **4**, ultimately resulting in aldehyde formation. The latter steps in this process (**4** to **5**) may occur either via loss of NH_3 and H^+ from a hemiaminal derived from the alkylperoxy radical **4** or by loss of superoxide[16] from **4** to give an iminium ion, which is then hydrolyzed. This overall conversion occurs in minor amounts in reactions initiated by oxygen-centered radicals; and lysine side-chain radicals have been implicated in LDL aggregation, lipid peroxidation, and the microbicidal action of leukocytes.[17]

The presence of aldehyde groups in modified H1 was confirmed by a positive Tollen's test (Figure S5), which was not observed for nonirradiated histone control that had been incubated with **1**. The production of lysine-derived carbonyls was confirmed further by FAB-MS of H1 that was photolyzed with **1**, hydrolyzed, and treated with dinitrophenylhydrazine (DNPH); a peak was observed at *m*/*z* 347.3, for the sodium salt of the hydrazone of the amino acid corresponding to **5**. Attempts to identify specific modified residues by MS of intact or trypsin- or CNBr-digested samples were thwarted by the inability to volatilize the protein samples, even under MALDI conditions that we and others[18] have successfully used for unmodified H1.

The extent of protein carbonylation was determined by ligation to DNPH and spectrophotometric quantification of the resulting hydrazone.[19] The amount of hydrazone, and thus of total carbonyl formed across all residues, is dependent on the initial concentration of **1**(Figure 3). A linear relationship cannot be positively confirmed within the narrow concentration range possible for these experiments. Interestingly, carbonyl production is observed at **1**/protein ratios that are ten times lower than is required for H1 dissociation from DNA.

Further support for the involvement of methyl radical but not Cp-tungsten side products was the observation of DNA-H1 dissociation upon treatment with another methyl radical source, Co^{III}-(cyclam)$(H_2O)CH_3^{2+}$ (Figure S6).[10] Also consistent with the proposed reactivity are the results from similar experiments with polylysine/DNA complexes (Figure S7), in which photolysis of **1** in the presence of this protein/nucleic acid assembly also led to dissociation of the protein from the DNA. Controls for both sets of experiments again indicated that both light and the methyl radical source were necessary for the observed activity.

In summary, methyl radical production leads to the modification of lysine and other side chains of histone H1 in a concentration- and oxygen-dependent manner to cause protein-DNA dissociation, suggesting an additional mechanism by which carbon-centered radicals exert their biological activity. Considering the key roles that both histone charge and the level of chromatin condensation play in regulating gene expression, such protein modifications that lead to protein-DNA dissociation are likely to cause significant cellular effects.

Supporting Information Available: Experimental procedures, data, and figures showing results of control experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA073683S