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ELECTROSPRAY IONIZATION MASS SPECTROMETRY FOR NATURAL AND RADIATION-INDUCED MODIFICATIONS IN HISTONE PROTEINS

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The nucleus of the cell is densely packed with DNA and numerous proteins which, at nuclear division, arrange themselves in distinct structures, called chromosomes. A basic subunit of this chromatin structure is the nucleosome consisting of 146 base pairs of DNA wrapped around a core histone octamer. The nucleosome consists of core histones that are lysine and arginine rich proteins which participate in formation of the reversible complex with DNA.

Exposure of nucleosomes to free radicals generated by ionizing radiation results in a number of modifications inducing altered base and deoxyribose moieties and DNA protein cross-links. Acid hydrolysis of irradiated nucleosomes followed by trimethylsilylation and GC/MS has revealed the presence of DNA base-amino acids dimers between thymine and aliphatic (Gly, Ala, Val, Leu, Ile and Thr) (E. Gajewski, A. F. Fuciarelli, and M. Dizdaroglu, Int. J. Radiat. Biol. *54*, **1988**, 445-459), basic (Lys) (Dizdaroglu and Gajewski, Cancer Res. *49*, **1989**, 3463-3467) and aromatic (Tyr) (M. Dizdaroglu, D. Gajewski, P. Reddy, and S. A. Margolis, Biochemistry, *28*, **1989**, 3625-3628,) amino acid residues, but failed to reveal acid-labile species. Additionally, information on the location of these cross-links relative to the amino acid sequence was lost. We have sought to extend these studies employing electrospray ionization mass spectrometry (ESI-MS) of the intact species.

In preliminary experiments, check erythrocyte histone H2B was irradiated in the presence of thymine, the principle cross-linking base recognized in earlier studies, and the products were examined directly by ESI-MS. Following exposure to 5 Gy of ionizing radiation the relative abundance of two unique species were increased by nearly 50% in irradiated samples over background response at the same *m/z*. The first corresponds to a mass increment increase similar to the expected value for thymine-H2B adduct formation (126.1 Da measured, 125.1 Da calculated). The mass increment increase for the second component (140.7 Da) was less easily explained. Additional dose-yield data are needed to confirm the significance of these changes. Further experiments on the intact proteins and on the products of specific proteolysis are being undertaken to confirm and further elucidate the structures of these radiation induced modifications.

The nature and function of naturally occurring covalent post-translational modifications of histones which include acetylation, methylation, phosphorylation, mono- and poly(ADP)ribosylation and ubiquitination is the object of other ESI-MS experiments. Each of these frequently reversible modifications is known to have important associations with alternations in chromatin structure that accompany induced damage and its repair. It is our belief that the temporal and spatial patterns of these modifications, although complex, is distinctly nonrandom and predictably related to cellular responses. We have begun preliminary evaluation of the nature and extent of histone modification by ESI-MS. Using chick erythrocyte histones we observed a number of modifications which are consistent with the known post-translational modifications of histone (Figure 1). Included among these are acetylation, methylation and an array of various combinations of modifying species. Ongoing experiments are being conducted to further define the nature of these modifications, as well as to attempt to understand the relationship between these modifications and cellular behavior.

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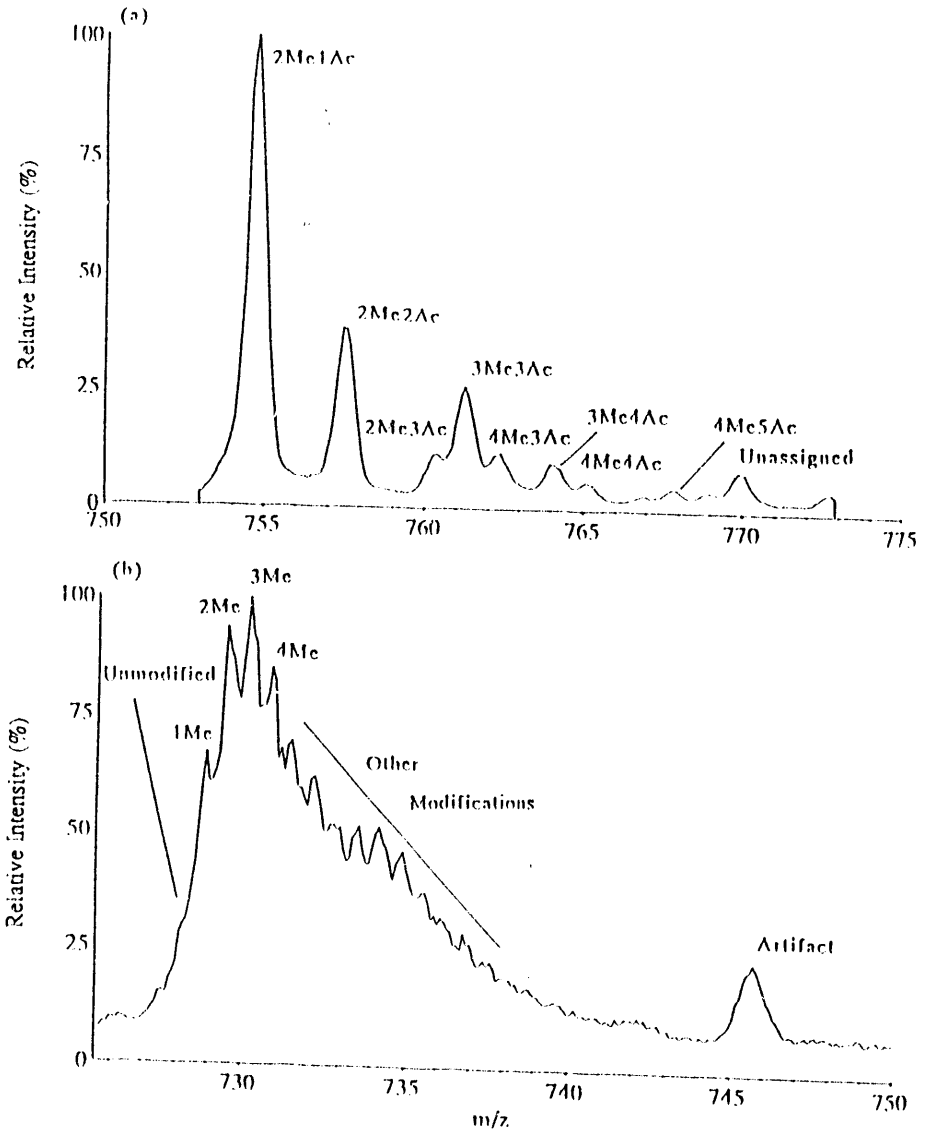

Figure 1. Electrospray ionization mass spectral profiles for the multiply protonated molecular ions of (a) chick erythrocyte histone H3, $(M+15H)^{15+}$, and (b) histone H4, $(M+21H)^{21+}$. Post-translational modifications are infered from differences in measured masses and the mass predicted from the protein gene sequence. Putative assignments are marked (Me = methylation, Ac = acetylation). Where nearly isobaric multiple modifications are possible the least multiplicity of modification is proposed.

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