ISOLATION AND CHARACTERIZATION OF STABLE PROTEIN–DNA ADDUCTS INDUCED IN CHROMATIN BY ULTRAVIOLET LIGHT

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ABSTRACT

The induction of protein–DNA adducts mediated by ultraviolet (uv) light was analyzed in two forms of chromatin isolated from cultured Chinese hamster cells: sheared, soluble chromatin, and defined, chromatin subunits (nucleosomes). Four methods of analysis were employed to quantify and qualify this photochemical reaction of stabilizing protein to DNA: (1) a membrane filter assay which retains both protein and protein–DNA complexes; (2) CsCl equilibrium density gradients in which stable complexes of protein and DNA band at densities other than their native buoyant densities; (3) gel filtration which allows separation of protein linked to DNA from the bulk, nonlinked protein; and (4) SDS-polyacrylamide gel electrophoretic analysis of the chromatin proteins. The following observations have been made: (1) the rate of linkage of protein-to-DNA is linear with uv light fluence in both forms of chromatin studied; (2) in sheared, soluble chromatin, the rate of linkage of nonhistone proteins exceeds the rate of linkage of histone proteins by two-fold (mass:mass); (3) in sheared, soluble chromatin, factors which increase condensation of chromatin (divalent metal ions, ionic strength, pH) enhance the photochemical addition reaction; (4) in both forms of chromatin, it appears as if all four core histones (H2A, H2B, H3, and H4) participate (to varying degrees) in the uv light-induced crosslinking reaction; and (5) uv light of 254-nm wavelength induces other photoproducts (protein–protein adducts and/or aggregates) besides protein–DNA adducts.
INTRODUCTION

Recent concerns about the potential depleting effects of certain technological waste products on the upper stratospheric ozone layer which acts as a protective shield against incoming, solar-originating, short wavelength, ultraviolet (uv) radiations have stimulated resurgent interests on the effects of uv light on biological systems. It is known that uv light can induce a variety of photoproducts in cells, including the classic and well-documented pyrimidine dimer in nucleic acids. Another rather unique photoproduct discovered simultaneously in uv-irradiated bacteria and mammalian cells is the protein-DNA adduct or complexes of protein and DNA stabilized evidently by a covalent linkage photochemically induced by the radiation (Alexander and Moroson, 1962; Smith, 1962). Although under certain conditions there exists a correlation between the occurrence of uv light-induced protein-DNA adducts and cell lethality in bacteria and mammalian cells (Ashwood-Smith et al., 1965; Smith and O'Leary, 1967; Habazin and Han, 1970; Han et al., 1975), the sublethal biological effects of this kind of photoproduct are not understood.

Several studies have been reported in an attempt to define the photochemical reaction involved in uv light-induced protein-DNA adducts. Various groups have reported the covalent linkage of various amino acids to DNA bases, to synthetic polynucleotides, and to native DNA (Smith and Aplin, 1966; Smith and Meun, 1968; Varghese, 1973; Schott and Shetlar, 1974). Furthermore, it has been shown that a variety of protein molecules which interact with nucleic acids can be photochemically induced to form covalent linkages with nucleic acids, including DNA polymerase (Markovitz, 1972), RNA polymerase (Strniste and Smith, 1974), aminoacyl tRNA synthetases (Schoemaker and Schimmel, 1974), ribosomal proteins (Gorelic, 1975a,b; Baca and Bodley, 1976), and chromatin proteins (Strniste and Rall, 1976a; Todd and Han, 1976). In some of these studies, uv light was used as a stabilizing agent to fix protein to DNA in order to determine the structural relationships or interacting sites of unique proteins and their nucleic acid counterparts.

The DNA of eucaryotic cells is heterogeneously complexed as chromatin with a variety of basic (histone) and acidic (nonhistone) proteins. Since protein-DNA adducts have been observed in uv-irradiated mammalian cells, we undertook this investigation to determine the role of chromosomal proteins in this particular photochemical process. In this report, we review some of our recent published studies concerning uv light-induced protein-DNA adducts in sheared, soluble chromatin (see Strniste and Rall, 1976a) and present some recent experimental findings concerning adduct formation in uv-irradiated, defined chromatin subunits or nucleosomes (for a detailed analysis and characterization of Chinese hamster cell nucleosomes, see Strniste et al., 1976b). In addition to