Available online at www.sciencedirect.com
SCIENCE DIRECT®
Radiation Physics and Chemistry 72 (2005) 265–270
Radiation Physics and Chemistry
www.elsevier.com/locate/radphyschem

Radiolysis of DNA–protein complexes

Marie Bĕgusová[a,*], Nathalie Gillard[b], Denise Sy[b], Bertrand Castaing[b], Michel Charlier[b], Melanie Spotheim-Maurizot[b]

[a]*Department of Radiation Dosimetry, Nuclear Physics Institute, Na Truhlářce 39/64, CZ-18086, Praha 8, Czech Republic*
[b]*Centre de Biophysique Moléculaire, CNRS, rue Charles-Sadron, F-45071 Orléans Cedex 2, France*

Received 16 October 2003; accepted 26 January 2004

Abstract

We discuss here modifications of DNA and protein radiolysis due to the interaction of these two partners in specific complexes. Experimental patterns of frank strand breaks (FSB) and alkali revealed breaks (ARB) obtained for DNA *lac* operator bound to the *lac* repressor and for a DNA containing an abasic site analog bound to the formamidopyrimidine-DNA glycosylase are reported. Experimental data are compared to predicted damage distribution obtained using the theoretical model RADACK.

Keywords: DNA radiolysis; Protein radiolysis; *lac* operator–*lac* repressor complex; Fpg protein; Abasic site

1. Introduction

Ionizing radiation induces DNA lesions, such as strand breaks, abasic sites, sugar and base modifications, in aerated solution mainly through the attack by $^{\bullet}$OH radicals produced *via* water radiolysis (von Sonntag, 1987; Cadet et al., 1997). The contribution of other radiolytic species such as hydrated electrons and hydrogen radicals to DNA damage can be neglected due to their fast reaction with oxygen (Ferradini and Pucheault, 1983). The strand breaks occurring at each nucleotide site along the DNA base sequence, experimentally observed by sequencing polyacrylamide gel electrophoresis at neutral pH, are called frank strand breaks (FSB). Some other DNA lesions can be revealed as strand breaks upon an alkaline treatment with hot piperidine. These lesions are called alkali revealed breaks (ARB) and they can be evaluated by subtracting from the breakage pattern of a DNA that underwent an alkaline treatment (FSB + ARB pattern) the breakage pattern of the same DNA without treatment (FSB pattern). The probability of DNA damage induction depends on DNA base sequence, on the local and global structure and on the presence of interacting ligands (Sy et al., 1997; Balasubramanian et al., 1998; Bĕgusová et al., 2001b). For instance, specifically bound proteins protect DNA against radiation induced damage at the binding sites. Radiolytic footprinting can thus be used as a method for studying specific DNA–protein interactions (Franchet-Beuzit et al., 1993; Charlier and Spotheim-Maurizot, 2000).

The present paper discusses results of experimental radiolytic footprinting measurements and theoretical predictions of radiolysis of partners within two prokaryotic DNA–protein complexes used as models of specific DNA–protein complexes in general. The first studied DNA–protein complex is the *lac* operator–*lac* repressor complex. The *lac* repressor is a tetrameric protein

*Corresponding author. Tel.: +420 283 842 791; fax: +420 283 842 788.
E-mail address: begusova@ujf.cas.cz (M. Bĕgusová).

0969-806X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.radphyschem.2004.01.009

formed by four identical 360 amino-acids (aa) protomers. There are two domains *per* protomer: the 60 aa N-terminal part, called the headpiece and the 300 aa C-terminal part, belonging to the tetrameric core. The specific protein–DNA interaction occurs via the binding of two headpieces to one operator DNA (about 25 base pairs (bp) long) leading to operator bending. The *Escherichia Coli* lactose operon to which such complexes participate is a system of gene expression and regulation whose function is well understood. Therefore different research groups have made efforts to solve the molecular structure of the *lac* repressor–*lac* operator complex. There exist several crystallography and NMR based models of natural or mutated repressor (Lewis et al., 1996; Spronk et al., 1999; Bell and Lewis, 2001; Kalodimos et al., 2001, 2002).

The second studied system is a complex between formamidopyrimidine-DNA glycosylase (Fpg protein) and a DNA oligomer containing one abasic site analogue. The bacterial Fpg protein is one of the enzymes initiating the base excision repair pathway. It removes damaged bases such as 8-oxoguanine (8-oxoG) and imidazole ring opened purines (Fapy residues) and further excise the resulting abasic site (Chetsanga and Lindahl, 1979; O'Connor and Laval 1989; Tchou et al., 1991; Graves et al., 1992). In order to study the Fpg catalytic mechanism, abortive complexes between the protein and substrates are investigated. Such substrates are analogues of an abasic site, the only high-affinity ligands for the enzyme, but are not cleaved by it. The Fpg protein requires for binding 5–6 nucleotides in the vicinity of the DNA damage and only the base opposite the lesion on the other strand (Castaing et al., 1999). Recently, the 2.5 Å resolution crystal structure of a non-covalent complex between Fpg and the DNA duplex containing 1,3-propanediol site (Pr), a high-affinity apurinic site analogue, has been published (Serre et al., 2002). Fpg binding triggers a strong bending of the DNA towards the major groove at the Pr site and thus alters the conformational parameters with respect to those of a B-DNA.

The radiation-induced breakage patterns for both DNA–protein complexes have been determined using the method of sequencing polyacrylamide gel electrophoresis. These experimental breakage profiles were then compared to the FSB and ARB probabilities calculated using the stochastic model RADACK (Bĕgusová et al., 2001b).

2. Materials and methods

2.1. Experimental

The 80 bp DNA fragment bearing the *lac* operator sequence has been prepared from a pOP203 plasmid as previously described (Franchet-Beuzit et al., 1993). The protein was prepared from the overproducing strain BMH 493 of *E. coli* (Culard and Maurizot, 1981). The *lac* operator–*lac* repressor complex was formed by incubation of the DNA fragment with the protein ([Rep]/[DNA] = 16) in 100 mM phospate buffer for 20 min at 0°C. The irradiation of samples was performed using a ^{137}Cs source of γ-rays (IBL 437, CISBio International). The irradiation dose of samples was 200 Gy for FSB and 150 Gy for FSB + ARB measurement, respectively.

The radiolytic breakage pattern due to the specific interaction with the Fpg protein was studied using a 59 bp oligonucleotide containing a 1,3-propanediol (Pr) abasic site analog in the central position, and a mutated *Lactoccocus lactis* Fpg (Castaing et al., 1999; Serre et al., 2002). The DNA oligomer was incubated with Fpg protein ([Fpg]/[DNA] = 50) in 10 mM KCl, 25 mM phosphate buffer for 15 min at room temperature. The samples were then irradiated with 150 Gy of γ-rays.

The DNA fragments were labeled at 5′-termini with ^{32}P, which allowed the analysis of samples by polyacrylamide sequencing gel electrophoresis (for the method, see, for example: Franchet-Beuzit et al., 1993; Eon et al., 2001).

2.2. Theoretical calculations

2.2.1. Molecular modeling

In our previous detailed studies (Bĕgusová et al., 2001a, 2003) on the radiolysis of *lac* repressor–*lac* operator complex, we have found the best agreement between experimental and calculated FSB patterns for a molecular structure deduced from crystallographic data of Bell and Lewis (2001) (PDB code 1JWL). We have added to that structure the missing side chains using the molecular modeling software SYBYL 6.4 (1998, TRIPOS, St. Louis, Missouri, USA). The position of the side chains was chosen according to the data of Spronk et al. (1999) (PDB code 1CJG). The molecular structure of the two *lac* repressor headpieces specifically bound to *lac* operator DNA was subsequently energy-minimized by the Powell's method (Powell, 1977). The energy calculations were performed with the AMBER force field using Kollman's charges with a cut-off distance of 10 Å. The solvent effect was implicitly considered by introducing a distance-dependent dielectric constant.

The molecular structure of the complex between the Fpg protein and a 13 bp DNA duplex containing a Pr abasic site analogue on one strand, derived from crystallography data, is available in the PDB databank [PDB code 1NNJ, Serre et al. (2002)]. Four lacking amino acids and all hydrogen atoms were added into the structure using the software SYBYL 6.4 and the energy minimization of the structure was performed as in the case of the *lac* repressor–*lac* operator complex.