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The DNA repair enzyme, CPD-photolyase restores the infectivity of UV-damaged fowlpox virus isolated from infected scabs of chickens

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Abstract

Fowlpox virus (FWPV), an important pathogen of poultry, replicates very efficiently in the featherless areas of skin, and persists in dried and desiccated scabs for prolonged periods. Although the molecular mechanisms underlying the stability of the virus are not completely known, we recently identified the presence of a virus-encoded novel DNA repair enzyme, CPD-photolyase, in FWPV. This enzyme repairs the ultraviolet (UV)-induced pyrimidine dimers, converting them to monomers using photons from white light as a renewable source of energy. In this study, we examined the role of photolyase in the pathogenesis of fowlpox. A comparison of pathogenesis of fowlpox in chickens infected with parental FWPV with that in chickens infected with photolyase-deficient FWPV (Phr^- FWPV) found no significant differences in terms of replication of virus or formation of secondary lesions. When the virions isolated from infected scabs were exposed to UV light, UV-damaged parental FWPV, unlike Phr^- FWPV, were rescued through the CPD-photolyase-mediated photoreactivation pathway by at least 48%. However, the mutant virus triggered host's immune response and conferred complete protection against subsequent challenge with virus similar to that conferred by the parental virus. Since the mutant virus is less stable than the parental virus in the infected scabs but is as immunogenic, Phr^- FWPV might be less persistent in the environment. Furthermore, this particular genetic locus can also be used to insert foreign genes for the development of FWPV recombinant vaccines.

Keywords: Photolyase; Fowlpox virus; Pathogenesis; Recombinant vaccines; Persistence; Virus stability

1. Introduction

A successful infection depends on three main factors, the etiologic agent, the susceptibility of the host and the environment, of which the role of environment is the least understood. Although the environment is an important source of infection, it also

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has the potential to damage pathogens. Organisms have evolved numerous strategies to counteract or protect against environmentally induced ill effects (Dulbecco, 1949; Nakabeppu and Sekiguchi, 1981; Martin et al., 1975). Large DNA viruses such as poxviruses, which are known to encode for numerous proteins in successful disease outbreaks, are no exception (Kotwal, 2000; Mahalingam and Karupiah, 2000). The gene repertoire of poxviruses, especially fowlpox virus (FWPV), a prototype member of genus *avipox* (Tripathy and Reed, 2003) in the development of their pathogenicity is well known (Afonso et al., 2000). Although the production of virus-encoded proteins as a means of modulating the host's immune response provides a favorable milieu for the virus within the host, the stability of FWPV in a hostile environment might be considered as another way by which the virus is perpetuated (Woods et al., 1992; Bergion and Dales, 1971).

Among the environmental insults encountered by various organisms is exposure to ultraviolet (UV) light either from sunlight or natural light UV irradiation is detrimental to all organisms, as it damages DNA by inducing cis–cyn pyrimidine (CPD) dimers and some (6–4) photo adducts (Taylor and Nadji, 1991; Taylor, 1994). To overcome this environmentally induced ill effect, organisms have evolved a variety of DNA repair mechanisms (Yu et al., 1999; Lloyd and Van Houten, 1995). The resilient FWPV that persist in the dried scabs and in the contaminated environment acts as a source of infection and threat to immunologically naïve chickens (Tripathy and Schnitzlein, 1999; Tripathy et al., 1974). Although the factors involved in the protection of the virus from environmental hazards are not well understood, we have identified a novel DNA repair enzyme in fowlpox virus, CPD-photolyase, that protects the viral DNA from UV-induced DNA damage (Srinivasan et al., 2001). CPD-photolyase is a unique DNA repair unienzyme that restores UV-induced pyrimidine dimers into monomers in the presence of white light (Hearst, 1995; Sancar, 1994). The chromophore bound to photolyase, absorbs photons from the white light at wavelengths of 300–500 nm. The excited energy is then transferred to a bound cofactor, which in turn transfers an electron to the pyrimidine–pyrimidine dimer. When the electron is transferred to the DNA lesion, the dimer is split into a monomer. The oxidized chromophore is converted back to reduced state by accepting an electron from the DNA lesion (Sancar, 1996; Carell et al., 2001). This light-dependent DNA repair activity catalyzed by CPD-photolyase is called photoreactivation (Hearst, 1995). Our initial studies with this novel DNA repair enzyme showed that the presence of CPD-photolyase in the virions confers a selective advantage for the virus, enabling it to remain viable even in the presence of UV light (Srinivasan et al., 2001).

In this study, we report the role of CPD-photolyase in the pathogenesis of fowlpox. We found that CPD-photolyase does not play a significant role in FWPV replication or in the formation of secondary lesions in the infected chickens. However, when photolyase deficient FWPV (Phr^- FWPV) isolated from infected scabs was exposed to UV light, infectivity was significantly reduced. We also determined that Phr^- FWPV and wild-type FWPV are equally immunogenic in protecting against fowlpox.

2. Materials and methods

2.1. Cells and viruses

FWPV, isolated from a naturally infected chicken at the University of Illinois poultry farm (Tripathy et al., 1974), was initially propagated on the chorioallantoic membranes of 9–11-day-old developing embryos obtained from a specific-pathogen-free chicken flock (Tripathy and Reed, 2003). Subsequently, the virus was passaged in QT-35 cells (Schnitzlein et al., 1988). We used this cell culture-adapted virus for our experimental studies. Phr^- FWPV was generated in our previous study by inserting a lacZ gene cassette at the photolyase gene locus by homologous recombination (Srinivasan et al., 2001). Both the parental and Phr^- FWPV were plaque purified and underwent five passages in cell culture.

2.2. Experimental chicken infection studies

Seventy 7-week-old specific pathogen-free chickens obtained from SPAFAS (North Franklin, CT) were randomly divided into seven groups of 10 chickens each. On day 1, chickens in each group except the control group were inoculated in the left wing by the wing-web method (Tripathy and Reed, 2003) with 1×10^4, 1×10^5 or 1×10^6 PFU of either parental or Phr^- FWPV virus. The