Functional Characterization of Partially Purified Epstein-Barr Virus DNA Polymerase Expressed in the Baculovirus System

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Abstract. The DNA polymerase gene of Epstein-Barr virus (EBV) was cloned into baculovirus transfer vector (pBlueBac). The recombinant baculovirus (AcEBP-15) was obtained by cotransfection of Spodoptera frugiperda (Sf9) cells with infectious DNA from Autographa californica multiple nuclear polyhedrin virus (AcMNPV) and pBlueBac plasmid carrying EBV polymerase gene. Infection of Sf9 cells with the recombinant virus produced substantial quantities of the EBV DNA polymerase protein of the expected size (110 kD). The identity of the EBV polymerase 110-kD polypeptide was determined by (a) immunoprecipitation and Western blot analyses with rabbit polyclonal antiserum specific for a synthetic peptide derived from the coding sequence of the polymerase gene; (b) identification of a polypeptide of identical size (110 kD) from EBV-infected cells; (c) measurement of DNA polymerase activity similar to that of the enzyme induced in EBV-infected cells; and (d) neutralization of the enzymatic activity by the rabbit antiserum and inhibition by phosphonoacetic acid. Our results indicate that the baculovirus expression system provides large quantities of functional polymerase suitable for biochemical and structural analyses, thereby furthering our understanding of the mechanism of viral DNA replication and its inhibition by antiviral drugs.

Key words: Epstein-Barr virus, DNA polymerase, expression in baculovirus

Introduction

The Epstein-Barr virus (EBV) DNA polymerase (pol) is inducible and is expressed in replicative, but not in latently infected, cells (1). This gene is important for deciphering the mechanisms of virus latency and reactivation. Studies on the regulation and function of the EBV pol gene can provide insight into the rational design of antiviral drugs for chemotherapy and understanding the mechanism underlying the control of EBV DNA synthesis and viral replication.

The initial assignment of the open reading frame (ORF) for EBV pol was based on DNA sequence comparison with the coding sequences for the herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV) DNA pol genes (2,3), and on the amino acid composition of partially purified EBV DNA pol (4). It is now established as the fifth leftward open reading frame in the BamHI A fragment of the EBV genome (BALF5) (5). We recently showed that BALF5 encodes a functional DNA pol by expression of the gene in vitro and translation in rabbit-reticulocyte lysates. The translated protein was enzymatically active in DNA polymerase assays (5). However, the activity of the in vitro translated enzyme was not stimulated by high concentrations of salt, as was the viral enzyme isolated from EBV-infected cells (5). In ad-
dition, the low levels of active enzyme expressed in that system have hampered further mechanistic studies. In this study we have used another approach to obtain large quantities of the enzyme by subcloning the EBV DNA pol gene into baculovirus transfer vectors. Subsequent selection of recombinant baculoviruses resulted in high-level expression of EBV pol protein. The expressed protein possesses elevated levels of DNA polymerase activity, similar to the levels of activity in EBV-infected cells, and is sensitive to antiviral drugs.

Materials and Methods

Cells and Viruses

The wild-type Autographa californica multiple nuclear polyhedrosis virus (AcMNPV) and the transfer vector (pBlueBac) were obtained from Invitrogen (San Diego, CA) and maintained according to the manufacturer’s protocol. The culture of Spodoptera frugiperda (Sf9) cells and the propagation of AcMNPV and recombinant baculoviruses were as described (6).

Strategy for Construction of Baculovirus Recombinant

The transfer vector pBlueBac contains twin promoters, the ETL promoter, which directs the synthesis of β-galactosidase, and the baculovirus polyhedrin promoter, which controls the synthesis of foreign gene products. To facilitate the insertion of the EBV pol gene at the Nhel cloning site in pBlueBac, we designed a primer pair (EBP5/EBP3) flanking Nhel linkers and the GAG sequence (Fig. 1) for amplification of the pol gene by polymerase chain reaction (PCR). This strategy enabled the PCR-amplified product to be inserted at the Nhel cloning site in pBlueBac. The 5’ primer (EBP5) (positions 156,757–156,738) includes only 11 nucleotides upstream of the first in-frame ATG, which has been implicated as the authentic initiation codon of pol message in vivo (5,7).

The full length (3.15 kbp) of pol DNA was amplified by PCR from pGEM3ZPOL2 (5) using primer pair EBP5/EBP3 and partially digested with Nhel restriction enzyme. The full-length fragment was then isolated and cloned into Nhel-cleaved pBlueBac vector to yield pBEP-12, in which the EBV pol ORF was properly oriented downstream of the baculovirus polyhedrin promoter. EBPRO was used as a probe to screen the positive recombinants.

![Fig. 1. Construction of pBEP-12.](image-url)