Mengo Virus 3C Proteinase: Recombinant Expression, Intergenus Substrate Cleavage and Localization in vivo

DAVID J. HALL & ANN C. PALMENBERG

Institute for Molecular Virology and Department of Animal Health & Biomedical Sciences, University of Wisconsin-Madison, 1655 Linden Drive, Madison, Wisconsin 53706

Received March 1, 1996; accepted May 14, 1996

Abstract. Mengo virus 3C proteinase was cloned and expressed to high levels in a bacterial vector system. The protein was solubilized from inclusion bodies then purified to homogeneity (>95%) by ion exchange chromatography. The recombinant enzyme was proteolytically active in cell-free processing assays with a Mengo capsid precursor substrate, L-P1-2A, correctly and proficiently cleaving it into L, 1AB, 1C, 1D and 2A protein products. Further analyses with synthetic peptide substrates encompassing the Mengo or rhinovirus-14 2C/3A cleavage sequences, showed the Mengo 3C could recognize and process specific glutamine-glycine sites within these peptides. The reactivity with the rhinovirus peptide was unexpected, because cross-reactivity between a picornavirus 3C enzyme and a protein substrate from different genus of this family has otherwise never been observed. In reciprocal reactions, a rhinovirus-14 3C preparation was unable to cleave the Mengo-derived synthetic peptide substrate. The recombinant Mengo 3C reactions were also characterized with regard to substrate Km, optimum pH and temperature. The protein was additionally used to raise monoclonal antibodies (mAbs) in mice, which in turn localized natural 3C, 3ABC, 3CD and P3 in immunoblots, immunoprecipitations and indirect immunofluorescence assays of Mengo-infected HeLa cells. The monoclonals showed cross-reactivity with 3C and 3C-containing precursors from encephalomyocarditis virus (EMCV), but did not react with 3C proteins from rhinovirus-14 or poliovirus-1M.

Key words: protease, picornavirus, enzyme purification, cardiovirus

Introduction

The RNA genome of picornaviruses encodes a large polyprotein which is proteolytically processed in co-translational and post-translational reactions by catalytic centers encoded within the polyprotein. The majority of these cleavages are catalyzed by the 3C proteinase, an unusual enzymatic sequence located in the carboxyl third of the polyprotein. The 3C protein takes a conformation similar to serine protease chymotrypsin, but the reactive nucleophile is cysteine rather than serine (1,2). During translation, the 3C sequence is synthesized in an enzymatically active form, and even while still part of the polyprotein, it rapidly catalyzes an ordered series of mono- and bi-molecular scissions within the parental sequence to release mature viral proteins and precursor combinations (3,4). Each released fragment is presumed to contribute in a vital way to the viral replication cycle, and thus, 3C and its activities are key to understanding the picornavirus infectious process.

Among the characteristics of this viral enzyme is a strong preference for its own polyprotein substrates rather than for exogenous cellular proteins (5). The consensus cleavage sites in most viral polyproteins are at Gln-Gly dipeptide pairs, but not all conforming viral sequences are cleaved, and in many viral strains, alternative combinations like Gln-Ser, Gln-Ala, Glu-Gly or Glu-Ser, occur at the natural sites (Fig. 1). Ex-
Experiments with partially purified 3C enzymes and synthetic or mutated substrates, show an even broader substrate repertoire than is evident from natural sequences. With cardioviruses like encephalomyocarditis (EMCV) and Mengo, the 3C enzymes will cleave Gln-Cys and Gln-Ala substitutions engineered into normal Gln-Gly or Gln-Ser polyprotein sites (6,7).

The origin of this specificity is unclear. The atomic structures of recombinant 3C from rhinovirus-14 (HRV14) and hepatitis A virus (HAV) show shallow elongated substrate pockets that could easily accommodate 8 amino acids (+P4 to −P4) (8,9). The models suggest that Gln in the P1 position and Ala at the +P4 position may convey substrate specificity, especially for