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Purification and partial characte rization of coxsakievi rus B3 2A protease expressed in *Escherichia coli*

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ABSTRACT

Reported here is the overexpression, purifica tion a nd partial cha racteriza tion of recom binan t coxsa kievirus 83 2A protease (CVB3 2AP'°) from bacterial cells transformed with a plasmid con tai ni ng the CVB3 2AP'° cDNA seq uences. The structural investiga tion showed tha t the protein contai ns mostly [3-stra nd elements a nd requ i res Zn^{2+} ions as a structu ral com ponent which a ppea red to be in hi bi tory i fadded exogenously.The pu rified enzyme activi ty was opti mal a t 4 C a nd had a short half-life at physi-ological tem peratu re. This featu re ca n be the resul t of the presence of a high con ten t of [3-structu re a nd also hyd rophobic resid ues i n i ts structu re.

1. Introd uction

Coxsackievi rus 83 (CVB3) is considered as the most i m porta nt i nfectious agent i nvolved i n vi ral hea rt muscle disease [1]. CVB3 may i nd uce acu te a nd ch ron ic forms of h u ma n myoca rdi tis. which ca n be a l i fe-th rea teni ng d isease wi th adverse acute a nd long-term cl i n ical ou tcome [2]. I n addi tion, persisti ng CVB3 genomes may lead to d ila ted card iomyopathy [3,4]. Besides i ts clinically impor- ta nt ad verse effects on the hea rt, CVB3 has also been impl icated as an infectious agent involved i n the pathogenesis of extracardiac d iseases such as hepa ti tis [5]. pancrea titis [6], and aseptic menin- gi tis [7]. I t has been shown tha t acute as well as persisti ng CVB3 i n fection of ca rd iac cells ca uses a direct cytopa thic effect i n both cu l tu red h u ma n myoca rd ial fibroblasts [8] and myocytes [9] and i n va rious a n i mal models [4,5,10]. I n the i ntact myocard i u m, cleavage of dyst roph i n by coxsackievi rus 2A protease (2AP'°) is considered a n i m porta n t mecha nism for CVB3-i nd uced card iac i nju ry [11]. Add i tiona ll y, there is evidence for CVB3-med ia ted apoptotic cell d ea t h. On entry i nto the cell, coxsackievi rus releases i ts messenger- li ke pl us st ra nded RNA, wh i ch is subseq uen tly tra nslated i nto a monocistronic polyprotei n. The fi rst step i n subsequent poly pro-tei n processi ng is cleavage by the viral 2AP'° [12]. This protease is also involved i n cytopa t hic processes, media ted by either cleavage of dystrophi n and /or shu toff of host cell tra nslational mecha- nisms [11,13]. Th us, coxsackieviral 2AP'° comprises an i mportant nonstructu ral viral gene essential for matu ration and cytopa thic processes and represents an idea l ta rget for an tiviral chemother-apy. For this aspect, the identi fication of the enzyme's structure serves as an im portant prerequisi te for a ntiviral d rug design.

To approach this goal, it is desirable to pu rify and characterize this enzyme, the purpose tha t has been followed in the present i nvestigation a nd should prove useful for high-th roughput screen-ing of 2AP'° inhi bitors.

2. Materials and methods

2.1. Materials

HeLa and Vero cells were purchased from Na tional Cell Ba n k of Ira n (NCBI ClIS). E. coli *BL-21, BLR (0£3), BLR (DE3) plysS,* pET22 b(+) vector were obtained from Novagen. RPM!, fetal ca lf serum and agarose were from Gibco and isopropa nol ch loroform was from Merck (Germany). Random Hexa mer, Reverse Transcriptase, Tag polymerase, EcoRI, Ndel, T4 Ligase, ampicili n, tetracycli ne, were purchased from Roche, LB., Scha rlau; I<LH (Keyhole Limpet Hemo-cya nin) were from Sigma Chem (USA), polyvi nylidene fl uori de (PVDF) was obtained Fluka; peptides 17CA, 17CV, 20CS and 20Pn

Abbreviations: 2N'°. 2A protease; CD. circular dich roism; CVB3, coxsackievirus B3; DAB. diaminobenzidine; GuHCI, guanidinium hydrochloride; HRP. horse radish pe,oxidase: KLH, Keyhole Li mpet Hemocya nin; PVDF. polyvi nylidene fluoride; SDS, sodium dodecyl sulfate

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