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Inhibition of the aspartic proteinase from HIV-2

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Kinetic constants were determined for the interaction of the HIV-2 aspartic proteinase with a synthetic substrate and a number of inhibitors at several pH values. Acetyl-pepstatin was more effective towards HIV-2 proteinase than the renin inhibitor, H-261; this effect is exactly the opposite from that observed previously for the proteinase from the HIV-1 AIDS virus.

HIV-2 proteinase; Acetyl-pepstatin; Renin inhibitor, H-261; Tight binding inhibitor; HIV-1 proteinase

1. INTRODUCTION

The life cycle of retroviruses including HIV, the pathogen of AIDS, involves processing of precursor gag and pol polyproteins into the mature virion components. The proteolytic enzyme encoded by the virus which facilitates this processing thus becomes a potential target for therapeutic intervention to block HIV infection [1]. The crystal structure of this enzyme from HIV-1 has been solved at medium resolution [2] and molecular modelling of its substrate-binding site [3] together with biochemical studies with various inhibitors [4] have shown that the HIV-1 proteinase has the molecular topography characteristics of an aspartic proteinase.

The genomic organisation of the HIV-2 virus has been determined [5] and the nucleotide sequence of the pol region in which the proteinase is encoded predicts that the proteinase will be homologous to but not identical with the corresponding enzyme from HIV-1. It has been demonstrated that the proteinase from HIV-2 will catalyse the maturation of chimaeric HIV-1/2 polyproteins in an *E. coli* expression system [6] but otherwise, relatively little information is available concerning the enzyme from HIV-2. Since it would clearly be desirable to attempt to develop proteinase inhibitors that would be effective against enzymes from different strains and species of virus, it was thus considered of interest to examine in detail the susceptibility of proteinase from HIV-2 to a variety of available inhibitors of aspartic proteinases.

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Abbreviations and convention: Iva, isovaleryl; Sta, 4-amino-3-hydroxy-6-methylheptanoic acid; HIV, human immunodeficiency virus; the * in the sequence containing Phe*Pro indicates the scissile peptide bond in the substrate

2. MATERIALS AND METHODS

The peptide substrate Tyr-Val-Ser-Gln-Asn-Phe*Pro-Ile-Val-Gln-Asn-Arg was synthesized as described previously [4]. Acetyl-pepstatin, lactoyl-pepstatin and H-261 were generously supplied by Dr K. Oda, Dr T. Aoyagi and Professor M. Szelke as detailed previously [4]. Purified HIV-1 proteinase was kindly provided by Dr Mary Graves (Roche Nutley, USA).

E. coli containing the plasmid p2 PROT L [6] was grown at 25°C and induced for 3 h with IPTG. HIV-2 proteinase was isolated from the soluble fraction obtained by lysing the cells with lysozyme and Tween-20 and purified by ammonium sulphate precipitation followed by chromatography on hydroxyapatite and Sephadex G-75 (a fuller account will be described elsewhere). The proteinase thus obtained migrated as a single band (of apparent M_r 11000) on SDS-polyacrylamide gel elec-

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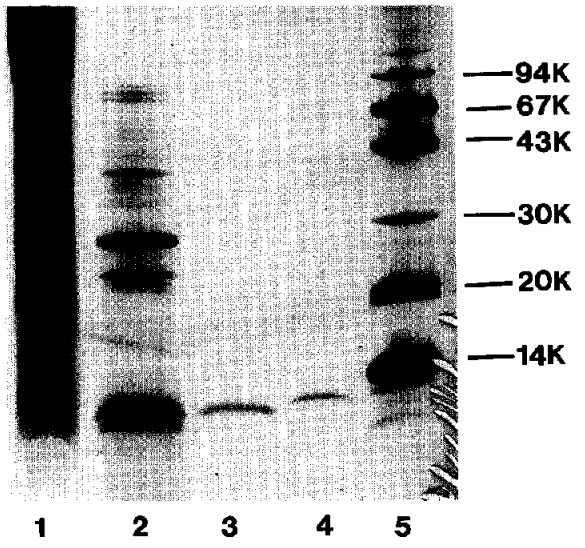

Fig.1. SDS-PAGE electrophoresis. Samples from HIV-2 proteinase purification were applied to a 20% Phast gel. Lanes: (1) extract after ammonium sulphate precipitation; (2) eluate from hydroxyapatite column; (3) G-75 fraction; (4) HIV-1 proteinase; (5) molecular mass markers.

trophoresis (fig.1 – the HIV-2 enzyme appeared slightly smaller than the proteinase from HIV-1, consistent with the sequence data).

Incubation of this purified HIV-2 proteinase with substrate was carried out at a variety of pH values and monitored by reverse-phase FPLC as described previously [4].

3. RESULTS AND DISCUSSION

Kinetic parameters for the hydrolysis of Tyr-Val-Ser-Gln-Asn-Phe*Pro-Ile-Val-Gln-Asn-Arg were determined at several pH values (table 1). Values of k_{cat} were derived from the equation $V_{max} = k_{cat} \cdot [E]$, (it was possible to derive values for [E] by titration with acetyl-pepstatin, see below). As observed previously for HIV-1 proteinase [4], the values of K_m diminished dramatically at pH 7.0, whereas the k_{cat} value appeared to increase (an estimated value is given because saturation of the enzyme with substrate could not readily be achieved at this pH). However, the value of K_m (160 μM) determined for HIV-2 proteinase (at pH 4.7 for example) was approx. 2.5-fold higher than the corresponding value reported previously [4] for the HIV-1 proteinase interaction with this substrate.

Similarly, determination of IC_{50} values for inhibition of HIV-2 enzyme by isovaleryl-pepstatin (= Iva-Val-Val-Sta-Ala-Sta) and lactoyl-pepstatin

Table 1

Kinetic constants for the hydrolysis of Tyr-Val-Ser-Gln-Asn-Phe*Pro-Ile-Val-Gln-Asn-Arg by HIV-2 proteinase at several pH values

pH	K_m (μM)	k_{cat} (s^{-1})
4.7	160	1
6.0	220	1
7.0	~4500	~12

All measurements were carried out at 30°C in buffers consisting of 0.1 M sodium acetate, pH 4.7; 0.02 M Mes, pH 6.0; 0.1 M Mes, pH 7.0, all containing 1 M NaCl (to keep the final ionic strengths the same) plus 4 mM EDTA and 5 mM mercaptoethanol. Samples were removed at 3 appropriate time points and quenched by addition of 5% (v/v) trifluoroacetic acid before analysis by RP-FPLC. The estimated precision of the values given is in the range ± 30% except at pH 7.0

(= lactoyl-Val-Sta-Ala-Sta) under pH 4.7 conditions resulted in values of 150 and 750 nM being obtained, respectively. Again, these are slightly poorer than the corresponding values [4] for HIV-1 proteinase inhibition at pH 4.7 (100 and 400 nM, respectively). The inhibition of HIV-2 enzyme by both compounds was, however, much weaker at pH 7.0 (where IC_{50} values of 1500 and 16000 nM, respectively, were measured).

It was reported previously that the acetyl-derivative of pepstatin (= Ac-Val-Val-Sta-Ala-Sta) was an effective inhibitor of HIV-1 enzyme [4] with a K_i value of 20 nM at pH 4.7 (but of >1000 nM at pH 7.0). Acetyl-pepstatin was an even better inhibitor of HIV-2 proteinase, binding sufficiently tightly for it to be used as an active site titrant with which to determine the active concentration of preparations of HIV-2 proteinase (fig.2). Inhibition constants for the HIV-2 interaction are summarised in table 2.

In contrast, the renin inhibitor H-261 = tBoc-His-Pro-Phe-His-Leuψ[CHOH-CH_2]Val-Ile-His was reported [4] to be a stronger inhibitor than acetyl-pepstatin of HIV-1 proteinase. Conversely, this was found to be a poorer inhibitor (than acetyl-pepstatin) towards HIV-2 enzyme (table 2). However, in complete contrast to the effect found previously with the HIV-1 proteinase, the potency of H-261 towards HIV-2 enzyme was not maintained at pH 7.0. The inhibitory potency of acetyl-pepstatin at pH 7.0 diminished substantially towards HIV-2 enzyme (table 2), as was found previously with HIV-1 proteinase [4].

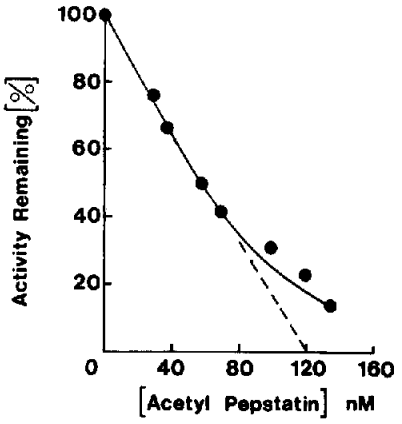

Fig.2. Inhibition of HIV-2 proteinase by acetyl-pepstatin. HIV-2 proteinase was incubated with various concentrations of acetyl-pepstatin at a fixed concentration (127 μM) of substrate peptide in 100 mM sodium acetate buffer, pH 4.7, containing 1 M NaCl, 4 mM EDTA, 5 mM mercaptoethanol at 30°C. Initial rates were determined from analysis of time courses of the reactions.

Table 2

Inhibitory constants for the interaction of acetyl-pepstatin and H-261 with HIV-2 proteinase at several pH values

pH	K_i (nM)	
	Acetyl-pepstatin	H-261
4.7	5	35
6.0	10	90
7.0	750*	>1000*

Reactions were carried out at the three pH values as described in the legend to table 1. The K_i values given at pH 4.7 and 6.0 are the means of at least three separate determinations. The estimated precision for each was in the range ± 30%. At pH 7.0, the value given (*) for acetyl-pepstatin is an IC_{50} determined at a substrate concentration of 127 μM (because of the essentially linear dependence of v with [S] at this pH). Similarly, for H-261 at pH 7.0, only 40% inhibition was observed at a [H-261] = 1 μM under these conditions

Thus, while it is clear that HIV-2 proteinase has a similar topography in its active site cleft to that of the enzyme from HIV-1, nevertheless, small but significant differences in the respective interactions with substrate and inhibitors can be readily distinguished.

From the 3-dimensional structure of the HIV-1 enzyme [2] it was predicted which residues in the enzyme might make contact with residues in individual positions in a substrate or inhibitor [2,3]. Comparison of the HIV-1 and HIV-2 (predicted) amino acid sequences [5] indicates that, with the exception of 3 conservative replacements (two Val → Ile; one Leu → Met), all of these (presumed) contact residues are identical in the two enzymes. Thus, molecular explanation of the interaction of substrates/inhibitors with the active sites of the two enzymes are subject to further considerations, possibly for example in the positioning of the two subunits which comprise the catalytic dimer of the viral proteinases. Subtle changes in specificity may also result from spatial reorientation of the conserved contact residues by changes in adjacent residues in either linear or spatial terms. However, the finding that the enzymes from HIV-1 and HIV-2 are both (relatively) susceptible to inhibition by the acetyl-derivative of pepstatin which contains the acetyl moiety in the P4 position is exactly in keeping with the structural findings that a smaller residue would bind preferentially in the S_4 position [3]. It would seem feasible that a single inhibitor would be effective towards both viral enzymes and that such a potentially therapeutic inhibitor should contain an acetyl moiety in P4 (if indeed such a large inhibitor should be necessary).

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