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Epitope mapping of a monoclonal antibody which binds HIV-1 Gag and not the Gag-derived proteins

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Monoclonal antibody (MAb) 1G12 binds the uncleaved HIV-1 Gag polypeptide (p55), but fails to recognize the final products of the proteolytic processing [Sarubbi, E. et al. (1991) FEBS Lett. 279, 265–269]. In this report we show that binding of MAb 1G12 to a 110-residue Gag fragment containing the p17–p24 cleavage site prevents proteolysis of this site by the HIV-1 protease. Competition studies with synthetic peptides have been performed to map the binding site of MAb 1G12 on Gag. The antibody recognizes a sequential epitope that spans the HIV-1 protease cleavage site; determinants located on both p17 and p24 are required for antibody binding. MAb 1G12 is also shown to lack any cross-reactivity with other HIV-1 protease cleavage sites.

Human immunodeficiency virus; Aspartic protease; Gag polyprotein; Monoclonal antibody; Epitope mapping

1. INTRODUCTION

In the life cycle of the human immunodeficiency virus (HIV) the polypeptidic precursors endoded by the *gag*, *pol* and *env* genes are processed by proteolytic cleavage to yield the mature forms of structural proteins and enzymes [1–4]. Even though the products of the *pol* and *env* genes have thus far attracted most interest as targets for anti-AIDS therapy [5], several groups have focused their attention on the Gag polypeptide. This protein is interesting not only for its ability to form virus-like particles when expressed in different host cells [6–11], but also for the recently reported possibility to use it as a target in a new approach to the chemotherapy of AIDS [12]. For these reasons the mechanism and the cellular compartments in which the virally-encoded aspartic protease (HIV-1 protease) processes the Gag polypeptide are the subject of extensive investigation [13–17]. Any specific reagent that might be used in these studies to discriminate between unprocessed and processed proteins would be extremely interesting.

In a Western Blot analysis of a total HIV-1 lysate, monoclonal antibody 1G12 (MAb 1G12) immunostains the whole Gag precursor (p55), but fails to bind the Gag-derived mature proteins [18]. Also, MAb 1G12 binds the Gag fragment contained in Gal-gag110, a protein fusion in which 110 amino acid residues from HIV-1 Gag were fused to *E. coli* β-galactosidase. This Gag portion contains the p17–p24 cleavage site which is recognized and efficiently cleaved by the HIV-1 protease. After this treatment, however, the fusion protein is not bound by MAb 1G12 any longer [18]. These data suggest that MAb 1G12 recognizes either a conformational epitope destroyed upon cleavage by HIV-1 protease or a contiguous sequence encompassing the p17–p24 cleavage site.

In this study, MAb 1G12 is shown to prevent the HIV-1 protease-mediated cleavage of the p17–p24 site on the Gag precursor. Besides, competition studies with synthetic peptides show that the inhibition of proteolytic cleavage is not due to sterical hindrance, but to overlapping between the antibody epitope and the cleavage site. Similar experiments also provide indications about the specificity of this interaction.

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Abbreviations: HIV-1, human immunodeficiency virus type 1; AIDS, acquired immunodeficiency syndrome; ELISA, enzyme-linked immunosorbent assay; BSA, bovine serum albumin; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

2. MATERIALS AND METHODS

2.1. *Proteins and peptides*

Recombinant HIV-1 protease and Gal-gag110 fusion protein were expressed in *E. coli*, as previously described [18]. MAb 1G12 was produced using Gal-gag110 for immunization and screening, as described [18]. All the peptides used in the competition studies were synthesized and purified by Chiron Mimotopes (Clayton, Australia).

2.2. *HIV-1 protease assays*

For the ELISA-like assay shown in Fig. 1, microtiter wells were coated overnight with 50 μl of MAb 1G12 or anti-β-galactosidase monoclonal antibody (Promega), both as 20 μg/ml in PBS (50 mM sodium phosphate, pH 7.3, 150 mM sodium chloride). Unreacted sites were blocked with 200 μl of 3% bovine serum albumin (BSA) in PBS for 1 h. After washing with PBS, 50 μl of 100 μg/ml Gal-gag110 fusion protein were added and plates were incubated for 2 h at room temperature. Unbound protein was then discarded and plates were washed with PBS. 50 μl of different concentrations of HIV-1 protease in PBS

containing 0.3% BSA were added to each well and plates were incubated for 1 h at room temperature. After washing with PBS, the amount of immobilized and uncleaved Gal-gag110 was determined by adding an anti-p24 polyclonal antibody (1:100 dil.) made in sheep (Biochrom, Germany) and then a peroxidase-labelled anti-sheep secondary antibody (Dakopatts). 1 mg/ml *o*-phenyl-diamine (OPD) was used for the chromogenic reaction.

For the Western blot analysis of Fig. 2, 100 μg/ml of Gal-gag110 were incubated with different concentrations of MAb 1G12 or anti-β-galactosidase monoclonal antibody (Promega) in PBS for 1 h at room temperature. HIV-1 protease was added at a final concentration of 0.5 μg/ml and samples were incubated for 30 min at 37°C. SDS-PAGE loading buffer was added and samples were boiled for 5 min. Gel electrophoresis in a 7.5% acrylamide homogeneous gel and blotting on nitrocellulose were performed using a PhastSystem apparatus (Pharmacia) according to the manufacturer's instructions. Nitrocellulose filters were blocked with 3% BSA and then incubated for 2 h with the anti-β-galactosidase monoclonal antibody (2 μg/ml) followed by anti-mouse AuroProbeEM (Amersham) and IntenseBL silver enhancement (Janssen).

2.3. *Competition assays*

60 μl of different peptide concentrations in PBS containing 0.3% BSA and 2.5% DMSO were added to MAb 1G12-coated microtiter wells (coating, blocking and washing were performed as for the ELISA). After 10 min 15 μl of 100 μg/ml Gal-gag110 were added and plates were incubated for 2 h at room temperature, solutions were removed and plates were washed with PBS containing 0.05% Tween-20. The amount of Gal-gag110 bound to the MAb 1G12-coated wells was determined by assaying its β-galactosidase activity as described [18].

3. RESULTS AND DISCUSSION

3.1. *Inhibition of proteolysis*

In order to assess whether binding of MAb 1G12 to Gag had an effect on the cleavage of the p17–p24 site by HIV-1 protease, two types of experiments were conducted.

In ELISA-like assays Gal-gag110 was bound to (a) an immobilized anti-β-galactosidase monoclonal antibody or (b) immobilized MAb 1G12 (Fig. 1). It was then challenged with HIV-1 protease and the extent of cleavage determined in the two cases. From two independent experiments, each run in duplicate, the amount of residual, uncleaved Gal-gag110, expressed as percentage of the respective controls (no HIV-1 protease), was (33 ± 4)% for (a) and (94 ± 5)% for (b). Hence, Gal-gag110 was substantially cleaved by the HIV-1 protease when it was bound by the control antibody. On the contrary, binding of MAb 1G12 to Gal-gag110 prevented the proteolytic reaction.

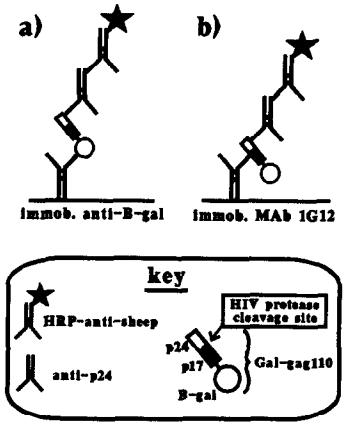


Fig. 1. Simplified scheme of the ELISA-like experiment showing the protection of the p17–p24 cleavage site by MAb 1G12. The two cases are depicted, i.e. (a) the anti-β-galactosidase monoclonal antibody or (b) MAb 1G12 on the solid phase. The detailed procedure is described in section 2.2.

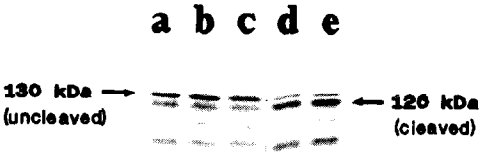


Fig. 2. Protection of the p17–p24 cleavage site by MAb 1G12, as shown by Western blot analysis. Gal-gag110 (100 μg/ml) was incubated with MAb 1G12 or the anti-β-galactosidase monoclonal antibody (Promega) before digestion with HIV-1 protease. The anti-β-gal antibody was also used as primary antibody for detection (see section 2.2.). Lanes: (a) no HIV-1 protease, no antibodies; (b) and (c) both + HIV-1 protease, + 100 and 400 μg/ml MAb 1G12, respectively; (d) + HIV-1 protease, + 800 μg/ml anti-β-galactosidase antibody; (e) + HIV-1 protease, no antibodies.

In the second set of experiments all reagents were in solution and the HIV-1 protease-mediated cleavage of Gal-gag110 was assayed by immunoblotting. Fig. 2 shows that the conversion of intact Gal-gag110 (about 130 kDa) into the cleaved product (about 120 kDa) was prevented when the protein was preincubated with MAb 1G12, but not when the control antibody was used. This result rules out the possibility that the interference in the activity of HIV-1 protease in the ELISA with MAb 1G12 was due to the presence of the solid phase.

Therefore, these experiments indicate that MAb 1G12 can effectively protect the p17–p24 cleavage site from HIV-1 protease-mediated proteolysis.

3.2. *Epitope mapping and specificity*

To distinguish whether MAb 1G12 recognizes a contiguous sequence or a conformational epitope on the Gag polypeptide, different peptides covering the p17–p24 boundary region were tested for their ability to compete in the binding of Gal-gag110 to immobilized MAb 1G12. In these experiments the amount of bound Gal-gag110 was quantitated assaying directly its β-galactosidase activity. The results shown in Table I, expressed as peptide concentrations causing 50% inhibition of binding (IC_{50}), indicate that competition was indeed observed. Peptide A, a 15-mer made of 7 residues from p17 and 8 residues from p24, was the most efficient competitor. Sequences of the same size, but shifted by 3 residues to the right (as in peptide B) or to the left (as in peptide C) exhibited lower affinity, with a more dramatic effect in the latter case. Peptide D, a 9-mer lacking

Table I

Competition of MAb 1G12 / Gal-gag110 binding

Peptide	Sequence[a]	IC_{50} (μM)[b]
A	NQVSQNY*PIVQNIQG	0.18 ± 0.04
B	SQNY*PIVQNIQGQMV	0.70 ± 0.15
C	GHSNQVSQNY*PIVQN	12 ± 2
D	SQNY*PIVQN	100 ± 24
E	SQNY*PIV	> 2,000

[a] All sequences include the p17–p24 cleavage site. The scissile peptide bond is indicated by an asterisk.
[b] Mean ± S.E.M. Data from three independent experiments, each run in duplicate.

both 3 N-terminal and 3 C-terminal residues of peptide A, was still able to bind MAb 1G12, even though the affinity was in the order of 10^4 M. Conversely, the ability to compete was completely lost by the 7-mer peptide E which instead has been reported to be efficiently recognized and cleaved by the HIV-1 protease [19].

Therefore, these data indicate that MAb 1G12 recognizes a contiguous sequence on Gag, located around the p17–p24 cleavage site, thereby protecting this site from the action of HIV-1 protease. They also indicate that, if compared with the seven-residue minimal sequence required by HIV-1 protease [19], MAb 1G12 requires, for full binding, additional determinants located on both sides of that sequence.

To verify the possibility that MAb 1G12 might recognize other HIV-1 protease cleavage sites on Gag, various peptides encompassing different cleavage sites were tested in the competition assay. As shown in Table II, none of these peptides exhibited any significant affinity for MAb 1G12. This antibody is therefore specific for the p17–p24 cleavage site.

3.3. *Conclusions*

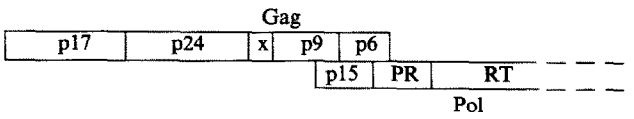
In summary, the binding epitope and the specificity of MAb 1G12 have been characterized. The peculiarity of its binding site renders this antibody a very interesting reagent. Indeed, it may be helpful in different areas of AIDS research, e.g. detection and quantitation of unprocessed vs. processed Gag protein for diagnostic purposes, or in vitro studies on the mechanism of processing of viral proteins into their mature forms. In this area, the possibility to selectively protect a specific HIV-1 protease cleavage site is undoubtedly the most interesting feature of this monoclonal antibody.

Table II

Competition of MAb 1G12/Gal-gag110 binding

Peptide	Junction	Sequence[a]	IC_{50} (μM)[b]
Gag125–140	p17–p24	NQVSQNY*PIVQNIQG	0.18 ± 0.04
Gag357–371	p24–X	GHKARVL*AEAMSQVT	> 1,000
Gag371–385	X–p9	TNTATIM*MQRGNFRN	> 1,000
Gag442–456	p9–p6	KGRPGNF*LQSRPEPT	> 1,000
Pol62–76	p15–PR	GTVSFNF*PQITLWQR	> 100[c]
Pol162–176	PR–RT	GCTLNF*PISPIETVP	> 1,000

[a] The scissile peptide bond is indicated by an asterisk.
[b] Mean ± S.E.M. Data from three independent experiments, each run in duplicate.
[c] Insoluble at higher concentrations.



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