Volume 286, number 1,2, 233–236 FEBS 09953 July 1991
© 1991 Federation of European Biochemical Societies 00145793/91/$3.50
ADONIS 001457939100662G

Tryptase TL_2 in the membrane of human $T4^+$ lymphocytes is a novel binding protein of the V3 domain of HIV-1 envelope glycoprotein gp120

Hiroshi Kido, Aiko Fukutomi and Nobuhiko Katunuma

Division of Enzyme Chemistry, Institute for Enzyme Research, The University of Tokushima, Tokushima 770, Japan

Received 21 May 1991

A novel membrane-bound serine esterase in cultured human $T4^+$ lymphocytes, recently purified and named tryptase TL_2, binds specifically to the external envelope protein gp120 of HIV-1, interacting with its V3 domain. This binding was selectively blocked by inhibitors of tryptase TL_2 with a GPCR sequence in their reactive site, synthetic peptides corresponding with the sequences of the V3 domains of various HIV-1 strains with the GPGR sequence, and antibody against tryptase TL_2, or neutralizing antibody against the V3 domain of $HTLV\text{-}III_B$. These findings suggest that tryptase TL_2 is a binding protein of the V3 domain of HIV-1 envelope glycoprotein.

AIDS; HIV-1 gp120; V3 domain; Serine esterase; Binding protein

1. INTRODUCTION

Human immunodeficiency virus (HIV-1) utilizes the CD4 antigen as a receptor for binding to the cell surface [1–3]. Although little is understood about the sequence of events occurring between CD4 binding and viral internalization, several observations suggest that some factor(s) involved in the events following binding to CD4 effects membrane fusion and internalization [4–8].

We have demonstrated that trypstatin [9] and anti-rat mast cell tryptase antibodies markedly inhibited multinucleated cell-to-cell fusion (syncytia) induced by HIV-1 [10]. We recently purified a novel membrane-bound serine esterase, named tryptase TL_2, from human $T4^+$ lymphocytes immunologically reactive with the antibody inhibiting syncytial formation [11]. The amidase activity of tryptase TL_2 was strongly inhibited by gp120 of human T-lymphotropic virus (HTLV) type III_B, by synthetic peptides with a central Gly-Pro-Gly-Arg (GPGR) motif, corresponding to the V3 domains of gp120s of various HIV-1 strains, and by Kunitz-type inhibitors, such as trypstatin and HI30 [12], containing the sequence GPCR in their reactive site [11]. These results suggest that gp120 of HIV-1 binds to tryptase TL_2 at a site other than the CD4 receptor. In this report, we demonstrated that gp120 of HIV-1 specifically binds to tryptase TL_2 in the region of the V3 domain.

Abbreviations: HIV, human immunodeficiency virus; HTLV, human T-lymphotropic virus; mmab, mouse monoclonal antibody; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate

Correspondence address: H. Kido, Division of Enzyme Chemistry, Institute for Enzyme Research, The University of Tokushima, Tokushima 770, Japan. Fax: (81) (886) 330771

2. MATERIALS AND METHODS

2.1. *Materials*

The compounds used were as follows: porcine pancreatic trypsin, leupeptin and aprotinin (Sigma), gp120 of $HTLV\text{-}III_B$ (MicroGenesys), mouse monoclonal antibody (mmab)-anti-HIV-1 gp120 and neutralizing mmab-anti-HIV-1 gp120N (Dupont), mmab-anti-Leu 3a (Becton Dickinson), and ^{125}I as sodium salts (3.7 GBq/ml) (Amersham). Tryptase TL_2 and trypstatin were purified as described [9,11]. HI30 and recombinant Arg-15, Glu-52 aprotinin were gifts from Dr H. Fritz, University of Munich.

2.2. *Assay of gp120 binding to tryptase TL_2*

$HTLV\text{-}III_B$ gp120 and purified tryptase TL_2 were radioiodinated by the method of Bolton and Hunter [13]. Radioiodinated gp120 (0.4 μg) was incubated with 0.4 μg of tryptase TL_2 in 40 μl of 100 mM Tris-HCl, pH 8.5, at 37°C for 30 min. The reaction was stopped by addition of 1 mM leupeptin and then 40 μl of 50% polyclonal antibody against tryptase TL_2 coupled with Sepharose 4B was added. After incubation at room temperature for 1 h with vigorous shaking, the Sepharose 4B was washed three times with 1 ml of 10 mM Tris-HCl, pH 7.0, containing 0.5 M NaCl and 0.05% Tween 20, and once with 1 ml of 10 mM Tris-HCl, pH 7.0 and its radioactivity was counted. Conversely, [^{125}I]tryptase TL_2 was incubated with gp120 and immunoprecipitated with mmab-gp120-coupled Sepharose 4B. Non-specific binding was determined by incubation of [^{125}I]gp120 or [^{125}I]tryptase TL_2 with 40 μl of 50% uncoupled Sepharose 4B. Bound proteins were eluted with 20 μl of sample loading buffer at 100°C for 5 min and analyzed by 10–20% polyacrylamide gradient gel electrophoresis (PAGE) under reducing conditions in sodium dodecyl sulphate (SDS) [14] followed by autoradiography.

The effects of inhibitors, peptides and antibodies other than anti-tryptase TL_2 on the binding of gp120 to tryptase TL_2 were tested by incubation of [^{125}I]gp120 (0.4 μg) with purified tryptase TL_2 (0.4 μg) in the presence or absence of inhibitors, peptides and antibodies and then with 40 μl of 50% anti-tryptase TL_2 IgG coupled with Sepharose 4B. The effect of anti-tryptase TL_2 was tested by incubation of [^{125}I]tryptase TL_2 (0.4 μg) with gp120 (0.4 μg) in the presence or absence of the antibody and then with mmab-gp120 coupled with Sepharose 4B. The Sepharose 4B was then precipitated and washed and its radioactivity was counted.

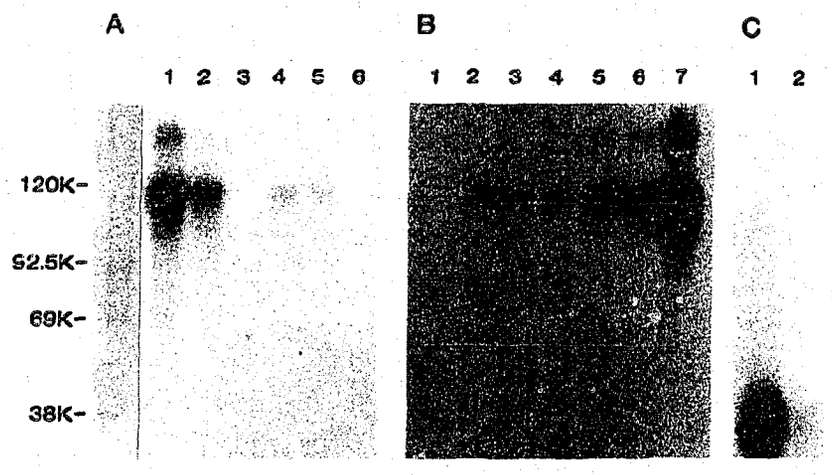

Fig. 1. Specific binding of HTLV-III$_B$ gp120 to tryptase TL$_2$. (A) (Lane 1) [^{125}I]gp120 (0.4 μg) incubated without tryptase TL$_2$ following incubation with mmab-gp120 IgG-Sepharose 4B; (lane 2) [^{125}I]gp120 incubated with 0.4 μg of tryptase TL$_2$, then with anti-tryptase TL$_2$ IgG-Sepharose 4B; (lane 3) [^{125}I]gp120 incubated with 0.4 μg of trypsin, then with anti-trypsin IgG-Sepharose 4B; (lane 4) [^{125}I]gp120 incubated with 0.4 μg of tryptase TL$_1$, then with anti-tryptase TL$_1$ IgG-Sepharose 4B; (lane 5) [^{125}I]gp120 incubated with 0.4 μg of tryptase TL$_2$, then with non-immunized rabbit IgG-Sepharose 4B; (lane 6) [^{125}I]gp120 incubated without tryptase TL$_2$, then with anti-tryptase TL$_2$ IgG-Sepharose 4B. Bound proteins were eluted, analyzed by SDS-PAGE and autoradiographed as described in section 2. (B) [^{125}I]gp120 (0.4 μg) incubated without tryptase TL$_2$ (lane 1), or with 0.05 μg (lane 2), 0.1 μg (lane 3), 0.2 μg (lane 4), 0.3 μg (lane 5), 0.4 μg (lane 6) or 0.6 μg (lane 7) of tryptase TL$_2$, then with anti-tryptase TL$_2$ IgG-Sepharose 4B. (C) [^{125}I]Tryptase TL$_2$ (0.4 μg) incubated with (lane 1) or without (lane 2) 0.4 μg of gp120 and then with 40 μl of mmab-gp120 IgG-Sepharose 4B.

3. RESULTS AND DISCUSSION

3.1. *Specific binding of gp120 to tryptase TL$_2$*

To determine whether HTLV-III$_B$ gp120 binds to tryptase TL$_2$, we incubated [^{125}I]gp120 with purified tryptase TL$_2$ and then treated the mixture with anti-tryptase TL$_2$ IgG-Sepharose 4B. As shown in Fig. 1A, lane 2, [^{125}I]gp120 was co-immunoprecipitated with tryptase TL$_2$. [^{125}I]gp120 was not immunoprecipitated by the antibody in the absence of tryptase TL$_2$ (lane 6) and gave only a very faint band with non-immunized IgG-Sepharose 4B (lane 5). [^{125}I]gp120, used as a positive control, was almost completely immunoprecipitated by mmab-gp120 of HTLV-III$_B$ (lane 1). No [^{125}I]gp120 was immunoprecipitated from mixtures with trypsin (lane 3) or tryptase TL$_1$, which is another arginine-cleaving protease purified from the cytosol fraction of human T4$^+$ lymphocyte clone Molt-4, clone-8 cells (H. Kido et al., in preparation) (lane 4), and these proteases hydrolyzed the gp120 to fragments of various sizes (data not shown). Fig. 1B shows that immunoprecipitation of [^{125}I]gp120 increased with an increase in the concentration of tryptase TL$_2$ in the assay mixture. Conversely [^{125}I]tryptase TL$_2$ was co-immunoprecipitated by mmab-gp120 after incubation with gp120 (Fig. 1C, lane 1), but not without gp120 (Fig. 1C, lane 2). These results indicate that gp120 of HTLV-III$_B$ recognizes and binds specifically to tryptase TL$_2$.

3.2. *Inhibition of binding of gp120 to tryptase TL$_2$*

The effects of inhibitors of tryptase TL$_2$, synthetic peptides and antibody against tryptase TL$_2$ or neutralizing antibody against the V3 domain of gp120 on the binding of HTLV-III$_B$ gp120 to purified tryptase TL$_2$ are shown in Table I. Potential Kunitz-type protease inhibitors of tryptase TL$_2$, such as trypstatin [9] and HI30 [12] with the sequence GPCR in their reactive site and Arg-15, Glu-52 aprotinin, in which Lys-15 in the reactive site is replaced by Arg-15, inhibited the binding. However, aprotinin, which has lysine in the reactive site, did not inhibit the binding. Leupeptin, which does not contain the GPCR sequence, also did not inhibit the binding, although it inhibited the amidase activity of tryptase TL$_2$ [11]. Synthetic peptides that correspond to the V3 domains of various HIV-1 strains and can inhibit the activity of tryptase TL$_2$ [11], such as NNT23 of HTLV-III$_{NY5}$, NNT24 of HTLV-III$_B$ and YNK23 of HTLV-III$_{MN}$, also inhibited the binding.

To identify the amino acid sequences necessary for the inhibition, we tested the effects on the binding of several short peptides related to the NNT24 sequence. The peptides VTI07 and AFV07 without the GPGR sequence were not inhibitory, whereas the peptides GPG07, IQR10, and IRI12 with the GPGR sequence were inhibitory, though less inhibitory than NNT24. As a negative control, peptide ASD15, corresponding to Ala-61 to Trp-75 of gp120 of HTLV-III$_B$, which was arbitrarily chosen as a sequence in a different position from those for CD4 binding and the V3 domain, had no inhibitory effect. These results suggest that a central GPGR motif in the peptides is essential, but not enough on its own, for the inhibition. Anti-tryptase TL$_2$ and neutralizing antibody against the V3 domain of HTLV-

Table I

Inhibition of binding of HTLV-III_B gp120 to tryptase TL_2

Addition	Conc. (μM)	Number[a]	Sequence	Inhibition[b] (%)
None	100			0
Leupeptin	100			2.3 ± 2.0
Trypstatin	40	(1)	IAACNLPIVQ GPCR AFAELLAFDA	88.8 ± 16.2
HI30	40	(79)	VAACNLPVIR GPCR AFIQLWAFDA	88.3 ± 16.4
Arg-15, Glu-52 aprotinin	100	(1)	RPDFCLEPPYT GPCR ARIIRYFYNA	30.7 ± 5.6
Aprotinin	100	(1)	RPDFCLEPPYT GPCK ARIIRYFYNA	2.2 ± 2.6
NNT23 (HTLV-III_{NY5})	400	(307)	NNTKKGIAI GPGR TLYAREKIIG	72.6 ± 18.2
YNK23 (HTLV-III_{MN})	400	(307)	YNKRKRIHI GPGR AFYTTKNII	46.3 ± 13.6
NNT24 (HTLV-III_B)	400	(307)	NNTRKSIRIQR GPGR AFVTIGKIG	84.2 ± 20.1
VTI07 (HTLV-III_B)	400	(324)	VTIGKIG	0 ± 2.0
AFV07 (HTLV-III_B)	400	(322)	AFVTIGK	0 ± 1.6
GPG08 (HTLV-III_B)	400	(318)	GPGR AFVT	5.5 ± 2.1
IQR10 (HTLV-III_B)	400	(315)	IQR GPGR AFV	15.7 ± 2.6
IRI12 (HTLV-III_B)	400	(313)	IRIQR GPGR AFV	15.7 ± 2.3
ASD15 (HTLV-III_B)	400	(61)	ASDAKAYDTEV HNVW	0 ± 1.8
Anti-gp120N mmab	5 μg/ml	(314)	RIQR GPGR AFVTIGK[c]	94.1 ± 12.1
Anti-tryptase TL_2 IgG	50 μg/ml			83.2 ± 8.9
Anti-CD4 mmab	1.25 μg/ml			2.3 ± 1.8
Non-immunized IgG	50 μg/ml			4.8 ± 2.8

[a] Positions of amino acid residues are indicated. The sequences of the various strains of HIV-1 listed are aligned at the cysteine residue at amino acid 302 according to the numbering system of Ratner et al. [15]. The listed sequences have all been published [9,12,15,16]

[b] Percentage inhibition was calculated as (count with inhibitor, peptide or antibody − count of non-specific binding)/(count without inhibitor, peptide or antibody − count of non-specific binding) × 100. Values are means ± SD for 5 separate experiments

[c] Amino acid sequence of the antigen peptide of mmab anti-gp120N (DuPont)

III_B gp120 (anti-gp120N) also markedly blocked the binding, whereas non-immunized IgG and anti-CD4 antibody had no effect. These results support the conclusion that tryptase TL_2 interacts with gp120 in the region of the V3 domain.

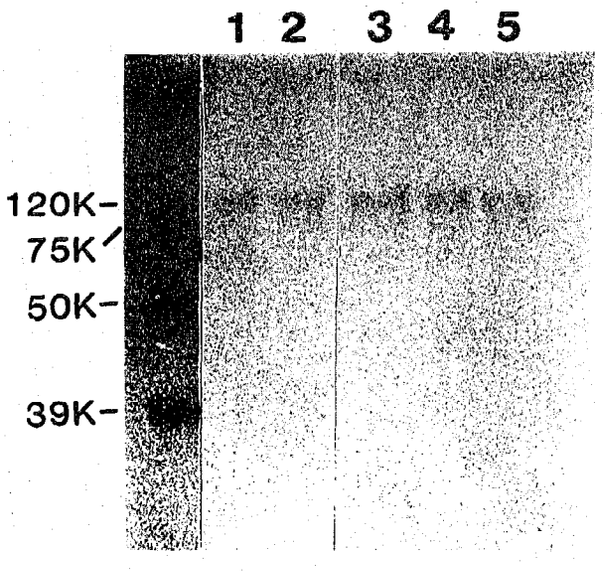

Fig. 2. Absence of cleavage of gp120 after binding to tryptase TL_2. gp120 (1.5 μg) was incubated with 0.05 μg of tryptase TL_2 in 20 μl of 100 mM Tris-HCl buffer, pH 8.5, at 37°C for 0 min (lane 1), 30 min (lane 2), 60 min (lane 3), 90 min (lane 4) or 180 min (lane 5) and then after reduction was analyzed by 10–20% SDS-PAGE. The gel was stained with Coomassie brilliant blue R-250, and destained.

3.3. *No cleavage of gp120 after binding to tryptase TL_2*

For determination of whether gp120 bound to tryptase TL_2 was cleaved proteolytically after binding, gp120 was incubated with tryptase TL_2 and then analyzed by SDS-PAGE. As shown in Fig. 2, tryptase TL_2 did not cleave gp120 during incubation for up to 180 min at 37°C. This result indicates that the selective binding of the V3 domain of HIV-1 gp120 to tryptase TL_2 may be one step in infection. A requirement for the binding of the V3 domain to cell surface tryptase TL_2 with a limited tissue distribution may also be an important mechanism for restricting the host cell tropism of HIV-1.

Acknowledgements: We thank Dr K. Hanada and Dr M. Yoshimoto for large-scale preparation of Molt-4, clone 8 cells and Dr K. Takatsuki for gifts of NNT24, VTI07, AFV07, GPG08, IQR10 and IRI12. This study was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.

REFERENCES

[1] Dalgleish, A.G., Beverley, P.C.L., Clapham, P.R., Crawford, D.H., Greaves, M.F. and Weiss, R.A. (1984) Nature 312, 763–766.

[2] Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Gluckman, J.-C. and Montagnier, L. (1984) Nature 312, 763–766.

[3] Sattentau, Q.J., Clapham, P.R., Weiss, R.A., Beverley, P.C.L., Montagnier, L., Alhalabi, M.F., Gluckman, J.-C. and Klatzmann, D. (1988) AIDS 2, 101–105.

[4] Bedinger, P., Moriarty, A., Von Borstel, R.C., Donovan, N.J., Steimer, K.S. and Littman, D.R. (1988) Nature 334, 162–165.
[5] Maddon, P.J., McDougal, J.S., Clapham, P.R., Dalgleish, A.G., Jamal, S., Weiss, R.A. and Axel, R. (1988) Cell 54, 865–874.
[6] Clapham, P.R., Blanc, D. and Weiss, R.A. (1991) Virology 181, 703–715.
[7] Linsley, P.S., Ledbetter, J.A., Kinney-Thomas, E. and Hu, S.-L. (1988) J. Virol. 62, 3695–3702.
[8] Skinner, M.A., Langlois, A.J., McDanal, C.B., McDougal, J.S., Bolognesi, D.P. and Matthews, T.J. (1988) J. Virol. 62, 4195–4200.
[9] Kido, H., Yokogoshi, Y. and Katunuma, N. (1988) J. Biol. Chem. 263, 18104–18107.
[10] Hattori, T., Koito, A., Takatsuki, K., Kido, H. and Katunuma, N. (1989) FEBS Lett. 248, 48–52.
[11] Kido, H., Fukutomi, A. and Katunuma, N. (1990) J. Biol. Chem. 265, 21979–21985.
[12] Wachter, E. and Hochstrasser, K. (1981) Hoppe-Seyler's Z. Physiol. Chem. 362, 1351–1355.
[13] Bolton, A.E. and Hunter, W.N. (1973) Biochem. J. 133, 529–539.
[14] Laemmli, U.K. (1970) Nature 227, 680–685.
[15] Ratner, L., Haseltine, W., Patarca, R., Livak, K.J., Starcich, B., Josephs, S.F., Doran, E.R., Ratalski, J.A., Whitehorn, E.A., Baumerster, K., Ivanoff, L., Petteway Jr., S.R., Pearson, M.L., Lautenberger, J.A., Papas, T.S., Ghrayeb, J., Chang, T.N., Gallo, R.C. and Wong-Staal, F. (1985) Nature 313, 277–284.
[16] Willey, R.L., Rutledge, R.A., Dias, S., Folks, T., Theodore, T., Buckler, C.E. and Martin, M.A. (1986) Proc. Natl. Acad. Sci. USA 83, 5038–5042.