Available online at www.sciencedirect.com
SCIENCE DIRECT
Antiviral Research 71 (2006) 69–72
Antiviral Research
www.elsevier.com/locate/antiviral

Short communication

The membranes' role in the HIV-1 neutralizing monoclonal antibody 2F5 mode of action needs re-evaluation

Ana Salomé Veiga, Miguel A.R.B. Castanho *

Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande Ed. C8, 1749-016 Lisboa, Portugal

Received 16 November 2005; accepted 3 February 2006

Abstract

2F5, a monoclonal antibody that neutralizes HIV-1 primary isolates, recognizes an epitope in the membrane proximal region of the glycoprotein gp41 ectodomain. It is believed that binding to the viral membrane is a step in the antibody mode of action, as usual in ligand membrane receptor interactions. We investigated the interaction of 2F5 with membrane model systems, namely large unilamellar vesicles, by means of fluorescence techniques. There were no significant interactions of 2F5 with model viral membranes or with model target cell membranes. Thus, the usual three-step 'membrane catalysis' method is not followed by 2F5 in its mode of action.

Keywords: MAb 2F5; HIV-1; Membrane; Fluorescence

Human immunodeficiency virus type 1 (HIV-1) entry into target cells occurs through a mechanism mediated by the envelope glycoprotein. Expressed on the viral membrane surface as trimer, this glycoprotein is composed of two noncovalently associated subunits, gp120 (surface glycoprotein) and gp41 (transmembrane glycoprotein) (Chan and Kim, 1998; Wyatt and Sodroski, 1998; Eckert and Kim, 2001). The gp120 binding to CD4 and chemokine receptors at the target cell surface induces conformational changes in the glycoprotein complex. Subsequent changes within the gp41 ectodomain involve the formation of a six-helix bundle structure (hairpin structure) allowing membrane fusion and viral entry (Chan and Kim, 1998; Wyatt and Sodroski, 1998; Eckert and Kim, 2001; LaBranche et al., 2001; Cooley and Lewin, 2003).

A few human monoclonal antibodies (mAbs) that neutralize a broad range of HIV-1 primary isolates, targeting different envelope glycoprotein epitopes, have been identified (Trkola et al., 1995; D'Souza et al., 1997; Zwick et al., 2001). The mAb 2F5 is one of these and recognizes an epitope, containing the sequence ELDKWA, in the membrane proximal region of the gp41 ectodomain (Muster et al., 1993; Parker et al., 2001). It has been proposed that 2F5 can bind its epitope in the prehairpin structure, a fusion intermediate (Gorny and Zolla-Pazner, 2000; Follis et al., 2002; de Rosny et al., 2004), although there are also indications that binding can occur with the native form of gp41 (Gorny and Zolla-Pazner, 2000; Barbato et al., 2003; de Rosny et al., 2004). Additionally, 2F5 binding affinity for the epitope is enhanced when gp41 is in a lipidic environment (Grundner et al., 2002; Ofek et al., 2004). The interactions between the 2F5 third complementarity-determining region of the heavy chain (CDR H3) and the epitope in gp41 occur predominantly with residues at the CDR H3 base. Thus, there are CDR H3 residues that remain unbound to gp41, including at the apex (Ofek et al., 2004). Ofek et al. (2004) suggested the existence at the apex of a hydrophobic surface composed by the hydrophobic amino acids Leu, Phe, Val, and Ile. Given the viral membrane proximity to the 2F5 epitope, this hydrophobic surface may be responsible for 2F5 binding to the membrane facilitating the epitope binding (Ofek et al., 2004).

We investigated mAb 2F5 membrane interactions using membrane model systems (large unilamellar vesicles, LUV) of pure POPC (1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine; Avanti Polar-Lipids, Alabaster, AL) and POPC/Cholesterol 67:33 (mol%). Cholesterol was purchased from Sigma (St. Louis, MO). POPC is a lipid with packing density and fluidity properties similar to biological membranes and the mixture POPC/Cholesterol mimic the viral membrane, which is rich in

* Corresponding author. Tel.: +351 21 7500931; fax: +351 21 7500088.
E-mail address: castanho@fc.ul.pt (M.A.R.B. Castanho).

0166-3542/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2006.02.002

cholesterol (Aloia et al., 1993; Campbell et al., 2002; Raulin, 2002).

2F5 is intrinsically fluorescent due to the presence of aromatic amino acids (tryptophan, tyrosine and phenylalanine), which enable the study of this molecule using fluorescence techniques. A spectrofluorimeter SLM Aminco 8100 with double monochromators and 450 W Xe lamp was used. Human HIV-1 gp41 monoclonal antibody 2F5 (IgG1 isotype, kappa chain) was provided by the EU Programme EVA Centralised Facility for AIDS Reagents, National Institute for Biological Standards and Control, UK, and from Dr. Hermann Katinger. 2F5 stock solutions (100 μg of purified antibody in 2 mM acetic acid (pH 4.0) + 10% maltose) were diluted with buffer (10 mM Hepes buffer pH 7.4, 150 mM NaCl) to the final desired concentrations.

Initially, an evaluation of the Fab 2F5 3D structure (PDB entry 2F5B) revealed that some of the aromatic residues are exposed at the mAb surface. As Trp and Tyr residues have propensity to insert into membranes and a tendency to locate at the membrane–water interface (White and Wimley, 1998; Killian and von Heijne, 2000), they may potentially help the interaction of the 2F5 with the viral membrane, in addition to the hydrophobic surface proposed by others (Ofek et al., 2004).

As the Trp residues' emission is sensitive to their local environment (Lakowicz, 1999), the measured emission maximum wavelength of ~330 nm (320 nm in the corrected spectrum compared to 350nm of free Trp in aqueous environment) of 2F5 1.5 μM (Fig. 1A) reveals that most Trp residues are buried inside the protein in the absence of membranes. This result was confirmed with a solution quenching study with acrylamide (Fig. 1B). The very small quenching efficiency shows that the aqueous quencher does not contact the Trp residues.

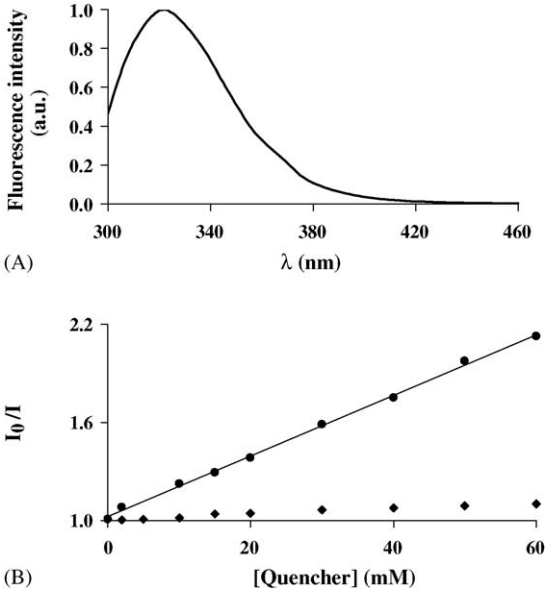

Fig. 1. (A) 2F5 (1.5 μM) emission spectra in aqueous solution. (B) Stern–Volmer plot for 2F5 (♦) and free Trp in solution (●) fluorescence quenching by acrylamide. Small amounts of quencher were added (in a range of 0–60 mM) to a 2F5 sample (1.5 μM) with 10 min incubation interval. The excitation and emission wavelengths used were 290 and 330 nm, respectively. The data were corrected with the correction factor *C* (Coutinho and Prieto, 1993) accounting for both inner filter effect and light absorption by the quencher.

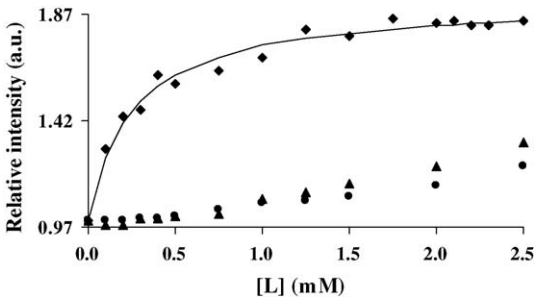

Fig. 2. Partition plots of 2F5 (1.5 μM) to LUV of POPC (●) and POPC/Chol (▲). For the sake of comparison, the partition plot of T-1249 (a molecule that interacts moderately with membranes (Veiga et al., 2004)) to LUV of POPC is presented (♦). [*L*] is the concentration of the lipid available to 2F5 or T-1249 (i.e., the outer hemilayer of the vesicles).

Large unilamellar vesicles (~100 nm diameter) were used to study the interaction of 2F5 with membranes. POPC alone or mixed with cholesterol (in chloroform) were placed in a round bottom flask, and the solution was dried under a stream of nitrogen. Solvent removal was completed in vacuum, overnight. Finally, the LUV were prepared by extrusion techniques (Mayer et al., 1986). Membrane partition studies were achieved by successive additions of small aliquots of LUV (15 mM) to the mAb samples (1.5 μM) with 10 min incubation intervals. The excitation and emission wavelengths used were 280 and 330 nm, respectively. Fluorescence intensity data was corrected for the dilution effect. The results obtained show no occurrence of shifts of the emission spectra upon addition of POPC or POPC/Chol vesicles (the spectra are similar to those in aqueous solution—data not shown). No concomitant increase in 2F5 fluorescence intensity was observed (Fig. 2). These findings suggest that 2F5 does not partition to the membranes through an extensive anchoring of a significant number of Trp residues.

However, the hydrophobic surface proposed to interact with membranes does not contain Trp residues and it could be argued that a mild interaction with the membrane could take place strictly involving this restricted surface. One Phe residue is present but Phe fluorescence is less sensitive to environment polarity than Trp fluorescence and the interaction with the lipids could thus remain undetected. To test this hypothesis we performed studies with di-8-ANEPPS (4-[2-[6-(dioctylamino)-2-naphthalenyl]ethenyl]-1-(3-sulfopropyl)-pyridinium; Molecular Probes, Eugene, OR), a dye sensitive to changes in the membrane dipole potential, which has been shown to detect protein membrane interaction (O'Shea, 2003) and can thus be used to probe the 2F5 interaction with membranes. Changes on the membrane dipole potential magnitude, incited by membrane binding and insertion of molecules, may be monitored by means of spectral shifts of the fluorescence indicator di-8-ANEPPS (Cladera and O'Shea, 1998; Cladera et al., 2001). POPC, and cholesterol when required, and di-8-ANEPPS (from a stock solution in ethanol) were used to prepare LUV by extrusion techniques as mentioned before. Di-8-ANEPPS excitation spectra were obtained with emissions set at 600 nm (for POPC membranes) or 578 nm (for POPC/Chol membranes). The concentrations used were 200 μM for lipids, 10 μM for di-8-ANEPPS and

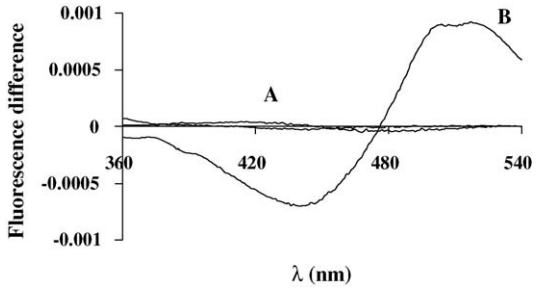

Fig. 3. Fluorescence difference spectra of di-8-ANEPPS-labeled POPC and POPC/Chol membranes in the presence of 2F5 (A). Both spectra overlap and were obtained by subtracting the excitation spectrum before the addition of 2F5 from the excitation spectrum after 2F5 (2 μM) addition, with $\lambda_{em} = 600$ nm and $\lambda_{em} = 578$ nm for POPC and POPC/Chol membranes, respectively. Before subtraction, the spectra were normalized to the integrated areas to reflect only the spectral shifts. Dye and lipid concentrations used were 10 μM and 200 μM, respectively. The difference spectrum obtained with di-8-ANEPPS-labeled POPC membranes in the presence of T-1249 (a molecule that interacts moderately with membranes (Veiga et al., 2004)) is depicted for comparison (B).

2 μM for 2F5. Fig. 3 shows the fluorescence difference spectra of di-8-ANEPPS-labeled POPC or POPC/Chol membranes, which were obtained by subtracting the excitation spectrum before the addition of 2F5 from the excitation spectrum in the presence of 2F5. Before subtraction the spectra were normalized to the integrated areas to reflect only spectral shifts (Cladera and O'Shea, 1998; Cladera et al., 2001). Difference spectra intensity obtained for both studied cases indicates that there are no interactions of 2F5 with the membranes, which is reinforced by comparing with the fluorescence difference spectrum of T-1249 (Fig. 3), a molecule known to interact moderately with membranes (Veiga et al., 2004, 2006).

In conclusion, no interaction occurs between the mAb 2F5 and both the cell and viral model membranes. Thus 2F5 does not use membrane interaction prior to gp41 docking, i.e., does not follow the classical 'membrane catalysis' scheme (Sargent and Schwyzer, 1986; Castanho and Fernandes, 2006). In the classical 'membrane catalysis' scheme the local concentrating effect of the membrane is expected to increase the occupied-to-unoccupied binding sites in the membrane by a factor that equals the molar ratio partition coefficient (Castanho and Fernandes, 2006). Probably, membranes play a role simply by inducing the best epitope conformation for binding (Grundner et al., 2002; Ofek et al., 2004), but not through a preparation of 2F5 for better docking. Taking together the results in this study with the others on the molecular level action of 2F5 (Ofek et al., 2004), it seems reasonable to speculate that 2F5 binds to the protein epitope, which in turn causes the CDR H3 region to bind to the membrane and stabilize docking.

Acknowledgments

This project was partially funded by FCT-MCIES (Portugal), including a grant (SFRH/BD/14336/2003) under the program POCTI to A.S.V. Monoclonal antibody 2F5 was provided by Dr. Hermann Katinger through the NIBSC Centralised Facility for AIDS Reagents supported by EU Programme EVA and the UK Medical Research Council.

References

Aloia, R.C., Tian, H., Jensen, F.C., 1993. Lipid composition and fluidity of the human immunodeficiency virus envelope and host cell plasma membranes. Proc. Natl. Acad. Sci. USA 90, 5181–5185.

Barbato, G., Bianchi, E., Ingallinella, P., Hurni, W.H., Miller, M.D., Ciliberto, G., Cortese, R., Bazzo, R., Shiver, J.W., Pessi, A., 2003. Structural analysis of the epitope of the anti-HIV antibody 2F5 sheds light into its mechanism of neutralization and HIV fusion. J. Mol. Biol. 330, 1101–1115.

Campbell, S.M., Crowe, S.M., Mak, J., 2002. Virion-associated cholesterol is critical for the maintenance of HIV-1 structure and infectivity. AIDS 16, 2253–2261.

Castanho, M.A.R.B., Fernandes, M.X., 2006. Lipid membrane-induced optimization for ligand-receptor docking: recent tools and insights for the 'membrane catalysis' model. Eur. Biophys. J. 35, 92–103.

Chan, D.C., Kim, P.S., 1998. HIV entry and its inhibition. Cell 93, 681–684.

Cladera, J., Martin, I., O'Shea, P., 2001. The fusion domain of HIV gp41 interacts specifically with heparan sulfate on the T-lymphocyte cell surface. EMBO J. 20, 19–26.

Cladera, J., O'Shea, P., 1998. Intramembrane molecular dipoles affect the membrane insertion and folding of a model amphiphilic peptide. Biophys. J. 74, 2434–2442.

Cooley, L.A., Lewin, S.R., 2003. HIV-1 cell entry and advances in viral entry inhibitor therapy. J. Clin. Virol. 26, 121–132.

Coutinho, A., Prieto, M., 1993. Ribonuclease T_1 and alcohol dehydrogenase fluorescence quenching by acrylamide. J. Chem. Educ. 70, 425–428.

de Rosny, E., Vassell, R., Jiang, S., Kunert, R., Weiss, C.D., 2004. Binding of the 2F5 monoclonal antibody to native and fusion-intermediate forms of human immunodeficiency virus type 1 gp41: implications for fusion-inducing conformational changes. J. Virol. 78, 2627–2631.

D'Souza, M.P., Livnat, D., Bradac, J.A., Bridges, S.H., the AIDS clinical trials group antibody selection working group, and collaborating investigators, 1997. Evaluation of monoclonal antibodies to human immunodeficiency virus type 1 primary isolates by neutralization assays: performance criteria for selecting candidate antibodies for clinical trials. J. Infect. Dis. 175, 1056–1062.

Eckert, D.M., Kim, P.S., 2001. Mechanisms of viral membrane fusion and its inhibition. Annu. Rev. Biochem. 70, 777–810.

Follis, K.E., Larson, S.J., Lu, M., Nunberg, J.H., 2002. Genetic evidence that interhelical packing interactions in the gp41 core are critical for transition of the human immunodeficiency virus type 1 envelope glycoprotein to the fusion-active state. J. Virol. 76, 7356–7362.

Gorny, M.K., Zolla-Pazner, S., 2000. Recognition by human monoclonal antibodies of free and complexed peptides representing the prefusogenic and fusogenic forms of human immunodeficiency virus type 1 gp41. J. Virol. 74, 6186–6192.

Grundner, C., Mirzabekov, T., Sodroski, J., Wyatt, R., 2002. Solid-phase proteoliposomes containing human immunodeficiency virus envelope glycoproteins. J. Virol. 76, 3511–3521.

Killian, J.A., von Heijne, G., 2000. How proteins adapt to a membrane–water interface. Trends Biochem. Sci. 25, 429–434.

LaBranche, C.C., Galasso, G., Moore, J.P., Bolognesi, D.P., Hirsch, M.S., Hammer, S.M., 2001. HIV fusion and its inhibition. Antiviral Res. 50, 95–115.

Lakowicz, J.R., 1999. Principles of Fluorescence Spectroscopy, 2nd ed. Kluwer Academic/Plenum Publishers, New York.

Mayer, L.D., Hopes, M.J., Cullis, P.R., 1986. Vesicles of variable sizes produced by a rapid extrusion procedure. Biochim. Biophys. Acta 858, 161–168.

Muster, T., Steindl, F., Purtscher, M., Trkola, A., Klima, A., Himmler, G., Ruker, F., Katinger, H., 1993. A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1. J. Virol. 67, 6642–6647.

Ofek, G., Tang, M., Sambor, A., Katinger, H., Mascola, J.R., Wyatt, R., Kwong, P.D., 2004. Structure and mechanistic analysis of the anti-human immunodeficiency virus type 1 antibody 2F5 in complex with its gp41 epitope. J. Virol. 78, 10724–10737.

O'Shea, P., 2003. Intermolecular interactions with/within cell membranes and the trinity of membrane potentials: kinetics and imaging. Biochem. Soc. Trans. 31, 990–996.

Parker, C.E., Deterding, L.J., Hager-Braun, C., Binley, J.M., Schülke, N., Katinger, H., Moore, J.P., Tomer, K.B., 2001. Fine definition of the epitope on the gp41 glycoprotein of human immunodeficiency virus type 1 for the neutralizing monoclonal antibody 2F5. J. Virol. 75, 10906–10911.

Raulin, J., 2002. Human immunodeficiency virus and host cell lipids. Interesting pathways in research for a new HIV therapy. Prog. Lipid Res. 41, 27–65.

Sargent, D.F., Schwyzer, R., 1986. Membrane lipid phase as catalyst for peptide–receptor interactions. Proc. Natl. Acad. Sci. USA 83, 5774–5778.

Trkola, A., Pomales, A.B., Yuan, H., Korber, B., Maddon, P.J., Allaway, G.P., Katinger, H., Barbas III, C.F., Burton, D.R., Ho, D.D., Moore, J.P., 1995. Cross-clade neutralization of primary isolates of human immunodeficiency virus type 1 by human monoclonal antibodies and tetrameric CD4-IgG. J. Virol. 69, 6609–6617.

Veiga, A.S., Santos, N.C., Loura, L.M.S., Fedorov, A., Castanho, M.A.R.B., 2004. HIV fusion inhibitor peptide T-1249 is able to insert or adsorb to lipidic bilayers. Putative correlation with improved efficiency. J. Am. Chem. Soc. 126, 14758–14763.

Veiga, S., Yuan, Y., Li, X., Santos, N.C., Liu, G., Castanho, M.A.R.B., 2006. Why are HIV-1 fusion inhibitors not effective against SARS-CoV? Biophysical evaluation of molecular interactions. Biochim. Biophys. Acta 1760, 55–61.

White, S.H., Wimley, W.C., 1998. Hydrophobic interactions of peptides with membrane interfaces. Biochim. Biophys. Acta 1376, 339–352.

Wyatt, R., Sodroski, J., 1998. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science 280, 1884–1888.

Zwick, M.B., Labrijn, A.F., Wang, M., Spenlehauer, C., Saphire, E.O., Binley, J.M., Moore, J.P., Stiegler, G., Katinger, H., Burton, D.R., Parren, P.W.H.I., 2001. Broadly neutralizing antibodies targeted to the membrane-proximal external region of human immunodeficiency virus type 1 glycoprotein gp41. J. Virol. 75, 10892–10905.