

# Biochemical characterization of derivatives of MA-T12D11, a TAFI neutralizing antibody

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## Introduction

- Thrombin Activatable Fibrinolysis Inhibitor (TAFI) provides a link between the coagulation and the fibrinolytic cascade.<sup>1</sup>
- TAFI can be activated to TAFIa by the thrombin-thrombomodulin complex (T/TM).
- TAFIa has carboxypeptidase activity and exerts an antifibrinolytic effect.

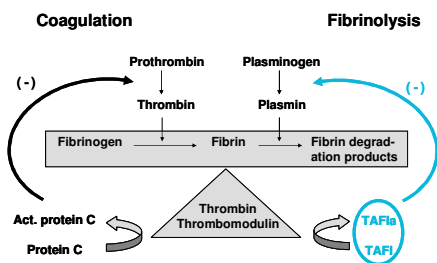


Figure 1 Scheme of blood coagulation and fibrinolysis

- TAFI/TAFIa is a risk factor for thrombosis and related diseases.<sup>2</sup>
- TAFI inhibitors can be a powerful tool in thrombosis models.
- Already known inhibitors of TAFI(a) are not selective or powerful enough:
  - Chelating agents (e.g. EDTA)
  - Reduction of disulfide bridges (e.g. DTT)
  - Arginine and lysine analogs (e.g. GEMSA)
  - Naturally occurring inhibitors (e.g. Carboxypeptidase inhibitor or CPI)
- Monoclonal Antibodies (MA) towards human TAFI were raised in our lab.
- MA-T12D11 inhibits the activation of TAFI by T/TM.

## Objective

To characterize the biochemical properties of antibody fragments derived from MA-T12D11.

## Materials & Methods

- Fab-T12D11 was generated by papain digestion of MA-T12D11, followed by protein A purification.
- The scFv-T12D11 fragment was constructed by isolation of the cDNA of MA-T12D11 producing hybridomas, followed by amplification and assembling of V<sub>H</sub> and V<sub>L</sub> with a (Gly<sub>4</sub>Ser)<sub>3</sub> linker.
- Affinity between TAFI and the antibody (fragments) was measured with surface plasmon resonance technique.
- The inhibitory effect of MA (derivatives) was tested in a chromogenic assay by activating TAFI with T/TM. Residual TAFIa activity was determined using a chromogenic assay in which hippuryl-arginine is used as a substrate.
- The profibrinolytic effect of MA (derivatives) was investigated with an *in vitro* clot lysis assay in which CaCl<sub>2</sub> was used to start clot formation and tPA to initiate clot lysis.

## Results

- The amino acid sequence of the V<sub>H</sub> and V<sub>L</sub> region of MA-T12D11 is shown in Figure 2. Based on this sequence, a model of scFv-T12D11 was obtained using the AbM software (Figure 3).

**V<sub>H</sub>:**  
 QVQLQQSGAELVKPGSSVKISCKASGYTFT **DHAIH**WVKQKPE  
 QGLEWIG **YISPGNGDVKYNEKFKG**KATLTADKSSSTAYMQLN  
 SLTSEDSAVYFCHH **GNWAAWFAY**WGQGTTVTSS  
  
**V<sub>L</sub>:**  
 DIVLTQSPASLAVSLGQRATISCK**ASQSV**YD**GGD**YLMWYQQ  
 RGPQPKLLIY **AASNLES**GIPARFSGSGSGTDFTLNIHPVEEE  
 DAATYYC**HQSNEDP**FTFGSGTKLEIKR

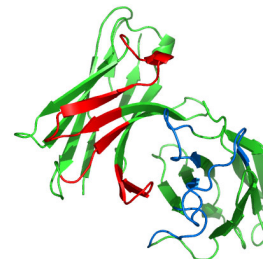


Figure 2: V<sub>H</sub> and V<sub>L</sub> chain of MA-T12D11 (CDR regions of V<sub>H</sub> and V<sub>L</sub> in red and blue, respectively)

Figure 3: Model of scFv-T12D11, obtained by modeling with AbM software (WAM server)

- The rate constants for association (k<sub>a</sub>) and dissociation (k<sub>d</sub>) and the affinity constant (K<sub>A</sub>) as well as the inhibitory effect of the antibody fragments on TAFI activity was compared to the parental MA-T12D11.

Table 1: Binding parameters (k<sub>a</sub>, k<sub>d</sub> and K<sub>A</sub>) of MA-T12D11 and its derivatives for binding to TAFI

T12D11	MA	Fab	scFv
k <sub>a</sub> (1/Ms)	1.3 ± 0.32 × 10 <sup>6</sup>	1.2 ± 0.38 × 10 <sup>6</sup>	5.4 ± 2.8 × 10 <sup>5</sup>
k <sub>d</sub> (1/s)	2.5 ± 0.28 × 10 <sup>-4</sup>	4.3 ± 0.33 × 10 <sup>-4</sup>	3.2 ± 0.46 × 10 <sup>-4</sup>
K <sub>A</sub> (1/M)	5.4 ± 1.5 × 10 <sup>9</sup>	2.8 ± 1.0 × 10 <sup>9</sup>	1.7 ± 0.75 × 10 <sup>9</sup>

The results represent mean ± SD (n ≥ 3)

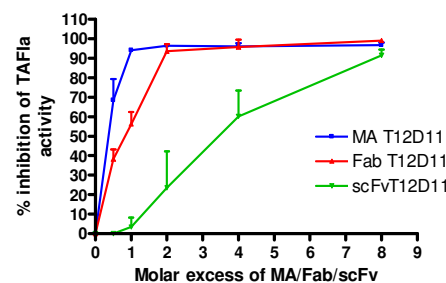


Figure 4: % inhibition of TAFIa activity by MA/Fab/scFv. TAFI was activated with T/TM in presence of MA, Fab or scFv. The results represent mean ± SD (n ≥ 3).

- Fab-T12D11 and scFv-T12D11 show a similar maximum inhibitory effect as MA-T12D11 i.e. 99.0 ± 0.72 % and 91.4 ± 3.0 % (Fab and scFv, respectively) vs. 96.7 ± 1.3 % for MA.

- The inhibitory effect of Fab-T12D11 and especially that of scFv-T12D11 shows a shift towards higher concentrations, in accordance to a slightly decreased affinity towards TAFI (table 1).

- Effect of MA-T12D11 and Fab-T12D11 in clot lysis. This effect is compared to that of CPI (a non-specific TAFIa inhibitor).

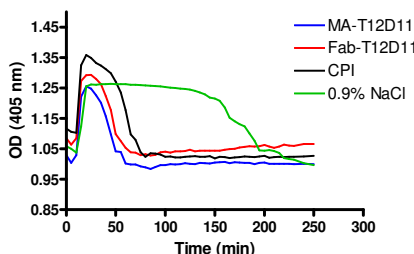


Figure 5: Profibrinolytic effect of MA, Fab and CPI during *in vitro* clot lysis. Clot formation and dissolution is shown in presence of 8-fold molar excess of MA-T12D11 (●), 8-fold molar excess of Fab-T12D11 (●), 45-fold molar excess of CPI (●) and 0.9% NaCl (negative control, ●). All concentrations are final concentrations. (n = 3). Note: concentration of scFv-T12D11 was too low to test it in a clot lysis assay.

- MA, Fab and CPI shorten clot lysis time significantly (i.e. 28.3 ± 2.9 min, 29.2 ± 10 min and 34.3 ± 1.2 min, respectively) vs. 137 ± 25.2 min for the negative control, demonstrating the powerful effect of both the MA and Fab fragment.

## Conclusions

- scFv-T12D11 as well as Fab-T12D11 have similar affinity constants as the parental MA.
- Fab-T12D11 has similar functional effects in the chromogenic assay and in the clot lysis.
- scFv-T12D11 has a similar functional effect in the chromogenic assay.