Dissolution of bi-component fibrin clots with plasmin: quantitation of the modulating effect of myosin

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Beginning of Pre-incubation :

fluid		V_{10}
gel	F_0', M_0'	V_{20}

$F_0' = F_0 + FM_0$ (1)

$M_0' = M_0 + FM_0$ (2)

Equilibrium condition:

$$k_a\left(\frac{F_0}{h_{20}}\right)\left(\frac{M_0}{h_{20}}\right) = k_d\left(\frac{FM_0}{h_{20}}\right)$$

$$FM_0^2 - (F_0' + M_0' + h_{20}.k_d/k_a).FM_0 + F_0'.M_0' = 0$$

$$FM_0 = \frac{F_0' + M_0' + h_{20}.k_d/k_a - \sqrt{\left(F_0' + M_0' + h_{20}.k_d/k_a\right)^2 - 4.F_0'.M_0'}}{2} \quad (3)$$

End of Pre-incubation:

fluid	E_0	V_{10}
V_R	M_0, F_0, FM_0	*fluid* *gel*
gel	F_0, M_0, FM_0	V_{20}

Current moment:

fluid	E_u, P_u, Q_u	V_1
V_R	M_x, EM, E_x, P_x, Q_x, F_x, FM_x, EFM, EF	*fluid* *gel*
gel	F_d, M_d, FM_d.	V_2

Table1. Species of molecules in the separate volumes

Volume	Height [m]	Initial Height [m]
V_1 (fluid phase)	h_1	h_{10}=2.6×10^{-3}
V_x (reactive layer)	r=4×10^{-5}	r_0=r=4×10^{-5}
V_2 (gel phase)	h_2–r	h_{20}–r_0=5.2×10^{-3} – 4 ×10^{-5}

Time t_e[s] is measured when $(F_x+F_d+EF+EFM+FM)$ reaches F_t[nmol/m^2].

First problem: model of the process in order to predict t_m as an estimate of t_e.

$E=E_x+E_u$ (4a)

$P=P_x+P_u$ (4b)

$Q=Q_x+Q_u$ (4c)

$F=F_x+F_d$ (4d)

$M=M_x+M_d$ (4e)

$FM=FM_x+FM_d$ (4f)

Convection dependencies

<u>Assumption 1.</u>: $w \times c_{V1} = c_{Vx}$ for the plasmin:

$$\frac{\mathbf{w.E_u}}{\mathbf{h_1}} = \frac{\mathbf{E_x + EF + EM + EFM}}{\mathbf{r}} \Rightarrow$$

$$E_x = \frac{w.r.E - h_1.(EF + EM + EFM)}{h_1 + w.r} \tag{5}$$

<u>Assumption 2.</u>: $c_{V2} = c_{Vx}$ for the fibrin, for the myosin and for the fibrin- myosin complex:

$$\frac{\mathbf{F_d}}{\mathbf{h_2 - r}} = \frac{\mathbf{F_x}}{\mathbf{r}} + \frac{\mathbf{EF}}{\mathbf{r}} \Rightarrow F_x = \frac{r.F - (h_2 - r).EF}{h_2} \tag{6}$$

$$\frac{\mathbf{M_d}}{\mathbf{h_2 - r}} = \frac{\mathbf{M_x}}{\mathbf{r}} + \frac{\mathbf{EM}}{\mathbf{r}} \Rightarrow M_x = \frac{r.M - (h_2 - r).EM}{h_2} \tag{7}$$

$$\frac{\mathbf{FM_d}}{\mathbf{h_2 - r}} = \frac{\mathbf{FM_x}}{\mathbf{r}} + \frac{\mathbf{EFM}}{\mathbf{r}} \Rightarrow FM_x = \frac{r.FM - (h_2 - r).EFM}{h_2} \tag{8a}$$

$$FM_d = \frac{(h_2 - r).FM + (h_2 - r).EFM}{h_2} \tag{8b}$$

<u>Assumption 3.</u>: $c_{V1} = c_{Vx}$ for fibrin degradation product, and for myosin degradation product

$$\frac{\mathbf{P_u}}{\mathbf{h_1}} = \frac{\mathbf{P_x}}{\mathbf{r}} \Rightarrow P_x = \frac{r}{h_1 + r} P \tag{9a}$$

$$P_u = \frac{h_1}{h_1 + r} P \tag{9b}$$

$$\frac{\mathbf{Q_u}}{\mathbf{h_1}} = \frac{\mathbf{Q_x}}{\mathbf{r}} \Rightarrow Q_x = \frac{r}{h_1 + r} Q \tag{10a}$$

$$Q_u = \frac{h_1}{h_1 + r} Q \tag{10b}$$

Process I: $$E_x + F_x \underset{k_{-1}}{\overset{k_1,\,s}{\rightleftarrows}} EF \xrightarrow{k_2} E_x + P_x$$

Process II: $$E_x + M_x \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} EM \xrightarrow{k_4} E_x + Q_x$$

Process III: $$E_x + FM_x \underset{k_{-5}}{\overset{k_5,\,g}{\rightleftarrows}} EFM \begin{cases} \xrightarrow{k_6} E_x + P_x + F_x \\ \xrightarrow{k_7} E_x + Q_x + M_x \end{cases}$$

Process IV : $$F_x + M_x \underset{k_d}{\overset{k_a}{\rightleftarrows}} FM_x$$

Process V: $$F_d + M_d \underset{k_d}{\overset{k_a}{\rightleftarrows}} FM_d$$

Rate equations for Process I: $E_x + F_x \underset{k_{-1}}{\overset{k_1,\, s}{\rightleftarrows}} EF \xrightarrow{k_2} E_x + P_x$

Formation of EF: $E_x + F_x \xrightarrow{k_1,\, s} EF$

$$\left(\frac{dEF}{dt}\right)_{I,f} = k_1\left(\frac{E_x}{r}\right)^s .F_x \qquad (12a)$$

$$\left(\frac{dE_x}{dt}\right)_{I,f} = -k_1\left(\frac{E_x}{r}\right)^s .F_x \qquad (12b)$$

$$\left(\frac{dF_x}{dt}\right)_{I,f} = -k_1\left(\frac{E_x}{r}\right)^s .F_x \qquad (12c)$$

Dissociation of EF: $E_x + F_x \xleftarrow[k_{-1}]{} EF$

$$\left(\frac{dEF}{dt}\right)_{I,d} = -k_{-1}.EF \qquad (13a)$$

$$\left(\frac{dE_x}{dt}\right)_{I,d} = k_{-1}.EF \qquad (13b)$$

$$\left(\frac{dF_x}{dt}\right)_{I,d} = k_{-1}.EF \qquad (13c)$$

Degradation of EF: $EF \xrightarrow{k_2} E_x + P_x$

$$\left(\frac{dEF}{dt}\right)_{I,dg} = -k_2.EF \qquad (14a)$$

$$\left(\frac{dE_x}{dt}\right)_{I,dg} = k_2.EF \qquad (14b)$$

$$\left(\frac{dP_x}{dt}\right)_{I,dg} = k_2.EF \qquad (14c)$$

Summarized equations for Process I :

$$\left(\frac{dEF}{dt}\right)_I = \left(\frac{dEF}{dt}\right)_{I,f} + \left(\frac{dEF}{dt}\right)_{I,d} + \left(\frac{dEF}{dt}\right)_{I,dg} \Rightarrow$$

$$\left(\frac{dEF}{dt}\right)_I = k_1\left(\frac{E_x}{r}\right)^s .F_x - k_{-1}.EF - k_2.EF \qquad (15a)$$

$$\left(\frac{dE_x}{dt}\right)_I = \left(\frac{dE_x}{dt}\right)_{I,f} + \left(\frac{dE_x}{dt}\right)_{I,d} + \left(\frac{dE_x}{dt}\right)_{I,dg} \Rightarrow$$

$$\left(\frac{dE_x}{dt}\right)_I = -k_1\left(\frac{E_x}{r}\right)^s .F_x + k_{-1}.EF + k_2.EF \qquad (15b)$$

$$\left(\frac{dF_x}{dt}\right)_I = \left(\frac{dF_x}{dt}\right)_{I,f} + \left(\frac{dF_x}{dt}\right)_{I,d} \Rightarrow \left(\frac{dF_x}{dt}\right)_I = -k_1\left(\frac{E_x}{r}\right)^s .F_x + k_{-1}.EF \qquad (15c)$$

$$\left(\frac{dP_x}{dt}\right)_I = \left(\frac{dP_x}{dt}\right)_{I,dg} \Rightarrow \left(\frac{dP_x}{dt}\right)_I = k_2.EF \qquad (15d)$$

Summarized equations for Process II : $E_x + M_x \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} EM \xrightarrow{k_4} E_x + Q_x$

$$\left(\frac{dEM}{dt}\right)_{II} = k_3\left(\frac{E_x}{r}\right).M_x - k_{-3}.EM - k_4.EM \qquad (16a)$$

$$\left(\frac{dE_x}{dt}\right)_{II} = -k_3\left(\frac{E_x}{r}\right).M_x + k_{-3}.EM + k_4.EM \qquad (16b)$$

$$\left(\frac{dM_x}{dt}\right)_{II} = -k_3\left(\frac{E_x}{r}\right).M_x + k_{-3}.EM \qquad (16c)$$

$$\left(\frac{dQ_x}{dt}\right)_{II} = k_4.EM \qquad (16d)$$

Rate equations for Process III: $$E_x + FM_x \underset{k_{-5}}{\overset{k_5,\,g}{\rightleftarrows}} EFM \begin{array}{l} \xrightarrow{k_6} E_x + P_x + F_x \\ \xrightarrow{k_7} E_x + Q_x + M_x \end{array}$$

Formation of *EFM*: $E_x + FM_x \xrightarrow{k_5,\,g} EFM$

$$\left(\frac{dEFM}{dt}\right)_{III,f} = k_5\left(\frac{E_x}{r}\right)^g .FM_x \quad (17a)$$

$$\left(\frac{dE_x}{dt}\right)_{III,f} = -k_5\left(\frac{E_x}{r}\right)^g .FM_x \quad (17b)$$

$$\left(\frac{dFM_x}{dt}\right)_{III,f} = -k_5\left(\frac{E_x}{r}\right)^g .FM_x \quad (17c)$$

Dissociation of *EFM*: $E_x + FM_x \xleftarrow[k_{-5}]{} EFM$

$$\left(\frac{dEFM}{dt}\right)_{III,d} = -k_{-5}.EFM \quad (18a)$$

$$\left(\frac{dE_x}{dt}\right)_{III,d} = k_{-5}.EFM \quad (18b)$$

$$\left(\frac{dFM_x}{dt}\right)_{III,d} = k_{-5}.EFM \quad (18c)$$

Degradation 1 of *EFM*: $EFM \xrightarrow{k_6} E_x + P_x + F_x$

$$\left(\frac{dEFM}{dt}\right)_{III,dg1} = -k_6.EFM \quad (19a)$$

$$\left(\frac{dE_x}{dt}\right)_{III,dg1} = k_6.EFM \quad (19b)$$

$$\left(\frac{dP_x}{dt}\right)_{III,dg1} = k_6.EFM \quad (19c)$$

$$\left(\frac{dF_x}{dt}\right)_{III,dg1} = k_6.EFM \quad (19d)$$

Degradation 2 of *EFM*: $EFM \xrightarrow{k_7} E_x + Q_x + M_x$

$$\left(\frac{dEFM}{dt}\right)_{III,dg2} = -k_7.EFM \quad (20a)$$

$$\left(\frac{dE_x}{dt}\right)_{III,dg2} = k_7.EFM \quad (20b)$$

$$\left(\frac{dQ_x}{dt}\right)_{III,dg2} = k_7.EFM \quad (20c)$$

$$\left(\frac{dM_x}{dt}\right)_{III,dg2} = k_7.EFM \quad (20d)$$

Summarized equations for Process III

$$\left(\frac{dEFM}{dt}\right)_{III} = \left(\frac{dEFM}{dt}\right)_{III,f} + \left(\frac{dEFM}{dt}\right)_{III,d} + \left(\frac{dEFM}{dt}\right)_{III,dg1} + \left(\frac{dEFM}{dt}\right)_{III,dg2} \Rightarrow$$

$$\left(\frac{dEFM}{dt}\right)_{III} = k_5\left(\frac{E_x}{r}\right)^g .FM_x - k_{-5}.EFM - k_6.EFM - k_7.EFM \qquad (21a)$$

$$\left(\frac{dE_x}{dt}\right)_{III} = \left(\frac{dE_x}{dt}\right)_{III,f} + \left(\frac{dE_x}{dt}\right)_{III,d} + \left(\frac{dE_x}{dt}\right)_{III,dg1} + \left(\frac{dE_x}{dt}\right)_{III,dg2} \Rightarrow$$

$$\left(\frac{dE_x}{dt}\right)_{III} = -k_5\left(\frac{E_x}{r}\right)^g .FM_x + k_{-5}.EFM + k_6.EFM + k_7.EFM \qquad (21b)$$

$$\left(\frac{dFM_x}{dt}\right)_{III} = \left(\frac{dFM_x}{dt}\right)_{III,f} + \left(\frac{dFM_x}{dt}\right)_{III,d} \Rightarrow$$

$$\left(\frac{dFM_x}{dt}\right)_{III} = -k_5\left(\frac{E_x}{r}\right)^g .FM_x + k_{-5}.EFM \qquad (21c)$$

$$\left(\frac{dP_x}{dt}\right)_{III} = \left(\frac{dP_x}{dt}\right)_{III,dg1} \Rightarrow \left(\frac{dP_x}{dt}\right)_{III} = k_6.EFM \qquad (21d)$$

$$\left(\frac{dF_x}{dt}\right)_{III} = \left(\frac{dF_x}{dt}\right)_{III,dg1} \Rightarrow \left(\frac{dF_x}{dt}\right)_{III} = k_6.EFM \qquad (21e)$$

$$\left(\frac{dQ_x}{dt}\right)_{III} = \left(\frac{dQ_x}{dt}\right)_{III,dg2} \Rightarrow \left(\frac{dQ_x}{dt}\right)_{III} = k_7.EFM \qquad (21f)$$

$$\left(\frac{dM_x}{dt}\right)_{III} = \left(\frac{dM_x}{dt}\right)_{III,dg2} \Rightarrow \left(\frac{dM_x}{dt}\right)_{III} = k_7.EFM \qquad (21g)$$

Rate equations for Process IV: $F_x + M_x \underset{k_d}{\overset{k_a}{\rightleftarrows}} FM_x$

Formation of FM_X**:** $F_x + M_x \xrightarrow{k_a} FM_x$

$$\left(\frac{dFM_x}{dt}\right)_{IV,f} = k_a\left(\frac{F_x}{r}\right).M_x \quad (22a)$$

$$\left(\frac{dF_x}{dt}\right)_{IV,f} = -k_a\left(\frac{F_x}{r}\right).M_x \quad (22b)$$

$$\left(\frac{dM_x}{dt}\right)_{IV,f} = -k_a\left(\frac{F_x}{r}\right).M_x \quad (22c)$$

Dissociation of FM_X**:** $F_x + M_x \xleftarrow[k_d]{} FM_x$

$$\left(\frac{dFM_x}{dt}\right)_{IV,d} = -k_d.FM_x \quad (23a)$$

$$\left(\frac{dF_x}{dt}\right)_{IV,d} = k_d.FM_x \quad (23b)$$

$$\left(\frac{dM_x}{dt}\right)_{IV,d} = k_d.FM_x \quad (23c)$$

Summarized equations for Process IV:

$$\left(\frac{dFM_x}{dt}\right)_{IV} = \left(\frac{dFM_x}{dt}\right)_{IV,f} + \left(\frac{dFM_x}{dt}\right)_{IV,d} \Rightarrow$$

$$\left(\frac{dFM_x}{dt}\right)_{IV} = k_a.\frac{F_x}{r}.M_x - k_d.FM_x \tag{24a}$$

$$\left(\frac{dF_x}{dt}\right)_{IV} = \left(\frac{dF_x}{dt}\right)_{IV,f} + \left(\frac{dF_x}{dt}\right)_{IV,d} \Rightarrow$$

$$\left(\frac{dF_x}{dt}\right)_{IV} = -k_a.\frac{F_x}{r}.M_x + k_d.FM_x \tag{24b}$$

$$\left(\frac{dM_x}{dt}\right)_{IV} = \left(\frac{dM_x}{dt}\right)_{IV,f} + \left(\frac{dM_x}{dt}\right)_{IV,d} \Rightarrow$$

$$\left(\frac{dM_x}{dt}\right)_{IV} = -k_a.\frac{F_x}{r}.M_x + k_d.FM_x \tag{24c}$$

Summarized equations for Process V: $F_d + M_d \underset{k_d}{\overset{k_a}{\rightleftarrows}} FM_d$

$$\left(\frac{dFM_d}{dt}\right)_{V} = k_a.\frac{F_d}{h_2 - r}.M_d - k_d.FM_d \tag{25a}$$

$$\left(\frac{dF_d}{dt}\right)_{V} = -k_a.\frac{F_d}{h_2 - r}.M_d + k_d.FM_d \tag{25b}$$

$$\left(\frac{dM_d}{dt}\right)_{V} = -k_a.\frac{F_d}{h_2 - r}.M_d + k_d.FM_d \tag{25c}$$

Summary of the differential rate-equations for basic substances:

$$\frac{dEF}{dt}=\left(\frac{dEF}{dt}\right)_{I}\Rightarrow\frac{dEF}{dt}=k_1\left(\frac{E_x}{r}\right)^{s}.F_x-k_{-1}.EF-k_2.EF \tag{26a}$$

$$\frac{dEM}{dt}=\left(\frac{dEM}{dt}\right)_{II}\Rightarrow\frac{dEM}{dt}=k_3\left(\frac{E_x}{r}\right).M_x-k_{-3}.EM-k_4.EM \tag{26b}$$

$$\frac{dEFM}{dt}=\left(\frac{dEFM}{dt}\right)_{III}\Rightarrow$$

$$\frac{dEFM}{dt}=k_5\left(\frac{E_x}{r}\right)^{g}.FM_x-k_{-5}.EFM-k_6.EFM-k_7.EFM \tag{26c}$$

$$\frac{dFM_x}{dt}=\left(\frac{dFM_x}{dt}\right)_{III}+\left(\frac{dFM_x}{dt}\right)_{IV}\Rightarrow$$

$$\frac{dFM_x}{dt}=-k_5\left(\frac{E_x}{r}\right)^{g}.FM_x+k_{-5}.EFM+k_a.\frac{F_x}{r}.M_x-k_d.FM_x \tag{26d}$$

$$\frac{dFM_d}{dt}=\left(\frac{dFM_d}{dt}\right)_{V}\Rightarrow\frac{dFM_d}{dt}=k_a.\frac{F_d}{h_2-r}.M_d-k_d.FM_d \tag{26e}$$

$$\frac{dQ_x}{dt}=\left(\frac{dQ_x}{dt}\right)_{II}+\left(\frac{dQ_x}{dt}\right)_{III}\Rightarrow\frac{dQ_x}{dt}=k_4.EM+k_7.EFM \tag{26f}$$

$$\frac{dQ_u}{dt}=0 \tag{26g}$$

$$\frac{dP_x}{dt}=\left(\frac{dP_x}{dt}\right)_{II}+\left(\frac{dP_x}{dt}\right)_{III}\Rightarrow\frac{dP_x}{dt}=k_2.EF+k_6.EFM \tag{26h}$$

$$\frac{dP_u}{dt}=0 \tag{26i}$$

$$\frac{dE_x}{dt} = \left(\frac{dE_x}{dt}\right)_I + \left(\frac{dE_x}{dt}\right)_{II} + \left(\frac{dE_x}{dt}\right)_{III} \Rightarrow$$

$$\frac{dE_x}{dt} = -k_1\left(\frac{E_x}{r}\right)^s.F_x + k_{-1}.EF + k_2.EF - k_3\left(\frac{E_x}{r}\right).M_x + k_{-3}.EM + \\ + k_4 EM - k_5\left(\frac{E_x}{r}\right)^g.FM_x + k_{-5}.EFM + k_6.EFM + k_7.EFM \tag{26j}$$

$$\frac{dE_u}{dt} = 0 \tag{26k}$$

$$\frac{dF_x}{dt} = \left(\frac{dF_x}{dt}\right)_I + \left(\frac{dF_x}{dt}\right)_{III} + \left(\frac{dF_x}{dt}\right)_{IV} \Rightarrow$$

$$\frac{dF_x}{dt} = -k_1\left(\frac{E_x}{r}\right)^s.F_x + k_{-1}.EF + k_6.EFM - k_a.\frac{F_x}{r}.M_x + k_d.FM_x \tag{26l}$$

$$\frac{dF_d}{dt} = \left(\frac{dF_d}{dt}\right)_V \Rightarrow \frac{dF_d}{dt} = -k_a \cdot \frac{F_d}{h_2 - r}.M_d + k_d.FM_d \tag{26m}$$

$$\frac{dM_x}{dt} = \left(\frac{dM_x}{dt}\right)_{II} + \left(\frac{dM_x}{dt}\right)_{III} + \left(\frac{dM_x}{dt}\right)_{IV} \Rightarrow$$

$$\frac{dM_x}{dt} = -k_3\left(\frac{E_x}{r}\right).M_x + k_{-3}.EM + k_7.EFM - k_a.\frac{F_x}{r}.M_x + k_d.FM_x \tag{26n}$$

$$\frac{dM_d}{dt} = \left(\frac{dM_d}{dt}\right)_V \Rightarrow \frac{dM_d}{dt} = -k_a \cdot \frac{F_d}{h_2 - r}.M_d + k_d.FM_d \tag{26o}$$

Summary of the differential rate-equations for the artificial substances (4):

$$\frac{dE}{dt} = \frac{dE_x}{dt} + \frac{dE_u}{dt} \Rightarrow$$

$$\frac{dE}{dt} = -k_1\left(\frac{E_x}{r}\right)^s .F_x + k_{-1}.EF + k_2.EF - k_3\left(\frac{E_x}{r}\right).M_x + k_{-3}.EM + $$
$$+ k_4 EM - k_5\left(\frac{E_x}{r}\right)^g .FM_x + k_{-5}.EFM + k_6.EFM + k_7.EFM \qquad (27a)$$

$$\frac{dP}{dt} = \frac{dP_x}{dt} + \frac{dP_u}{dt} \Rightarrow$$

$$\frac{dP}{dt} = k_2.EF + k_6.EFM \qquad (27b)$$

$$\frac{dQ}{dt} = \frac{dQ_x}{dt} + \frac{dQ_u}{dt} \Rightarrow$$

$$\frac{dQ}{dt} = k_4.EM + k_7.EFM \qquad (27c)$$

$$\frac{dF}{dt} = \frac{dF_x}{dt} + \frac{dF_d}{dt} \Rightarrow$$

$$\frac{dF}{dt} = -k_1\left(\frac{E_x}{r}\right)^s .F_x + k_{-1}.EF + k_6.EFM - k_a.\frac{F_x}{r}.M_x + $$
$$+ k_d.FM_x - k_a.\frac{F_d}{h_2 - r}.M_d + k_d.FM_d \qquad (27d)$$

$$\frac{dM}{dt} = \frac{dM_x}{dt} + \frac{dM_d}{dt} \Rightarrow$$

$$\frac{dM}{dt} = -k_3\left(\frac{E_x}{r}\right).M_x + k_{-3}.EM + k_7.EFM - k_a.\frac{F_x}{r}.M_x + $$
$$+ k_d.FM_x - k_a.\frac{F_d}{h_2 - r}.M_d + k_d.FM_d \qquad (27e)$$

$$\frac{dFM}{dt} = \frac{dFM_x}{dt} + \frac{dFM_d}{dt} \Rightarrow$$

$$\frac{dFM}{dt} = -k_5\left(\frac{E_x}{r}\right)^g FM_x + k_{-5}.EFM + k_a\left(\frac{F_x.M_x}{r} + \frac{F_d.M_d}{h_2 - r}\right) - k_d.FM \qquad (27f)$$

Mass dependencies

Mass dependence for the plasmin

$$\frac{dE}{dt}+\frac{dEF}{dt}+\frac{dEM}{dt}+\frac{dEFM}{dt}=0$$

$E\text{-}E_0+EF\text{-}EF_0+EM\text{-}EM_0+EFM\text{-}EFM_0=0$,
$EF_0=EM_0=EFM_0=0$,
$E=E_0\text{-}EF\text{-}EM\text{-}EFM$ (28)

Mass dependence for the fibrin

$$\frac{dF}{dt}+\frac{dFM}{dt}+\frac{dEF}{dt}+\frac{dEFM}{dt}+\frac{dP}{dt}=0$$

$F\text{-}F_0+FM\text{-}FM_0+EF\text{-}EF_0+EFM\text{-}EFM_0+P\text{-}P_0=0$,
$FM_0=EF_0=EFM_0=0$
$F_0'=F+FM+EF+EFM+P \Rightarrow$
$F=F_0'\text{-}FM\text{-}EF\text{-}EFM\text{-}P$ (29)

Mass dependence for the myosin

$$\frac{dM}{dt}+\frac{dFM}{dt}+\frac{dEM}{dt}+\frac{dEFM}{dt}+\frac{dQ}{dt}=0$$

$M\text{-}M_0+FM\text{-}FM_0+EM\text{-}EM_0+EFM\text{-}EFM_0+Q\text{-}Q_0=0$,
$FM_0=EF_0=EFM_0=0$,
$M_0'=M+FM+EM+EFM+Q \Rightarrow$
$M= M_0'\text{-}FM\text{-}EM\text{-}EFM\text{-}Q$ (30)

Geometric dependence for the layers' height

$$\frac{h_2}{F_o'-P}=\frac{h_{20}}{F_o'} \Rightarrow$$

$$h_2=h_{20}-\frac{P}{F_o'}h_{20} \quad (31a)$$

$$h_1+h_2=h_{10}+h_{20} \Rightarrow h_1=h_{10}+h_{20}-(h_{20}-\frac{P}{F_o'}h_{20}) \Rightarrow$$

$$h_1=h_{10}+\frac{P}{F_o'}h_{20} \quad (31b)$$

Model with myosin using the time *t* as independent variable

$$\begin{cases} \dfrac{dEF}{dt} = k_1\left(\dfrac{E_x}{r}\right)^s .F_x - k_{-1}.EF - k_2.EF \\ \dfrac{dEM}{dt} = k_3\left(\dfrac{E_x}{r}\right).M_x - k_{-3}.EM - k_4.EM \\ \dfrac{dEFM}{dt} = k_5\left(\dfrac{E_x}{r}\right)^g .FM_x - k_{-5}.EFM - k_6.EFM - k_7.EFM \\ \dfrac{dFM}{dt} = -k_5\left(\dfrac{E_x}{r}\right)^g FM_x + k_{-5}.EFM + k_a\left(\dfrac{F_x.M_x}{r} + \dfrac{F_d.M_d}{h_2 - r}\right) - k_d.FM \\ \dfrac{dQ}{dt} = k_4.EM + k_7.EFM \\ \dfrac{dP}{dt} = k_2.EF + k_6.EFM \end{cases} \tag{35a}$$

where:

$$\begin{cases} h_1 = h_{10} + \dfrac{P}{F_o^{'}} h_{20} \\ h_2 = h_{20} - \dfrac{P}{F_o^{'}} h_{20} \\ E_x = \dfrac{r}{r + \dfrac{h_1}{w}} E_0 - EF - EM - EFM \\ F_x = \dfrac{r}{h_2}\left(F_0^{'} - FM - EFM - P\right) - EF \\ M_x = \dfrac{r}{h_2}\left(M_0^{'} - FM - EFM - Q\right) - EM \\ FM_x = \dfrac{r.FM - (h_2 - r).EFM}{h_2} \\ F_d = \dfrac{h_2 - r}{h_2}\left(F_0^{'} - FM - EFM - P\right) \\ M_d = \dfrac{h_2 - r}{h_2}\left(M_0^{'} - FM - EFM - Q\right) \end{cases} \tag{35b}$$

Initial conditions at *t=0* :

$EF_{ini}=0$, $EM_{ini}=0$, $EFM_{ini}=0$, $FM_{ini}=FM_0$, $Q_{ini}=0$, $P_{ini}=0$ (35c)

Model with myosin using the product *P* as independent variable

$$\frac{dEF}{dP}=\frac{\frac{dEF}{dt}}{\frac{dP}{dt}}=\frac{k_1\left(\frac{E_x}{r}\right)^s.F_x-k_{-1}.EF-k_2.EF}{k_2.EF+k_6.EFM}$$

$$\frac{dEM}{dP}=\frac{\frac{dEM}{dt}}{\frac{dP}{dt}}=\frac{k_3\left(\frac{E_x}{r}\right).M_x-k_{-3}.EM-k_4.EM}{k_2.EF+k_6.EFM}$$

$$\frac{dEFM}{dP}=\frac{\frac{dEFM}{dt}}{\frac{dP}{dt}}=\frac{k_5\left(\frac{E_x}{r}\right)^g.FM_x-k_{-5}.EFM-k_6.EFM-k_7.EFM}{k_2.EF+k_6.EFM} \qquad (36a)$$

$$\frac{dFM}{dP}=\frac{\frac{dFM}{dt}}{\frac{dP}{dt}}=\frac{-k_5\left(\frac{E_x}{r}\right)^g FM_x+k_{-5}.EFM+k_a\left(\frac{F_x.M_x}{r}+\frac{F_d.M_d}{h_2-r}\right)-k_d.FM}{k_2.EF+k_6.EFM}$$

$$\frac{dQ}{dP}=\frac{\frac{dQ}{dt}}{\frac{dP}{dt}}=\frac{k_4.EM+k_7.EFM}{k_2.EF+k_6.EFM}$$

$$\frac{dt}{dP}=\frac{1}{\frac{dP}{dt}}=\frac{1}{k_2.EF+k_6.EFM}$$

where (35b) holds.

Initial conditions at *P=0*:

$EF_{ini}=0$, $EM_{ini}=0$, $EFM_{ini}=0$, $FM_{ini}=FM_0$, $Q_{ini}=0$, $t_{ini}=0$ (36b)

$$F_t=F_x+F_d+EF+EFM+FM=F+EF+EFM+FM=$$
$$=F_0^{'}-EF-EFM-FM-P_t+EF+EFM+FM=F_0^{'}-P_t\Rightarrow$$

$P_t=F_0'-F_t$ (37)

<u>Approach 1</u>: "precise and slow"

1) Integrate (35a) from $\boldsymbol{t=0}$ [s] to $\boldsymbol{t=\varepsilon}$ [s],

Initial conditions at $\boldsymbol{t=0}$:

$EF_{ini}=0$, $EM_{ini}=0$, $EFM_{ini}=0$, $FM_{ini}=FM_0$, $Q_{ini}=0$, $P_{ini}=0$

2) Integrate (36a) from $P=P_\varepsilon$ [nmol/m^2] to $P=P_t$ [nmol/m^2],

Initial conditions at $P=P_\varepsilon$:

$$EF_{ini}=EF_\varepsilon,\ EM_{ini}=EM_\varepsilon,\ EFM_{ini}=EMF_\varepsilon,$$
$$FM_{ini}=FM_\varepsilon,\ Q_{ini}=Q_\varepsilon,\ t_{ini}=\varepsilon \tag{39}$$

<u>Approach 2</u>: "quick and dirty"

1) Estimate feasible non-singular values for the initial complexes

$$EF_f = Min\left\{ \frac{r}{r+\frac{h_1}{w}} E_0 / 4; \quad \frac{r}{h_2}(F_0' - FM_0)/2 \right\} \tag{40a}$$

$$EM_f = Min\left\{ \frac{r}{r+\frac{h_1}{w}} E_0 / 4; \quad \frac{r}{h_2}(M_0' - FM_0)/2 \right\} \tag{40b}$$

$$EFM_f = Min\left\{ \frac{r}{r+\frac{h_1}{w}} E_0 / 4; \quad \frac{r}{h_2} FM_0 / 2 \right\} \tag{40c}$$

$$FM_f = FM_0 - EFM_f \tag{40d}$$

2) Integrate (36a) from $P=0$ [nmol/m^2] to $P=P_t$ [nmol/m^2],

Initial conditions at $P=0$:

$$EF_{ini}=EF_f,\ EM_{ini}=EM_f,\ EFM_{ini}=EMF_f,$$
$$FM_{ini}=FM_0 - EMF_f,\ Q_{ini}=0,\ t_{ini}=0 \tag{41}$$

40% faster

Model without myosin using the time *t* as independent variable

$M=M_x=Md=FM=FM_x=FM_d=EM=EFM=Q=Q_x=Q_u= 0$ (42a)

$M_0' =FM_0=0; F_0=F_0'$ (42b)

$$\begin{cases} \dfrac{dEF}{dt} = k_1\left(\dfrac{E_x}{r}\right)^s .F_x - k_{-1}.EF - k_2.EF \\ \dfrac{dP}{dt} = k_2.EF \end{cases} \quad (43a)$$

where:

$$\begin{cases} h_1 = h_{10} + \dfrac{P}{F_0'} h_{20} \\ h_2 = h_{20} - \dfrac{P}{F_0'} h_{20} \\ E_x = \dfrac{r}{r + \dfrac{h_1}{w}} E_0 - EF \\ F_x = \dfrac{r}{h_2}\left(F_0' - P\right) - EF \end{cases} \quad (43b)$$

Initial conditions in *t=0*:

$EF_{ini}=0, \qquad P_{ini}=0$ (43c)

Model without myosin using the product *P* as independent variable

$$\frac{dEF}{dP} = \frac{\frac{dEF}{dt}}{\frac{dP}{dt}} = \frac{k_1\left(\frac{E_x}{r}\right)^s .F_x - k_{-1}.EF - k_2.EF}{k_2.EF}$$

$$\frac{dt}{dP} = \frac{1}{\frac{dP}{dt}} = \frac{1}{k_2.EF} \qquad (44a)$$

where (43b) holds.

Initial conditions at *P=0* :

$EF_{ini}=0$, $t_{ini}=0$ (44b)

$$F_t = F_x + F_d + EF = F + EF = F_0^{'} - EF - P_t + EF = F_0^{'} - P_t \Rightarrow$$
$P_t = F_0' - F_t$ (45)

<u>Approach 3</u>: "precise and slow"

1) Integrate (43a) from $\boldsymbol{t=0}$ [s] to $\boldsymbol{t=\varepsilon}$ [s],

Initial conditions at $\boldsymbol{t=0}$:

$\boldsymbol{EF_{ini}=0}$, $\boldsymbol{P_{ini}=0}$

2) Integrate (44a) from $\boldsymbol{P=P_\varepsilon}$ [nmol/m^2] to $\boldsymbol{P=P_t}$ [nmol/m^2],

Initial conditions at $\boldsymbol{P=P_\varepsilon}$:

$$\boldsymbol{EF_{ini}=EF_\varepsilon}, \quad \boldsymbol{t_{ini}=\varepsilon} \tag{46}$$

<u>Approach 4</u>:"quick and dirty"

1) Estimate feasible settled value for the initial plasmin-fibrin complex

$$\frac{\boldsymbol{dEF}}{\boldsymbol{dt}} \approx 0 \Rightarrow$$

$$\boldsymbol{k_1}\left(\frac{\boldsymbol{E_0}}{\boldsymbol{r}+\frac{\boldsymbol{h_1}}{\boldsymbol{w}}}-\frac{\boldsymbol{EF_f}}{\boldsymbol{r}}\right)^{s}\cdot\left(\frac{\boldsymbol{r}}{\boldsymbol{h_2}}\boldsymbol{F_0'}-\boldsymbol{EF_f}\right)-\boldsymbol{k_{-1}}.\boldsymbol{EF_f}-\boldsymbol{k_2}.\boldsymbol{EF_f}=\boldsymbol{0} \tag{47}$$

2) Integrate (44a) from $\boldsymbol{P=0}$ [nmol/m^2] to $\boldsymbol{P=P_t}$ [nmol/m^2],

Initial conditions at $\boldsymbol{P=0}$:

$$\boldsymbol{EF_{ini}=EF_f}, \quad \boldsymbol{t_{ini}=0} \tag{48}$$

50% faster

Second problem: identify the parameters

k_1, k_{-1}, k_2, s, k_3, k_{-3}, k_4, k_5, k_{-5}, k_6, k_7 and ***g***

Given

Known: k_a=1.728×10^{-10} [nmol^{-1}.m^3.s^{-1}] , k_d=3.2×10^{-4} [s^{-1}], **w**=10[-]

Experiment: $t_e^{(i,j,k)}$ measured at E_0(i), M_0'(j), F_0' and F_t(k)
Replicas 3 for each point
E_0(i)× $\mathbf{h_{10}}$ ∈ {0.08, 0.16, 0.32, 0.64, 1.28, 2.56} [μM]
M_0'(j)× $\mathbf{h_{20}}$ ∈ {0, 0.42, 0.8, 1.26, 1.6, 3.2} [μM]
F_0'× $\mathbf{h_{20}}$=5.57 [μM]
F_t(k)/F_0' ∈ {59, 47, 43, 36, 25, 17, 14} [%]

Solution

Model: calculate $t_m^{(i,j,k)}$ measured at E_0(i), M_0'(j), F_0' and F_t(k)

Software: MATLAB

Working horse: ode15s for stiff differential equations, variable order method from the Shampine's ODE suit

Jacobiants: jacobiants of (35a), (36a), (43a), and (44a) are derived and used in ode15s

Identify:
k_1, k_{-1}, k_2, s
by χ^2 minimization for all experiments with M_0'(i)=0
MATLAB optimization toolbox

Identify:
k_3, k_{-3}, k_4, k_5, k_{-5}, k_6, k_7, g
by χ^2 minimization for all experiments with M_0'(i)>0
where ***k_1, k_{-1}, k_2, s*** are held fixed to the identified values
MATLAB optimization toolbox

Kinetic parameters calculated from the plasmin-catalysed dissolution of fibrin containing myosin

Parameter	Measurement unit	Value
rate constant for the formation of the plasmin-fibrin complex (k_1)	$m^{3s}.nmol^{-s}.s^{-1}$	0.14
rate constant for the dissociation of the plasmin-fibrin complex (k_{-1})	s^{-1}	98.87
rate constant for the degradation of fibrin in the plasmin-fibrin complex (k_2)	s^{-1}	0.14
exponent of the enzyme concentration in the gel phase fibrin reaction (s)	-	0.32
rate constant for the formation of the plasmin-myosin complex (k_3)	$m^3.nmol^{-1}.s^{-1}$	2.98×10^{-6}
rate constant for the dissociation of the plasmin-myosin complex (k_{-3})	s^{-1}	196.4
rate constant for the degradation of myosin in the plasmin-myosin complex (k_4)	s^{-1}	0.91
rate constant for the formation of the plasmin-myosin-fibrin complex (k_5)	$m^{3g}.nmol^{-g}.s^{-1}$	1.98×10^{-4}
rate constant for the dissociation of the plasmin-myosin-fibrin complex (k_{-5})	s^{-1}	0.71
rate constant for the degradation of fibrin in the plasmin-myosin-fibrin complex (k_6)	s^{-1}	2.90×10^{-3}
rate constant for the degradation of myosin in the plasmin-myosin-fibrin complex (k_7)	s^{-1}	0.038
exponent of the enzyme concentration in the gel phase fibrin-myosin reaction (g)	-	0.079
optimized value of the relative square error of the function (χ^2)	%	11.72

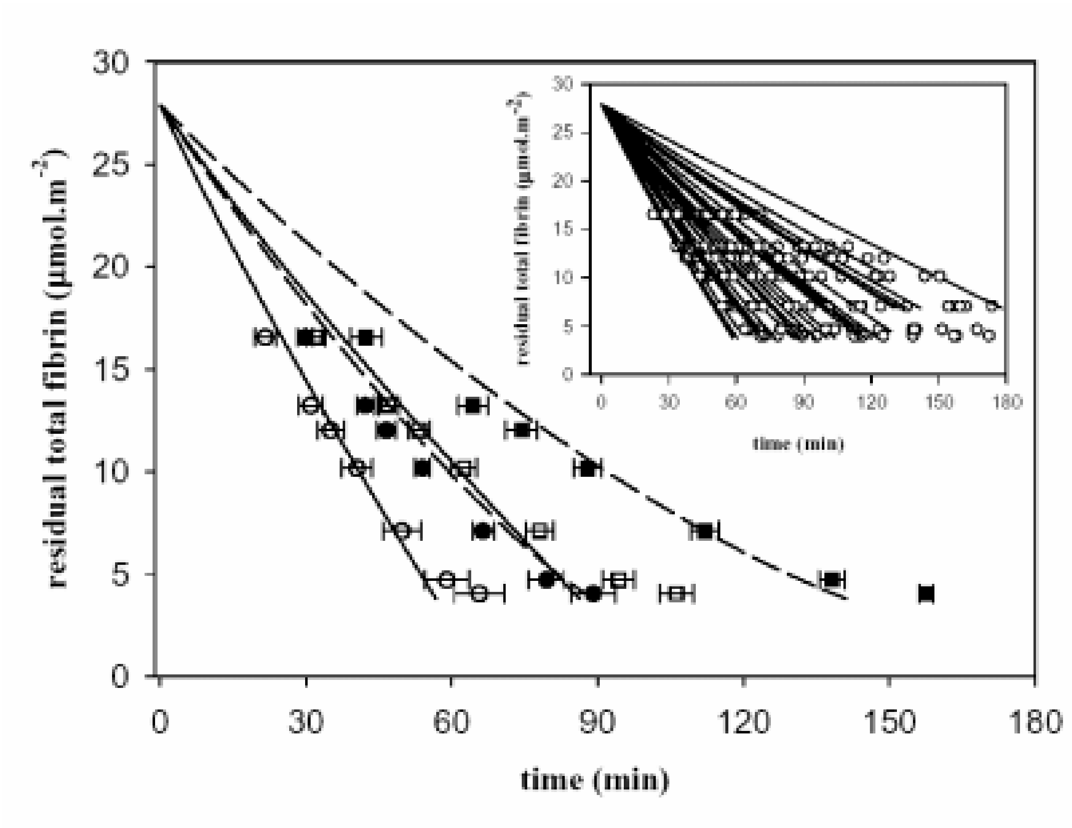

Figure 3

Legend

○ – mean experiment value $M_0' \times \mathbf{h_{20}}$=0 [μM], $E_0 \times \mathbf{h_{10}}$=2.56 [μM]

□ – mean experiment value $M_0' \times \mathbf{h_{20}}$=0 [μM], $E_0 \times \mathbf{h_{10}}$=0.32 [μM]

● – mean experiment value $M_0' \times \mathbf{h_{20}}$=3.2 [μM], $E_0 \times \mathbf{h_{10}}$=2.56 [μM]

■ – mean experiment value $M_0' \times \mathbf{h_{20}}$=3.2 [μM], $E_0 \times \mathbf{h_{10}}$=0.32 [μM]

|----| – standard deviation

Solid lines – without myosin

Dashed lines – with myosin

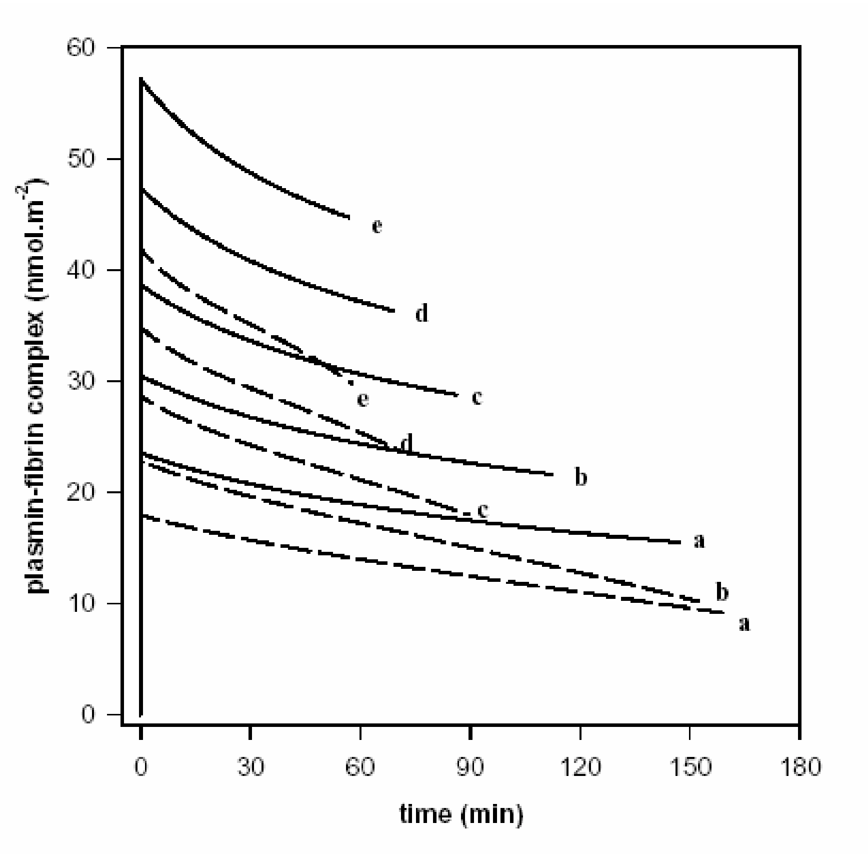

Figure 4A

Legend

Solid lines – $\boldsymbol{M_0}'\times\mathbf{h_{20}}$=0 [μM]

Dashed lines – $\boldsymbol{M_0}'\times\mathbf{h_{20}}$=3.2 [μM]

a – $\boldsymbol{E_0}\times\mathbf{h_{10}}$=0.16 [μM]

b – $\boldsymbol{E_0}\times\mathbf{h_{10}}$=0.32 [μM]

c – $\boldsymbol{E_0}\times\mathbf{h_{10}}$=0.64 [μM]

d – $\boldsymbol{E_0}\times\mathbf{h_{10}}$=1.28 [μM]

e – $\boldsymbol{E_0}\times\mathbf{h_{10}}$=2.56 [μM]

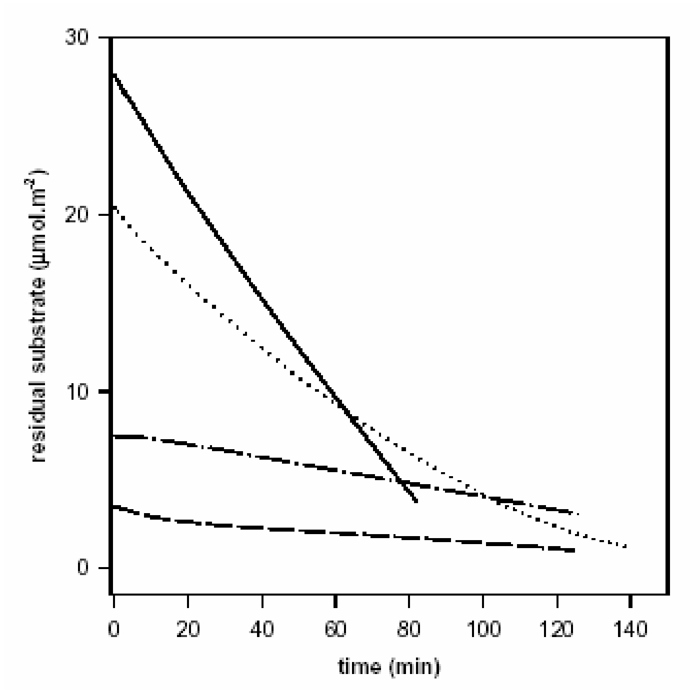

Figure 4B – Fibrin and myosin at 2.5 molar ratio digested with plasmin $\boldsymbol{E_0} \times \mathbf{h_{10}}$=0.32 [µM]

Legend

Dashed line – free fibrin

Dotted line – myosin

Dash-dotted line – fibrin-myosin complex

Solid line – monocomponent fibrin without myosin

Conclusions:

- *The substrate competition for the enzyme (fig. 4B) acts as inhibitor for fibrin digestion.*

- *In Initial period (first 10 min of the example) the fibrin degradation is blocked in the fibrin-myosin complex because of the low k_6 and can proceed efficiently only after the removal of the myosin (at the same time free myosin is preferentially degraded).*

- *The myosin forms a shield on the fibrin fibers, which is removed preferably through degradation of the free myosin followed by dissociation of the myosin-fibrin complex (k_4, k_7).*

- *Plasmin removes at first the myosin in the myosin-fibrin complex and only following this the fibrin network becomes susceptible for digestion (comparing k_6 and k_7).*

- *The myosin is worse co-factor of the plasminogen activator than the fibrin. That is the third inhibitor effect of the myosin over the fibrinolysis.*

General conclusion:

The delay of fibrinolysis in the presence of myosin can be attributed not only to the competing nature of the two substrates, but also to the formation of a complex that is relatively resistant to plasmin. The amount of plasmin in complex with fibrin varies in parallel with the changes in the availability of free fibrin, thus the enzyme-catalysed process is not in a steady-state.