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Letter to the Editor

Comments on "Sensitive immunoassays of nitrated fibrinogen in human biofluids" by Tang et al

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Dear Editor,

Tang et al. recently reported three sensitive new sandwich immunoassays for detection of nitrated biomarker with nitrated human fibrinogen as target [1]. The article is interesting not only because of new immunoassays of nitrated fibrinogen in human biofluids, but even more because it presents results of LC–MS/MS identified nitrotyrosine sites of *in vitro* nitrated fibrinogen. It may be helpful in explanation of biological functions of fibrinogen under nitration *in vivo*. Though there are some wrong assumptions considering quantity of tyrosines in fibrinogen's α and γ chains.

Fibrinogen is a complex blood coagulation protein composed of two sets of three non-identical polypeptide chains α, β (or usually Aα and Bβ underlining presence of fibrinopeptides A and B) and γ [2]. Fibrinogen is composed of dimers of α, β and γ $(\alpha\ \beta\ \gamma)_2$, but in human and other mammals two different isoforms of α and γ chains are expressed in the liver [3]. The fibrinogen α gene is spliced in mammals into two alternative isoforms—1 (alpha-E, P02671-1) and 2 (alpha, P02671-2). The isoform 2 or alpha is shorter, is lacking C-terminal fibrinogen like domian (FreD domain) and its expression predominates expression of the isoform 1. The C-terminus of isoform 1 shows high homology to β and γ C-termini (FreD domains with high content of tyrosines). The isoforms complicate tyrosine calculations. The both isoforms of α chain differ drastically in tyrosine content. The isoform 2 has only 9 tyrosines in human. This form is present in fibrinogen with molecular mass 340 kDa which constitute 98% of human plasma fibrinogen. The isoform 1 builds fibrinogen with molecular mass 420 kDa [4]. There is only about 2% of heavy fibrinogen in the whole fibrinogen pool [4]. The laboratory and commercial products isolated from plasma mostly consist of light fibrinogen with isoform 2 α chain [5]. Tang et al. claim that α chain has 25 tyrosines which is almost true for isoform 1 (it has 24 residues) but is absolutely wrong for isoform 2. There are only 9 tyrosines in predominant α chain. The γ gene is spliced into gamma-A (P02679-2) or gamma-B (gamma', P02679-1) isoforms. Mature forms of gamma-A and gamma-B chains have respectively 20 and 22 tyrosines but not 24. Two additional tyrosines are present in a signal peptide removed before fibrinogen is assembled in a hepatocyte. Gamma-A predominates gamma-B in human [6]. The authors are right with β chain which in human has only one known isoform with 21 tyrosines.

Sequences for human fibrinogen and information about isoforms are available at http://www.uniprot.org/uniprot/ (P02671, P02675, P02679). Tang et al. used P02671-1, P02675 and P02679-1 sequences according to the Table 2. It must be once again underlined that sequence P02671-2 and P02679-2 are predominant in human fibrinogen (molecular mass 340 kDa) [5].

The whole molecule of predominant fibrinogen 340 has $2 \times (9 + 21 + 20) = 100$ tyrosyl residues. Observed by authors stoichiometry "of 15 nitrotyrosines per mol of fibrinogen" (there is also a small mistake—should be rather 15 mol of nitrotyrosines per mole of fibrinogen or 15 nitrotyrosines per molecule of fibrinogen because 1 mol = Avogadro constant of molecules, so 15 tyrosines per 6.02×10^{23} of fibrinogen molecules would be 1.82×10^{-23}% of nitrated tyrosines or taking the proper value of fibrinogen's tyrosines it gives 2.49×10^{-23}% of nitrated tyrosines!) suggests that an average of 15% of the tyrosines are nitrated (not 11%). 15 mol of nitrotyrosines per 1 mol of fibrinogen seems reasonable as 1 mg/ml fibrinogen was nitrated by 1 mM peroxynitrite. In our previous studies 2 mg/ml (5.88 μM) fibrinogen nitrated with 1 mM peroxynitrite in similar conditions gave 8 mol nitrotyrosines per 1 mol of fibrinogen (or 8 nitrotyrosines per molecule) [7]. Fibrinogen nitration is dependant on fibrinogen–peroxynitrite ratio.

Table 2 with LC–MS/MS identified tyrosine sites provides brilliant information about nitration localisation. The numbers of sites start including signal peptides for all fibrinogen chains (19, 30 and 26 amino acid long respectively for α, β and γ, removed in posttranslational modifications). The signal peptides are absent in mature fibrinogen and are usually not counted in fibrinogen residue numeration (for instance in PDB structures like 3GHG or 1FZA) [1,8]. Numeration of authors may be a little confusing as it differs from native α, β and γ fibrinogen beginning respectively in 19, 30 and 26 residues (signal peptides amino acid length of α, β and γ).

It is well known that fibrinogen is susceptible to nitration *in vivo* which can alter its biological function. Interestingly, according to Table 2 some of nitrated tyrosines lie not far from fibrinogen fragments which are known that have important physiological functions. Beta chain Y 404 (434)[1] is in polymerization site "b", gamma chain Y 363 (389) is in polymerization site "a", gamma chain Y 18 (44) is in thrombin binding site, gamma chain Y 68 (94) is near a place of plasmin digestion of fibrinogen and alpha chain Y 178 (197) is close to a place of tissue plasminogen activator binding and plasminogen activation [9]. The role of mentioned tyrosines in biological function of fibrinogen actually is not studied at all. The work of Tang et al. is valuable (apart from their sensitive immunoassays) because it shows that listed tyrosines are nitrated by peroxynitrite *in vitro*, so it is also possible that they are nitrated *in vivo* under oxidative/nitrative stress and such modifications may alter phys-

[1] In parentheses values of Tang et al. (with signal peptide).

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iological function of fibrinogen. Further studies of this topic seem extremely interesting.

References

[1] Z. Tang, H. Wu, D. Du, J. Wang, H. Wang, W.J. Qian, D.J. Bigelow, J.G. Pounds, R.D.R.D. Smith, Y. Lin, Talanta 81 (2010) 1662.
[2] J.M. Kollman, L. Pandi, M.R. Sawaya, M. Riley, R.F. Doolittle, Biochemistry 48 (2009) 3877.
[3] Y. Fu, L. Weissbach, P.W. Plant, C. Oddoux, Y. Cao, T.J. Liang, S.N. Roy, C.M. Redman, G. Grieninger, Biochemistry 31 (1992) 11968.
[4] G.G. Grieninger, X. Lu, Y. Cao, Y. Fu, B.J. Kudryk, D.K. Galanakis, K.M. Hertzberg, Blood 90 (1997) 2609.
[5] R.F. Doolittle, J.M. Kollman, Proteins 63 (2006) 391.
[6] R.F. Doolittle, S. Hong, D. Wilcox, J. Thromb. Haemost. 7 (2009) 71431.
[7] P. Nowak, H.M. Zbikowska, M. Ponczek, J. Kolodziejczyk, B. Wachowicz, Thromb. Res. 121 (2007) 163.
[8] G. Spraggon, S.J. Everse, R.F. Doolittle, Nature 2 (1997) 455.
[9] W.J. Schielen, H.P. Adams, M. Voskuilen, G.I. Tesser, W. Nieuwenhuizen, Blood Coagul. Fibrinolysis 2 (1991) 465.

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