TITLE OF REPORT : Investigation of the Role of Post-translational Tyrosine Sulfation in Hemostasis

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During the three-week period (November 24 - December 14, 2017) funded by the JSPS Bridge Fellowship, I was fortunate to be able to perform a good deal of academic activities. Upon my arrival in Japan on November 24, I met with Dr. Yoichi Sakakibara, my host scientist, in Hiroshima. The next day, we attended the 68th Annual Meeting of the Japanese Electrophoresis Society. On November 26, Dr. Sakakibara and I moved to Kumamoto, where we met Dr. Shin Yasuda, a research collaborator of ours, and had a discussion about our future collaboration. The following day (November 27), I visited Dr. Yasuda's laboratory in the morning and gave a research seminar entitled "Biological Sulfation: Physiological and Pharmacological Implications" in the afternoon. Afterwards, Dr. Sakakibara and I traveled to Miyazaki. In the following five days (November 28-December 2), I stayed in Miyazaki and met with several professors (Drs. Masahito Suiko, Katsuhisa Kurogi, Yuichi Saeki, Kazuo Nishiyama, Nozomu Eto) and talked about research and worked on a couple of manuscripts. On December 3, I traveled with Drs. Masahito Suiko and Katsuhisa Kurogi to China, where we visited the North Sichuan Medical College and met with a couple of research collaborators (Drs. Chunyang Zhou and Lijun Luo). This side trip (which had been pre-approved by the JSPS Office in Tokyo) had a special purpose of probing the possibility of establishing a student/faculty exchange program between the University of Miyazaki and North Sichuan Medical College. The five-day visit in China turned out to be quite fruitful. A preliminary agreement to further develop an MOU in the coming year was reached between the two universities. On December 8, Dr. Suiko, Dr. Kurogi and I traveled back to Okinawa where we attended

the 24th Annual Meeting of the Japan Society for Biotechnology Kyushu Branch on December 9th. On December 10th, we moved back to Miyazaki, where I spent another four days talking about research collaboration, doing some *in silico* research, and working on manuscripts. I also presented a seminar entitled "A Tale of My Forty Years of Research Experience" on December 12th. On December 14th, I finally returned to the United States.

The research I performed while I was in Miyazaki was focused on the identification of hemostatic proteins that carry potentially sulfatable tyrosine residues. Post-translational tyrosine sulfation is known to have a widespread occurrence among proteins in multicellular eukaryotic organisms. The functional relevance of protein tyrosine sulfation, however, has remained poorly understood. An on-going collaborative research project between Dr. Sakakibara's lab and lab is focused on elucidating the functional role of tyrosine sulfation in hemostasis. Using a computer algorithm called "Sulfinator" (), we were able to systematically identified a number of hemostatic proteins that are potentially sulfatable on their tyrosine residues. The Table show below illustrates the potentially sulfatable tyrosine residues in the hemostatic proteins which we have identified.

Table 1. Potential tyrosine sulfation sites of some hemostatic proteins identified using Sulfinator[a]

SWISS-Prot Name	Description	Site(s)[b]	Sequence Surrounding Sulfatable Tyrosine Residue
FA9_HUMAN	Coagulation factor IX	201	FPDVD**Y**VNSTEAETILD
FA12_HUMAN	Coagulation factor XII	289	RLSWE**Y**CDLAQCQTPT
F13A_HUMAN	Coagulation factor XIII	70	HHTDK**Y**ENNKLIVRR
	A chain	195	HHTDK**Y**ENNKLIVRR
FA5_HUMAN	Coagulation factor V	693	DDEDS**Y**EIFEPPESTV
		1552	KDGTD**Y**IEIIPKEEV
		1593	EISWD**Y**SEFVQRETD

KLKB1_HUMAN	kallikrein	40,46	ASM**Y**TPNAQ**Y**CQMR
PROS_HUMAN	Protein S	80,82	DPETD**Y**F**Y**PKYLVC
GP1BA_HUMAN	Platelet glycoprotein Ib alpha chain	292, 294,295	GDTDL**Y**D**YY**PEEDT
PLMN_HUMAN	Plasminogen	173,175	DPEKR**Y**D**Y**CDILEC
		554	KLYD**Y**CDVPQC
UROK_HUMAN	Urokinase-type plasminogen activator	326	ENSTD**Y**LYPEQ
A2AP_HUMAN	Alpha-2-antiplasmin	484	EED**Y**PQFGSP
HEP2_HUMAN	Heparin cofactor 2	79	EEDDD**Y**LDLEKIFSEDD
		92	SEDDD**Y**IDIVDSLSVSP
TFPI1_HUMAN	Tissue factor pathway inhibitor	296	RKKQRVKI-A**Y**EEIFVK

[a]Only those that contain potentially sultatable tyrosine residue(s) among all known components of hemostatic pathways are shown.

[b]Potential tyrosine sulfation sites refer to the positions of the potential sulfatable tyrosine residues in the amino acid sequences of individual proteins deposited in SWISS-PROT database.

Based on these results, we plan to further verify the identity of these hemostatic proteins as being truly tyrosine-sulfated as they are produced by the liver cells in the body. The follow-up studies will be performed as a collaborative effort between my lab and Dr. Sakakibara's lab in the years to come.

Photo taken during the biotechnology meeting in Okinawa:

Photo taken during the seminar presented at the University of Miyazaki