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Study of reaction between 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) and tertiary amines by LC-MS

○ Xiaomin LI¹, Yayoi KANBAYASHI², Zekun YANG¹, Muneki ISOKAWA¹, Chao WANG^{1,3}, Takashi FUNATSU¹, Masanobu UCHIYAMA^{1,3}, Makoto TSUNODA¹ (¹Graduate School of Pharmaceutical Sciences, The University of Tokyo, ²Institute of Innovation, Ajinomoto Co., Inc., ³Riken)

[Introduction] 4-Fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), a pre-labeling reagent for HPLC analysis, has high reactivity to amines under mild conditions. Therefore, it has been widely used for analysis of amino acids and biogenic amines. It is generally believed that NBD-F is not reactive towards tertiary amines. However, as for the NBD-derivatization of ϵ -N, N-dimethyl-lysine (diMeLys) with both primary and tertiary amino groups, its di-NBD-derivative was obtained, indicating that tertiary amino group in diMeLys may be reactive to NBD-F. To reveal generality of reactivity between NBD-F and tertiary amines, reaction of several tertiary amines including diMeLys with NBD-F was investigated.

[Methods] Tertiary amines were reacted with NBD-F in alkaline borate buffer at 60 °C. The reaction solutions were analyzed by reversed-phase HPLC with fluorescence (ex. 470 nm, em. 530 nm) and ESI-MS detection.

[Results and Discussion] First, LC-MS analysis showed that NBD-F could react with diMeLys to form two products (mono- and di-NBD-diMeLys, m/z 338 and 501, respectively). The structure of di-NBD-diMeLys was investigated by LC-MS/MS. The fragmentation pattern indicated that two NBD moieties in di-NBD-diMeLys were bound to primary and tertiary amino group of diMeLys, respectively. Next, the formation of di-NBD-diMeLys from the reaction of mono-NBD-diMeLys with NBD-F was confirmed. Finally, reaction of other tertiary amines with NBD-F was investigated by LC-fluorescence-MS. Although no significant fluorescence signal was observed from NBD-derivatives of the tertiary amines studied, molecular weights corresponding to these NBD-derivatives were found by LC-MS analysis. It can be concluded that NBD-F reacts with tertiary amines and the derivatives basically have no fluorescence.