Formation of a flavin-linked cysteine.

Masayuki Morikawa, Takeshi Senda, Masayo Suzuki, Takanobu Kobayashi, Hiroshi Miyazawa, Katsuhito Kino*

School of Pharmaceutical Sciences, Tokushima Bunri University, 1314-1 Shido, Sanuki, Kagawa 769-2193, Japan; E-Mails: s110702@stu.bunri-u.ac.jp (M.M.); s068064@stu.bunri-u.ac.jp (T.S.); E-Mails: s120002@stu.bunri-u.ac.jp (M.S.); E-Mails: kobayashit@kph.bunri-u.ac.jp (T.K.); E-Mails: miyazawah@kph.bunri-u.ac.jp (H.M.)

* Author to whom correspondence should be addressed; E-Mail: kkino@kph.bunri-u.ac.jp; Tel.: +81-87-899-7463; Fax: +81-87-894-0181.

Abstract: The deposition of amyloid beta peptides (Aβ) in the formation of amyloid fibrils is believed to be causally linked to Alzheimer's disease. A short Aβ fragment (KLVFF; $A\beta_{16-20}$) can bind full-length Aβ. The Aβ recognition peptide including KLVFF was attached with hydrophilic groups, and then the product altered the Aβ aggregation pathways and inhibited Aβ toxicity (Tjernberg, L. O. et al. J. Biol. Chem. 1996, 271, 8545-8548.). Since flavins is widely known as biological oxidation reagents, the flavin-linked KLVFF is likely to directly hydroxylate aggregated Aβ fibrils and then disrupt the aggregated Aβ fibril. 2-Aminoethanethiol derivatives, such as cysteine, are reacted with aldehydes. Since formylmethylflavin (FMF) contains an aldehyde group, FMF is likely to react with cysteine. Then, it is possible that this methodology is applied to the synthesis of the flavin-linked KLVFF via the reaction between CKLVFF and FMF. As the preliminary experiment of this reaction, the reaction between FMF and cysteine was investigated. FMF (2.84 mg, 10 μmol) was suspended in phosphate buffer (pH 7, 10 ml), and cysteine (2.42 mg, 20 μmol) was added. The suspension was stirred at 65ºC for 1h. As a result, FMF was completely reacted, and flavin-linked cystein were detected using HPLC and ESI-MS.

Keywords: Alzheimer's disease, Amyloid beta peptides, Cysteine, Flavin

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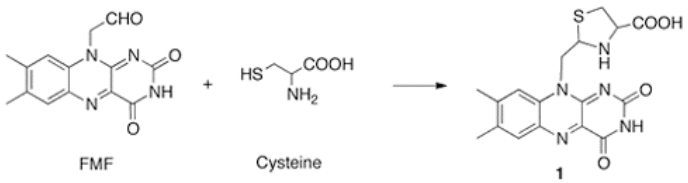
1. Introduction

Amyloid beta peptides (Aβ) is a 40- to 42-amino acid-long fragment, and the deposition of Aβ in the brain parenchyma and cerebro-vasculature is a critical step in the pathogenesis of Alzheimer's disease [1,2]. Aβ easily aggregates into fibrils with a β-sheet conformation, and Aβ toxicity is likely to be linked to the aggregational status of the Aβ peptides [3-5]. A previous report shows that a short Aβ fragment (KLVFF; $A\beta_{16-20}$) can bind full-length Aβ [6]. Then, hydrophilic groups-attached KLVFF was designed as the inhibitor of the Aβ toxicity, and the product altered the Aβ aggregation pathways and inhibited Aβ toxicity [6].

Since flavins is widely known as biological oxidation reagents, the flavin-linked KLVFF is likely to directly hydroxylate aggregated Aβ fibrils and then disrupts the aggregated Aβ fibril and inhibits Aβ toxicity. In this study, simple synthesis of the flavin-linked KLVFF was investigated.

2-Aminoethanethiol derivatives, such as cysteine, are reacted with aldehydes, and five-membered heterocyclic ring is formed via formation of imine. Since formylmethylflavin (FMF) contains an aldehyde group, FMF is likely to react with cysteine. Since cysteine in the N-terminal tail of CKLVFF contains an 2-aminoethanethiol group, it is possible that this methodology is applied to the synthesis of the flavin-linked KLVFF via the reaction between CKLVFF and FMF. As the preliminary experiment of this reaction, the reaction between FMF and cysteine was performed (Scheme 1).

Scheme 1. The reaction between FMF and cysteine.



2. Experimental Section

2.1. Chemicals

Riboflavin (RF) was purchased from Kishida Chemical Co., Ltd. Cysteine, DMSO, NaH_2PO_4, Na_2HPO_4, triethylamine and acetic acid were purchased from Wako Pure Chemical Industries, Ltd. CH_3CN was purchased from Kanto Chemical Co., Inc. Sep-Pak Plus C18 cartridge was purchased from Waters Co. Triethylammonium acetate buffer (TEAA) was prepared from triethylamine and acetic acid. Phosphate buffer was prepared from NaH_2PO_4 and Na_2HPO_4. FMF was prepared from RF, as previously described [7].

2.2. Formation of product 1 in Scheme 1

FMF (2.84 mg, 10 μmol) was suspended in phosphate buffer (pH 7, 10 ml), and cysteine (2.42 mg, 20 μmol) was added. The suspension was stirred at 65ºC for 1h. As a result, the yellowish solution was obtained. Then, product **1** was purified by adsorption onto a Sep-Pak Plus C18 cartridge, followed by washing with water and eluting with CH_3CN. Purified product **1** was dried under vacuum (95% total yield, brown solid). Product 1 ($C_{17}H_{17}N_5O_4S$) was confirmed by ESI-MS: m/z 388.10760 (388.10740, calculated for $[M+H]^+$). Product **1** was analyzed using HPLC with a Nacalai Tesque 5C18-ARII column (5 μm, 150 × 4.6 mm) (elution with a solvent mixture of 50 mM TEAA (pH 7), 12% (isocratic) CH_3CN/20 min, at a flow rate of 1.0 mL/min).

3. Results and Discussion

FMF and cysteine were reacted at 65ºC for 1h, and formation of product **1** was confirmed using ESI-MS (Figure 1). Then, the solution was analyzed using HPLC (Figure 2A). As a result, FMF was completely reacted, and the two peaks at 10.5 and 10.8 min in Figure 2A were detected using HPLC. The ratio of the peak areas at 10.5 and 10.8 min was about 2:1. Since product **1** contains chiral centers, the two isomers are likely to be diastereomers of product **1**.

The two isomers could not be completely isolated using HPLC, but the partial isolation of each isomer was performed. Then, the samples were stored for 1 hour and analyzed using HPLC, and the HPLC charts were shown in Figure 2B and 2D. As a result, the ratio of the peak areas at 10.5 and 10.8 min was about 6:1 in figure 2B, and the ratio of the peak areas at 10.5 and 10.8 min was about 4:5 in figure 2D. Furthermore, the samples were stored for 2 days and analyzed using HPLC (Figure 2C and 2E), and the both ratios of the peak areas at 10.5 and 10.8 min in Figure 2C and 2E were about 2:1. Therefore, the equilibrium between two isomers of product **1** was observed.

Figure 1. ESI-MS analysis of product **1**. FMF and cysteine were reacted at 65ºC for 1h, and the sample was analyzed using ESI-MS.

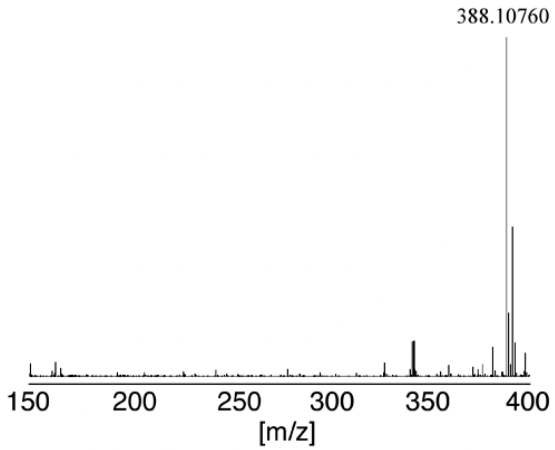
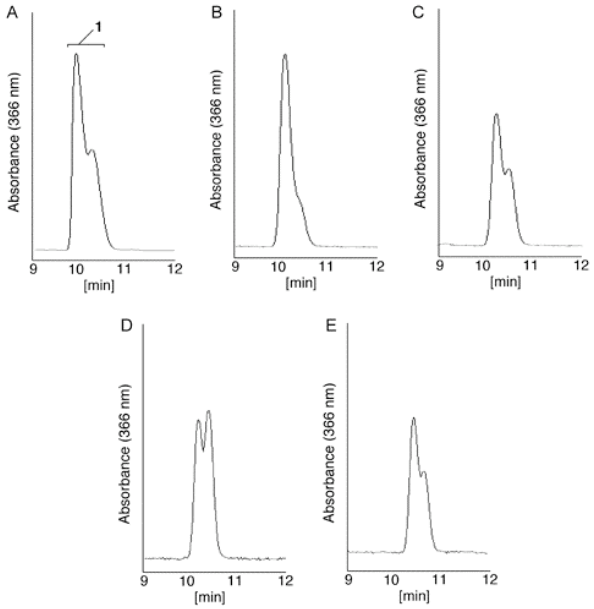


Figure 2. HPLC charts of product **1**. (A) FMF and cysteine were reacted, and the solution was analyzed using HPLC. The partial isolation of the peak at 10.5 and 10.8 min in Figure 2A were performed using HPLC. The sample at 10.5 min in Figure 2A was stored at room temperature for (B) 1h, and (C) 2 days. The sample at 10.8 min in Figure 2A was stored at room temperature for (D) 1h, and (E) 2 days.



4. Conclusions

We report the simple and practical synthesis of flavin-linked cysteine. FMF was completely reacted, and two isomers of product **1** were formed. The reaction can be exploited for the reaction between FMF and peptides including cytosine. Then, the formation of flavin-linked KLVFF will be investigated, and we would confirm whether the flavin-linked KLVFF disrupts the aggregated Aβ fibril and inhibits Aβ toxicity.

Acknowledgments

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