Figure 2. A pH-triggered "click peptide" based on the "*O*-acyl isopeptide method": production of Aβ1–42 (**9**) through the pH-dependent *O*–*N* intramolecular acyl migration of 26-AIAβ42 (**10**).

ingly, since isomerization of the peptide chain to the *O*-acyl isopeptide structure seems to increase the solubility of the insoluble original peptide with 42 residues drastically, this suggests that *O*-acyl isopeptides totally break the secondary structures responsible for the insolubility of the original peptide. In addition, HPLC analysis of **10** revealed a sharp peak even in a slow gradient system, while **9** was eluted as a broad peak under the same elution condition, as reported.[25] Recent solution-state NMR studies of Aβ1–40 and Aβ1–42 have indicated that the Ser26 residue produces turn- or bendlike structures that bring two β-sheets into contact and so cause hydrogen bonding interactions between peptide chains, which is associated with β-aggregation.[3d] As we have demonstrated that the use of *O*-acyl isopeptides allows the unfavorable natures of pentapeptides containing difficult sequences to be suppressed, permitting the synthesis of **10**. Thus, this method might be a powerful strategy for increasing the solubilities of even larger peptides.

As shown in Figure 3, purified **10** could be quantitatively converted into Aβ1–42 (**9**) by *O*–*N* intramolecular acyl migration in PBS (pH 7.4) with no side reactions such as hydrolysis of the ester bond. In PBS (pH 7.4) at 37 °C, this migration was very rapid, with a half-life of approximately 1 min, and the migration was complete after 30 min. This fast migration may be attributed to the presence of the less sterically hindered Gly25

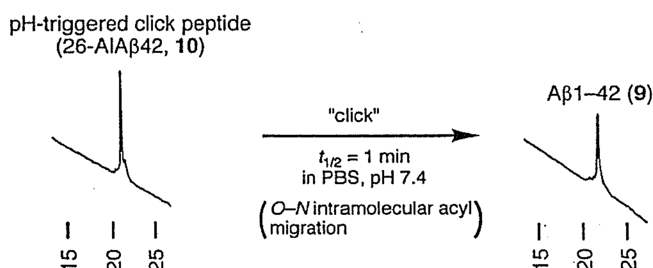

Figure 3. pH-triggered "click": HPLC profiles of the conversion of pH-triggered click peptide (26-AIAβ42, **10**) into the corresponding Aβ1–42 (**9**) in PBS (pH 7.4) at 37 °C.

residue. On the other hand, the TFA salt of **10** was stable at 4 °C either in the solid state or in DMSO solution. Moreover, slower migration was observed at pH 4.9, with a half-life of 3 h, with no migration at pH 3.5 after incubation for 3 h. This rapid migration under physiological conditions enables the production of an intact monomer Aβ1–42 in situ for investigation of the inherent biological function of Aβ1–42 in AD.

Not only is wildtype Aβ1–42 observed in AD, but missense mutations inside the Aβ-coding region in the APP gene are also well known. These mutations, known as Flemish- (A21G),[26] Arctic- (E22G),[27] Dutch- (E22Q),[28] Italian- (E22 K),[29] and Iowa-type (D23N)[30] are found at positions 21–23 in Aβ.

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Recently, a Japanese-Tottori-type (D7N) mutation has also been reported.[31] Recent studies have discussed several differences between Aβ mutants in amyloid formation, metabolism, and elimination that are related to the progression of AD-like diseases,[32] so more detailed studies comparing these features between Aβ mutants should afford crucial information for understanding the mechanism of the diseases. With the aid of this background information, novel water-soluble isopeptides of each Aβ1–42 mutant, "26-AIAβ42 mutant", were also successfully synthesized,[12] suggesting that the *O*-acyl isopeptide method is a universal strategy for increasing the water solubility of poorly soluble peptides. Moreover, these isopeptides were converted into their corresponding intact Aβ1–42 mutants with no significant differences in half-life values under physiological conditions.

We named these *O*-acyl isopeptides pH-triggered "click peptides", since the isopeptides had the capability for "quick and one-way conversion" to the parent Aβ1–42 through pH-dependent *O*–*N* intramolecular acyl migration (Figures 2 and 3). These pH-triggered click peptides should provide a novel tool for biological evaluation in AD research, with the click peptides being storable in a solubilized form before use and rapidly producing intact Aβ1–42 in situ during biological experiments.

4) A "Click Peptide" Based on the "*O*-Acyl Isopeptide Method"—Efficient Phototriggered Production of Aβ1–42 from an Aβ1–42 Analogue

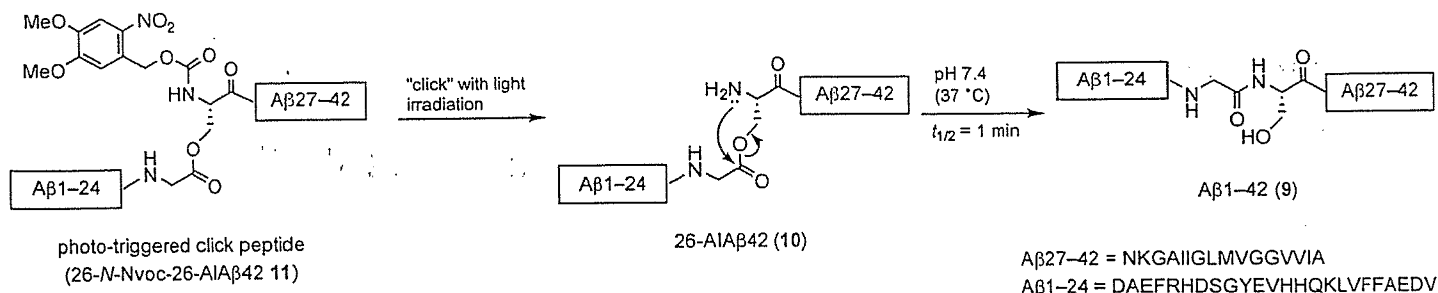
A clear understanding of the currently unexplained processes of pathological folding, self-assembly, and aggregation of Aβ1–42 would be of great significance in AD research. However, elucidation of these Aβ1–42 dynamic events is also a difficult issue due to uncontrolled polymerization.[22–24]

"Caged" compounds—synthetic molecules with their biological activities masked by covalently attached photocleavable protecting groups—are generally considered to be advantageous for the study of the dynamic processes of peptides or proteins, because, upon photoactivation, only a short duration of time is required to control the spatiotemporal dynamics of the native compounds.[33,34] However, the attachment of small photocleavable groups would be unlikely to be able to mask the spontaneous self-assembly potency of aggregative peptides, since this sort of potency is generally extremely strong and attributable to large sections of the peptide structure. To overcome this issue, Imperiali et al. introduced an additional cationic fibrilinhibitory unit, covalently attached through a photocleavable linker to an aggregative peptide derived from prion protein.[35] This analogue suppressed the self-assembling nature of the original aggregative peptide, although the fibrils formed from the original peptide released by photolysis were insufficiently dense because of a side effect involving the co-released fibril-inhibitory unit.

In a different approach to the development of a phototriggered Aβ1–42 analogue with effective inactivation of the self-assembling nature, a strategy based on an *O*-acyl isopeptide protected by a photocleavable group was planned. The *O*-acyl isopeptide was expected to be nonaggregative and to be able to convert into the inherent aggregative peptide by photo-irradiation "click" without the presence of any additional fibril-inhibitory unit. Consequently, we designed and synthesized a phototriggered "click peptide" of Aβ1–42 (**9**)—26-*N*-Nvoc-26-AIAβ42 (**11**)[13]—in which a photocleavable 6-nitroveratryloxycarbonyl (Nvoc) group[36] had been introduced at the α-amino group of Ser26 in **10**, to establish a novel biological evaluation system in which the activation of the self-assembly process could be readily controlled (Scheme 6). Mutter et al. have also presented a similar concept, in the form of a pH- or enzyme-triggered "switch-peptide" to control self-assembly of Aβ-derived peptides.[21c] These systems could be crucial in current AD-related research.

In size-exclusion chromatography, a peak corresponding to an oligomer (~octamer) of **9** (t_R = 15 min) increased with incubation time at the expense of the monomer peak (t_R = 27 min), while, in the click peptide **11**, the monomeric form was clearly retained even after 24 h incubation (Figure 4A). Similarly, Th-T fluorescence intensity, which corresponds to the extent of fibril formation,[37] increased with time in **9**, while fluorescence intensity in **11** remained unchanged during 24 h incubation (Figure 4B). These results clearly indicate that click peptide **11** is nonaggregative and that isomerization of the peptide backbone at only one position in the whole peptide sequence (i.e., the formation of a branched ester structure in **11**) had significantly changed the secondary structure of **9**, resulting in the complete masking of the aggregative nature of **9**.

Under nonphotolytic conditions, click peptide **11** demonstrated only slight hydrolysis (<2%) at the ester bond between Gly25 and Ser26 after 6 h incubation in PBS (pH 7.4) at



Scheme 6. Phototriggered click peptide (26-*N*-Nvoc-26-AIAβ42, **11**): the production of Aβ1–42 (**9**) by phototriggered click, followed by the *O*–*N* intramolecular acyl migration reaction of 26-AIAβ42 (**10**).

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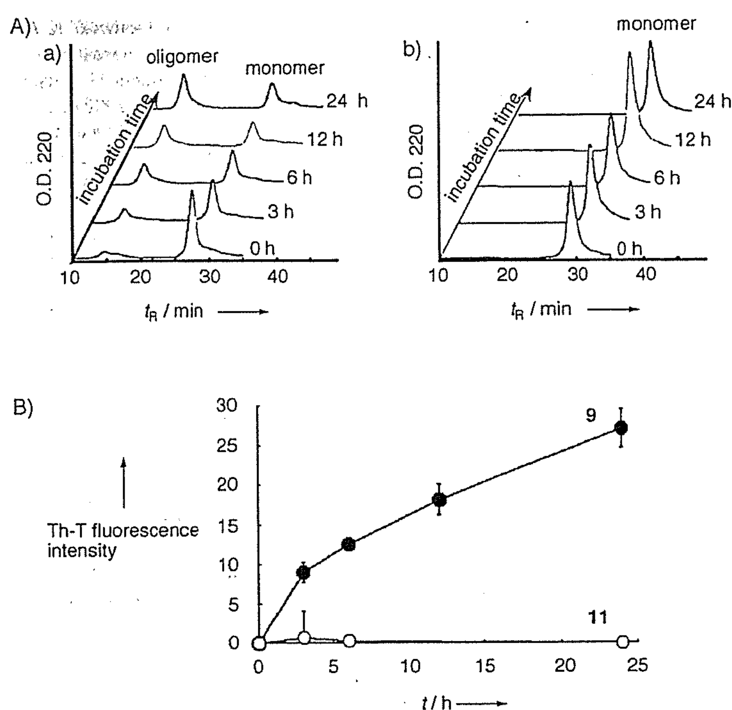

Figure 4. Peptide aggregation determined A) by size-exclusion chromatography—a) Aβ1–42 (**9**); b) click peptide **11**—and B) by Th-T assay.

37 °C; this suggests that click peptide **11** is sufficiently stable for use in biological assays under ambient light.[34] Peptide **11** was stable for at least 24 h in buffer (pH 7.4) solution at −20 °C and for three months either in the solid state or in DMSO solution at −20 °C. On the other hand, when a solution of **11** in PBS (pH 7.4, 20 μM peptide, 1 mM DTT) was photoirradiated with UV pulses for 15 min, the Nvoc group-derived absorption band at around 355 nm completely disappeared, indicating that the Nvoc group in **11** had been quantitatively removed by photolysis. We also confirmed that Aβ1–42 (**9**) was quantitatively recovered from **11** after photolysis followed by incubation at 37 °C for 30 min to induce migration. No by-products arising from 4,5-dimethoxy-2-nitrosobenzaldehyde co-released with **9** by photolysis were observed.

These results suggest that 1) click peptide **11** did not exhibit any selfassembling nature under physiological conditions, 2) photoirradiation of **11** and subsequent *O–N* intramolecular acyl migration rapidly afforded intact **9** in situ, while **11** was stable under nonphotolytic or storage conditions, and 3) no additional fibril-inhibitory auxiliaries were required. This method provides a novel system useful for investigation of the biological dynamism of Aβ1–42 in AD by inducible activation of Aβ1–42 self-assembly. Additionally, a fundamental drawback of the caged strategy for large peptides or proteins is that a small photocleavable group is not always able to mask their biological activities. This drawback would be overcome by our "click peptide" strategy in which the inherent properties can be masked by simple isomerization of the backbone structure from *N*-acyl peptide to *O*-acyl isopeptide at hydroxyamino acid residues such as Ser and Thr. This method should open doors for the development of novel and useful phototriggerable tools in chemical biology and medical science.

5) Conclusion

In 2003, when we conceived the idea that the synthesis of more hydrophilic "*O*-acyl isopeptides" derived from peptides containing difficult sequences might overcome the solubility problem in HPLC purification, we made the surprising discovery that the "*O*-acyl isopeptide" could improve not only the solubility in various media, but also coupling and deprotection efficacy during solid-phase peptide synthesis through the modification of the nature of the difficult sequence. The isomerization of the peptide backbone at only one position in the whole peptide sequence—that is, the formation of one single ester bond—could significantly change the unfavorable secondary structure of the peptide. This finding led to the discovery of the "*O*-acyl isopeptide method" as a novel synthetic method in the field of peptide chemistry whose efficacy has recently been confirmed by Mutter et al.[21a,c] and by Carpino et al.[21b] In research oriented towards chemical biology we have applied this method to the synthesis of a novel "click peptide" precursor for Aβ1–42. This click peptide did not exhibit any self-assembling nature under physiological conditions, because of the presence of a single ester moiety, but was able to undergo a migration to form the original Aβ1–42 in a quick and one-way conversion reaction (so-called "click"). Because the difficulties involved in handing Aβ1–42 in synthesis and in biological experiments, are an impediment in progress in Aβ1–42-related Alzheimer's disease research, we expect that the "click peptide" method should contribute to clarifying the currently unexplained processes of Alzheimer's disease. Moreover, it has recently been ascertained that the pathological self-assembly of inherent peptides or proteins and their subsequent aggregation into amyloidogenic deposits is associated with many diseases,[38] such as prion protein in prion disease, α-synuclein in Parkinson's disease, and islet amyloid polypeptide in type 2 diabetes, as well as Aβ1–42 in AD, so we hope that the "click peptide" strategy may be widely applied to these amyloid-related peptides or proteins.

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Keywords: Alzheimer's disease · amyloid peptide · click peptide · O-acyl isopeptide method · self-assembly

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'Click peptide': a novel 'O-acyl isopeptide method' for peptide synthesis and chemical biology-oriented synthesis of amyloid β peptide analogues

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Abstract: After over a decade of studies on aspartic protease inhibitors and water-soluble prodrugs, we have been developing a novel method, since 2003, called 'O-acyl isopeptide method', for the synthesis of peptides containing difficult sequences. With our recent discoveries of 'O-acyl isodipeptide unit' and the 'racemization-free segment condensation method', this method has further evolved as a general synthetic method for peptides. Moreover, 'Click Peptide', which could be a powerful tool for identifying the pathological functions of amyloid β peptides in Alzheimer's disease, represents a valuable use of the isopeptide method in Chemical Biology-oriented research. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: O-acyl isopeptide method; Alzheimer's disease; amyloid β peptide; click peptide; difficult sequence

INTRODUCTION

Since 2003, we have been developing a novel method, called 'O-acyl isopeptide method', for the synthesis of peptides containing difficult sequences in which a native amide bond at a hydroxyamino acid residue, such as Ser being isomerized to an ester bond, is followed by an O–N intramolecular acyl migration reaction [1–10]. Recently, the 'O-acyl isopeptide method' began to be widely utilized by several other groups [11–15]. In chemical biology-oriented research, we developed a novel 'Click Peptide' based on the O-acyl isopeptide method to study the inherent biological functions of native peptides or proteins (Figure 1) [3–7,9].

O-ACYL ISOPEPTIDE METHOD

Several years ago, when we tried to synthesize some peptide derivatives, including phenylnorstatine, for the study of aspartic protease inhibitors [16–18], some of the synthesized compounds could not be purified in preparative scale HPLC owing to their extremely low solubility in various solvents (Figure 2(A)). Thus, these peptide derivatives were considered to be the so-called 'difficult sequence'-containing peptides [19,20].

On the other hand, for over a decade, we studied the pH-dependent 'O–N intramolecular acyl migration [21,22]'-type water-soluble prodrugs of the peptide mimetic HIV-1 protease inhibitors [23,24]. These prodrugs, which are O-acyl isoforms of parent drugs possessing α-hydroxy-β-amino acids, had higher water solubility because of a newly formed and ionized amino group. Moreover, migration to the N-acyl parent drugs could be transacted with no side reaction under physiological conditions.

As a consequence, in 2003, we considered that the hydrophilic 'O-acyl isopeptides' derived from the phenylnorstatine-containing peptide derivatives would overcome the solubility problem in HPLC purification (Figure 2(B)). However, we had a surprising discovery in this research, which showed that not only did the 'O-acyl isopeptide' possess a higher solubility in various media, but also that the coupling and deprotection efficacy during solid-phase peptide synthesis (SPPS) was improved by modifying the nature of the difficult sequence [1,2]; namely, the isomerization of the peptide backbone from the N-acyl to O-acyl isopeptide structure, i.e. formation of one single ester bond, significantly changed the unfavorable secondary structure of the native peptides. Thus, this finding led to the development of the 'O-acyl isopeptide method' as a novel synthetic method in the field of Peptide Chemistry.

We also designed an 'O-acyl isodipeptide unit', e.g. Boc–Ser/Thr(Fmoc–Xaa)–OH. The use of O-acyl isodipeptide units, in which the racemization-inducing

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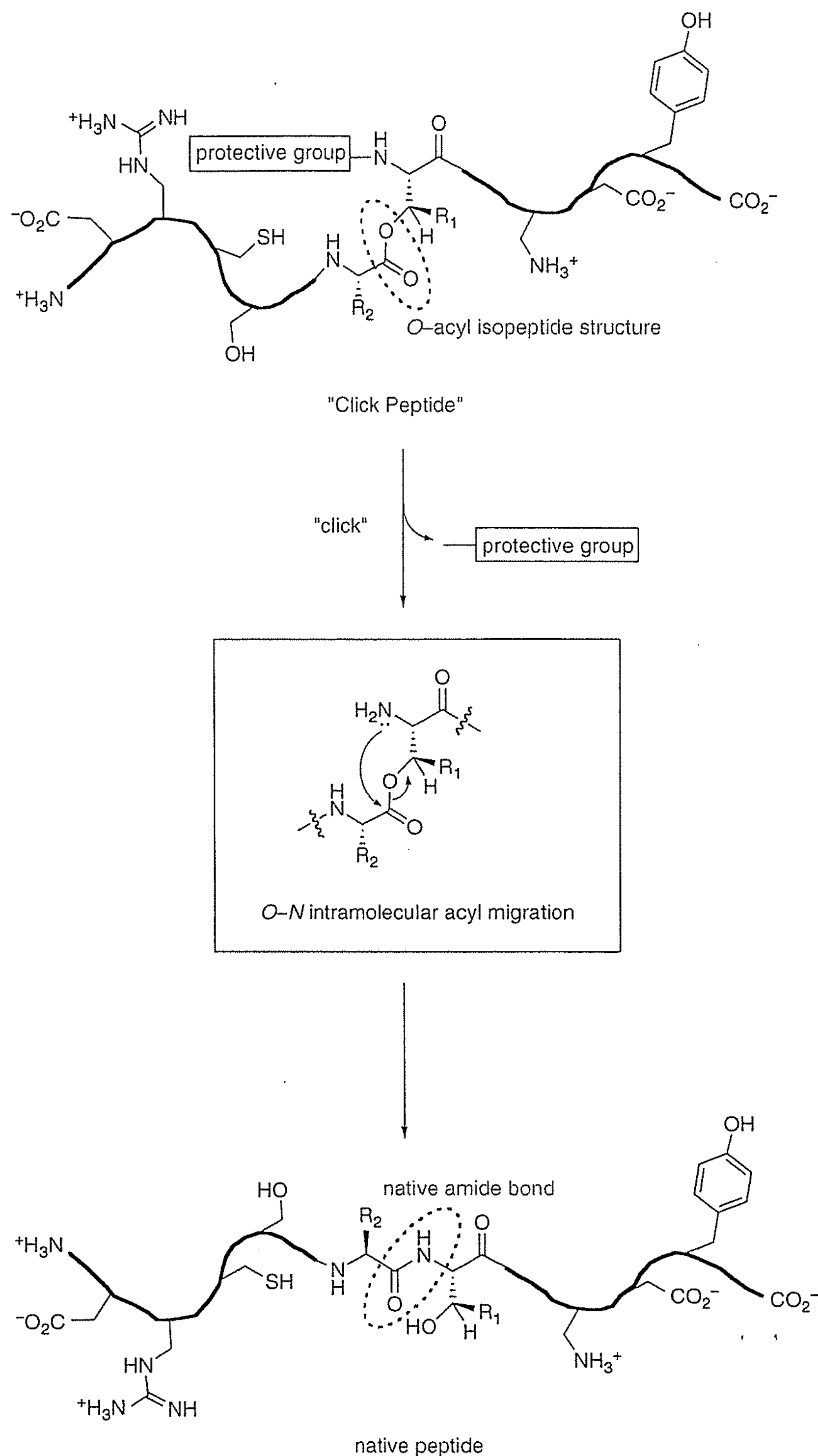

Figure 1 'Click Peptide' based on the '*O*-acyl isopeptide method'.

esterification reaction on the resin could be omitted, allows the application of the '*O*-acyl isopeptide method' to fully automated protocols for the synthesis of long peptides or proteins (Figure 3) [8]. Additionally, very recently, we developed a novel 'racemization-free segment condensation' based on the '*O*-acyl isopeptide method' [10].

The Application of *O*-Acyl Isopeptide Method

Moreover, we have successfully applied the '*O*-acyl isopeptide method' to the chemical biology-oriented synthesis of the Alzheimer's disease (AD)-related amyloid β peptide (Aβ) 1–42 analogues, leading to the development of pH- or photo-triggered 'Click Peptide'

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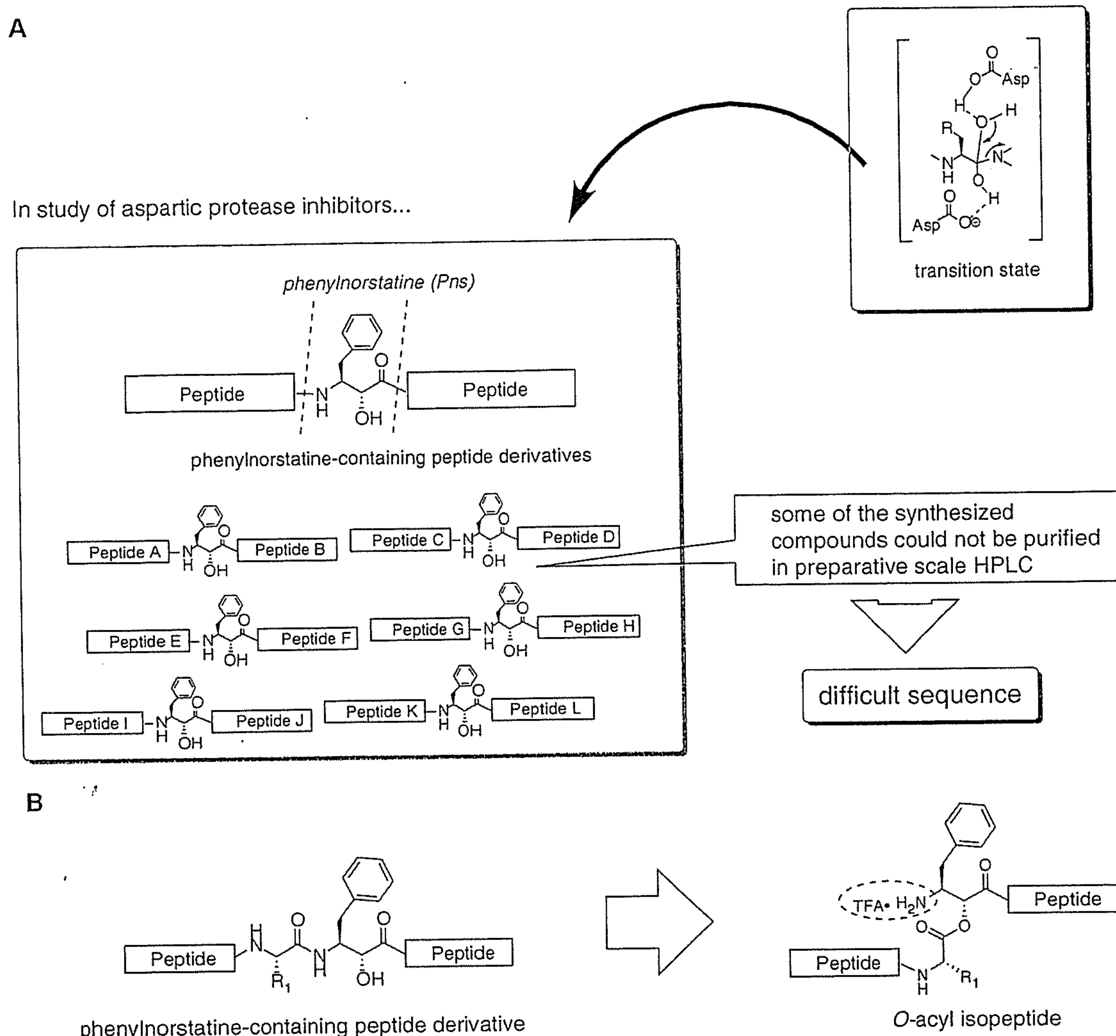

Figure 2 (A) 'Difficult sequence' in our studies of aspartic protease inhibitors, (B) design of *O*-acyl isopeptide derived from phenylnorstatine-containing peptide derivative.

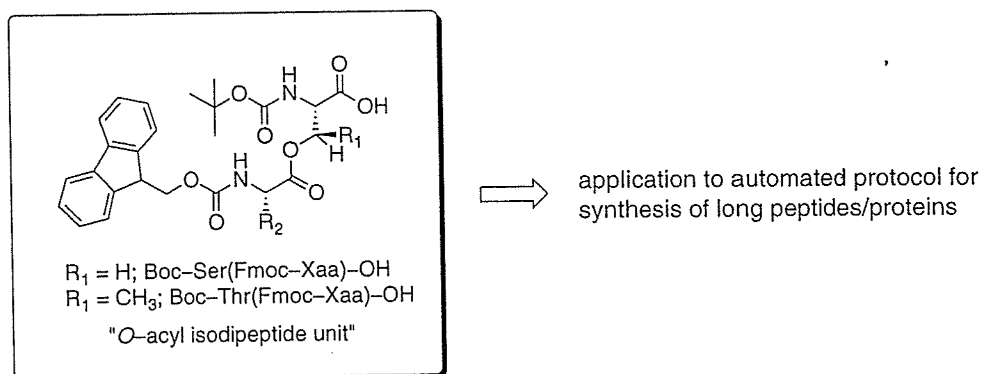

Figure 3 General structure of *O*-acyl isodipeptide unit: application of the '*O*-acyl isopeptide method' to fully automated protocol.

(Figure 1) [3–7,9]. The 'Click Peptide' did not exhibit the self-assembling nature under physiological conditions because of one single ester, and could migrate to the original Aβ1–42 with a quick and easy one-way conversion reaction (so-called 'click') via the *O–N* intramolecular acyl migration. A clear understanding of the pathological mechanism of Aβ1–42, a currently unexplained process, would be of great significance in the discovery of novel drug targets against AD [25–28]. Currently, the difficulties in handling Aβ1–42, because

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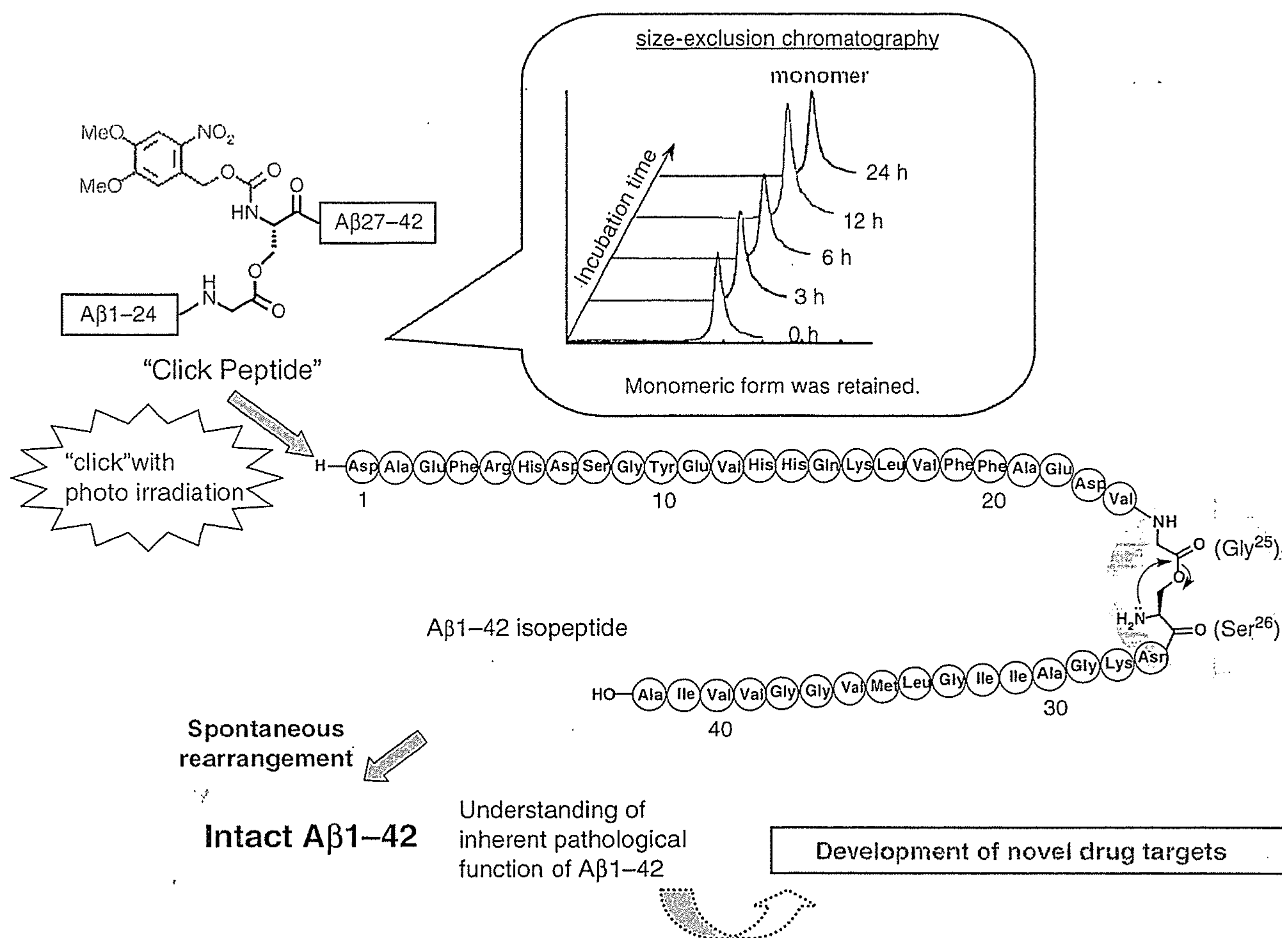

Figure 4 Photo-triggered click peptide. The production of Aβ1–42 by photo-triggered click followed by *O–N* intramolecular acyl migration reaction of Aβ1–42 isopeptide.

of its highly aggregative nature, hamper the progress of Aβ1–42-related AD research [29–32]. The 'Click Peptide' method would open the doors for investigation of the biological functions of Aβ1–42 in AD by inducible activation of Aβ1–42 self-assembly (Figure 4).

Interestingly, shortly after we disclosed the '*O*-acyl isopeptide method' [1,2], several other groups also confirmed the efficacy of this method. Carpino *et al.* synthesized the Jung–Redemann 26-residue peptide efficiently by utilizing the '*O*-acyl isopeptide method', whereas this peptide could not be synthesized by standard SPPS [12]. By carefully evaluating the appropriate protecting group, stability of the ester bond during assembly, and occurrence of side reactions, they concluded that the efficacy of the '*O*-acyl isopeptide method' was comparable to that of the pseudoproline method [15]. Börner *et al.* also synthesized the *O*-acyl isopeptide for efficient preparation of poly(ethylene oxide)–peptide conjugates [14]. Moreover, Mutter *et al.* confirmed by circular dichroism (CD)-based analyses that the secondary structure of *O*-acyl isopeptide structure is significantly different from that of the corresponding *N*-acyl native peptides [11,13], which agrees with our hypothesis. These reports indicate that the '*O*-acyl isopeptide method' is widely advantageous for peptide preparation by disrupting the unfavorable secondary structures of the native peptides.

CONCLUSION

Classical *O–N* intramolecular acyl migration was revived by our group as a powerful key reaction in the field of modern medicinal chemistry in the development of water-soluble prodrugs. After more than a decade of prodrug studies, we recently disclosed the '*O*-acyl isopeptide method' as a novel synthetic method in the field of peptide chemistry and its application to chemical biology-oriented synthesis of Aβ analogues, leading to the development of 'Click Peptide' (Figure 5). We hope that the strategy using the '*O*-acyl isopeptide method', in which a simple isomerization to an *O*-acyl isopeptide remarkably and temporarily changes the physicochemical properties of the native peptide and an *O–N* intramolecular acyl migration triggers the native amide bond formation under physiological conditions,

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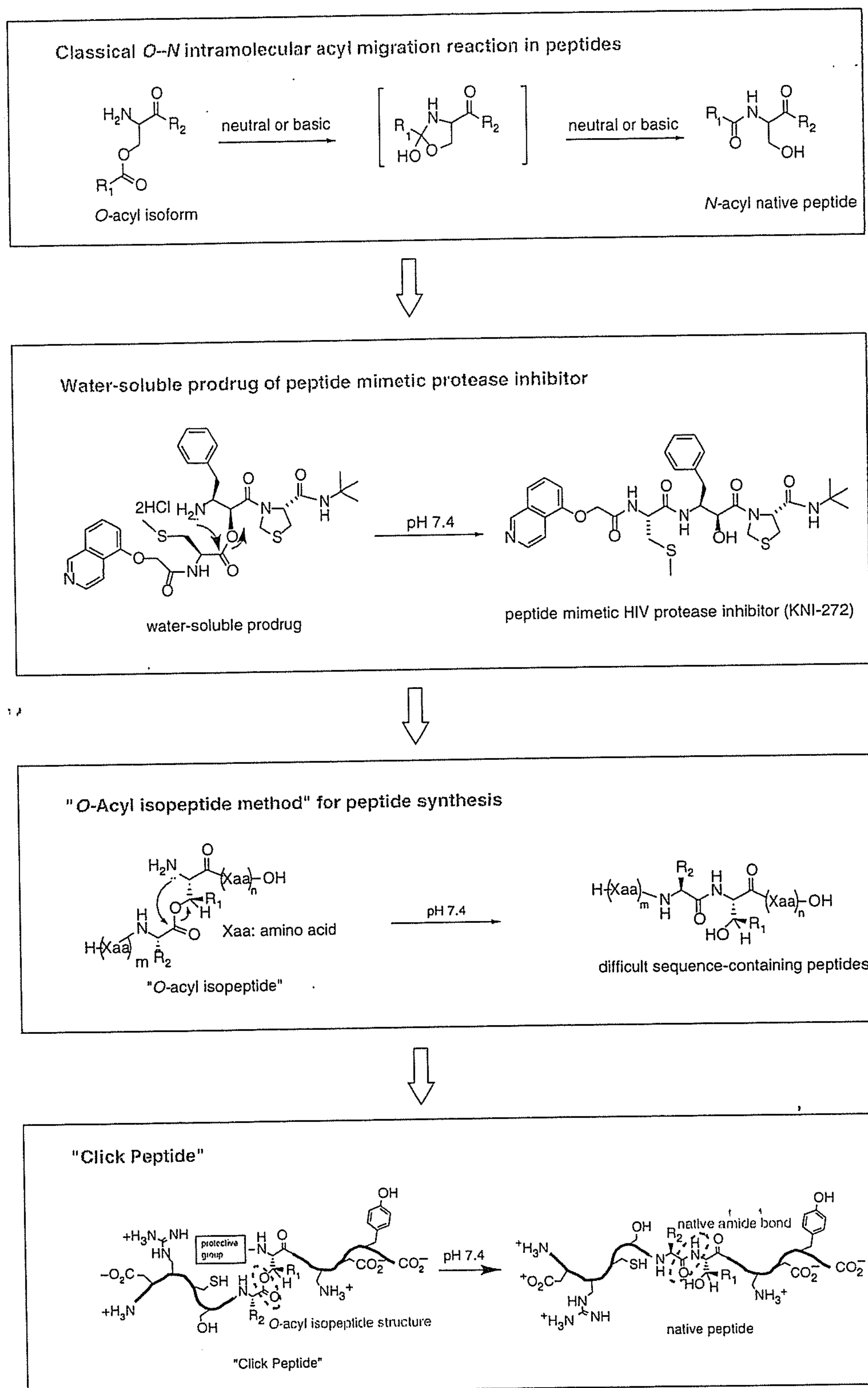

Figure 5 Our workflow on peptide science based on the '*O–N* intramolecular acyl migration reaction'.

will further contribute to the study of peptides and proteins. Examples of such studies include the studies of membrane peptides/proteins that are difficult to handle in various conditions because of their high self-assembling characters.

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1. シクロフィリンとHIV-1

足立 昭夫，鎌田 和弥，藤田 美歌子

はじめに

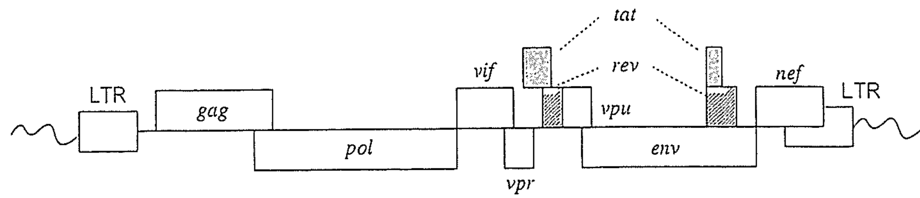
エイズの原因ウイルスであるヒト免疫不全ウイルス（HIV：human immunodeficiency virus）が発見されたのは1983年のことである。その後まもなく，新種のウイルスが見出されHIV2型(HIV-2：HIV type 2)と名付けられた。HIV-2はほぼ西アフリカ地方に限局して存在し伝播性や病原性が弱いとされているのに対し，1983年のウイルスHIV-1は全世界に蔓延し人類の脅威となっている[1]。感染爆発が凄まじいアジア・アフリカ地域では，HIV-1の制圧が最も緊急の医学的課題である。

1993年にHIV-1粒子にシクロフィリンが結合することが著名な科学雑誌Cellに掲載された[2]。HIV-1タンパク質と細胞タンパク質の相互作用を分子生物学的に証明した画期的論文である。この論文を契機としてシクロスポリンは抗HIV-1薬としても一躍脚光を浴び，作用機構などが詳細に解析された。その後，シクロフィリンAがHIV-1の複製や宿主域（トロピズム）に密接にかかわっていることも明らかにされ，現在も精力的に研究が行われている。ここでは，HIV-1複製とシクロフィリン / シクロスポリンとの関係をまとめるが，さらに，ウイルストロピズムに関する筆者らの最新の成果についても概説する。

1. HIV-1の基本的性状

1）ゲノムおよびタンパク質

図1に示したように，HIV-1ゲノムには9種類の遺伝子がある。これらの遺伝子は構造遺伝



ウイルスタンパク質		機能
構造タンパク質		
Gag	マトリックス（MA：matrix）	ウイルス粒子形成・放出，脱殻・逆転写
	カプシド（CA：capsid）	ウイルス粒子形成・放出，脱殻・逆転写
	ヌクレオカプシド（NC：nucleocapsid）	脱殻・逆転写
	p6	ウイルス粒子放出，Vpr/Vpxのウイルス粒子へのターゲッティング
Pol	プロテアーゼ（PR：protease）	Gag前駆体やGag-Pol前駆体の開裂による成熟ウイルス粒子（感染性ウイルス粒子）の生成
	逆転写酵素（RT：reverse transcriptase）	ウイルスDNA合成（含RNase H活性）
	インテグラーゼ（IN：integrase）	ウイルスDNAの細胞染色体DNAへの組込み
Env	gp120	標的細胞受容体への結合
	gp41	標的細胞への侵入
調節タンパク質		
Tat		転写の増強
Rev		構造およびアクセサリータンパク質（Nefを除く）の発現増強
アクセサリータンパク質		
Vif		抗ウイルス細胞因子APOBEC3Gの不活化
Vpr		ウイルスDNAの核移行
Vpu		ウイルス粒子の放出促進
Nef		細胞表面におけるMHC-Ⅰ発現の抑制

図1　HIV-1ゲノムとウイルスタンパク質

LTR：long terminal repeat（長末端反復配列）

HIV-1のゲノム構造の模式図を上に，ウイルスタンパク質の機能を下に示した。ゲノムはプロウイルス（図3）の構造である。機能は数多くある報告[1,3]のうち，主要なものだけを記載した。

子，調節遺伝子，アクセサリー遺伝子に分類され，その産物も同様に3群に分類されている[3]。構造タンパク質はウイルス粒子の主要構成成分であり，Tat，RevおよびVpuを除く他のウイルスタンパク質も粒子内に存在する（図2）。感染細胞内にのみ存在するこれら3種類のタンパク質は，ウイルス遺伝子やタンパク質の発現制御あるいはウイルス粒子の産生効率に関与している。構造タンパク質はすべてのレトロウイルスに共通に存在するが，調節タンパク質は霊長

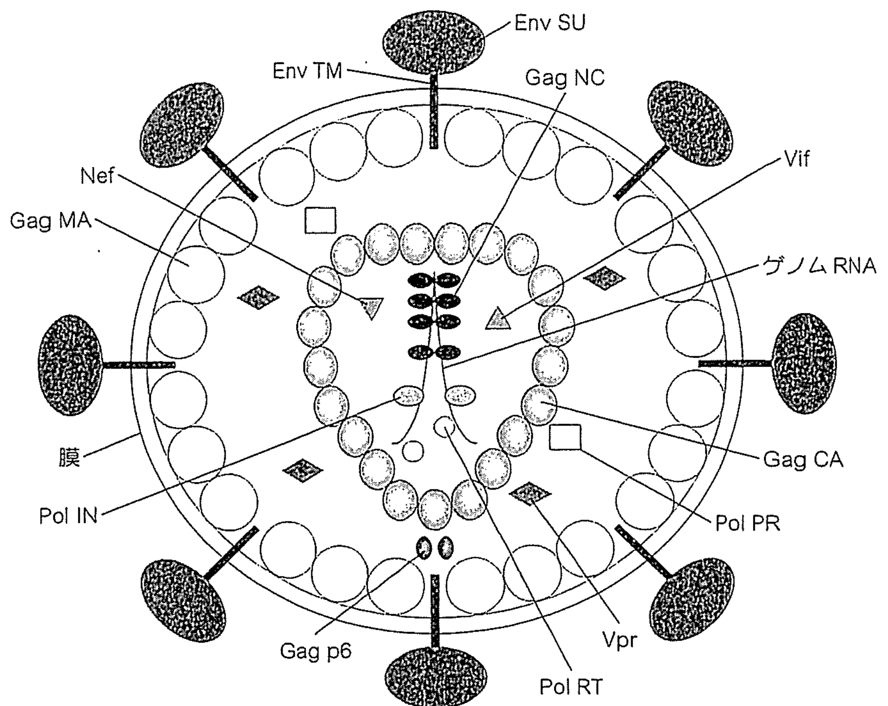


図2　HIV-1 粒子の構造

SU : surface protein（外膜タンパク質）gp120, TM : transmembrane protein（膜貫通タンパク質）gp41
HIV-1 粒子を構成するウイルスタンパク質を模式的に示した。Vif および Nef は粒子中に微量しか存在しないため，その生物学的意義については議論がある。ウイルスタンパク質の略称については図1参照のこと。

類レトロウイルス（白血病ウイルス，免疫不全ウイルス，スプーマウイルス）に特徴的で，アクセサリータンパク質はそれぞれの霊長類レトロウイルスに特異的である。

2）ウイルス複製機構

HIV-1 もレトロウイルスの一員であるから，基本的にはこのウイルス群特有の複製様式に従って増殖するが，構造遺伝子のみからなる単純なレトロウイルスに比べてはるかに複雑な複製制御機構がある。図3に感染細胞における HIV-1 複製の主な過程とそれに関与するウイルスタンパク質をまとめた。ウイルス粒子の細胞への吸着・侵入に始まり，プロウイルスの形成，プロウイルスからの遺伝子発現を経て，ウイルス粒子の放出，感染性粒子への成熟に至るのがレトロウイルス共通の生活環である。HIV-1 が特徴的であるのは，上記調節タンパク質とアクセサリータンパク質によって制御される複製過程である（図1および図3）。また，Nef の活性に代表されるように，細胞レベルでは解析困難な，個体内ウイルス複製や病原性に重要な機能もある。

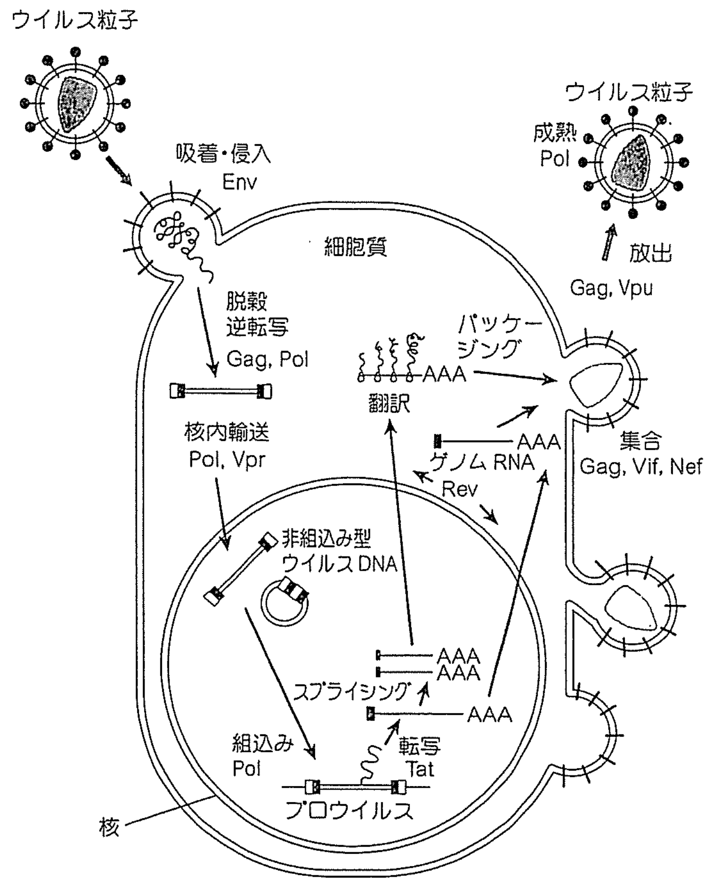


図３　HIV-1 の複製サイクル（文献１より一部引用改変）
HIV-1 の一複製サイクル（吸着・侵入からウイルス粒子の成熟まで）を模式的に示した。複製過程とそれに関与するウイルスタンパク質をまとめた。ウイルスタンパク質の略称および主要機能については図１参照のこと。

2．シクロフィリンと HIV-1 複製

シクロフィリン A は HIV-1 Gag（group specific antigen：群特異抗原）の CA（capsid：カプシド）領域（図１および図２）に特異的に結合する（HIV-2 など他の霊長類免疫不全ウイルスの Gag には結合しない。また，他のイムノフィリン FKBP〔FK-binding protein：FK 結合タンパク質〕は HIV-1 Gag に結合しない）ので，シクロスポリンは効率的にこれを阻害する[4,5]。シクロスポリンの持つ免疫抑制作用[6]はこの阻害効果に影響しない[5]。単に結合するだけでなく，このシクロフィリン A/Gag CA 相互作用はウイルス学的に極めて重要で，HIV-1 複製の初

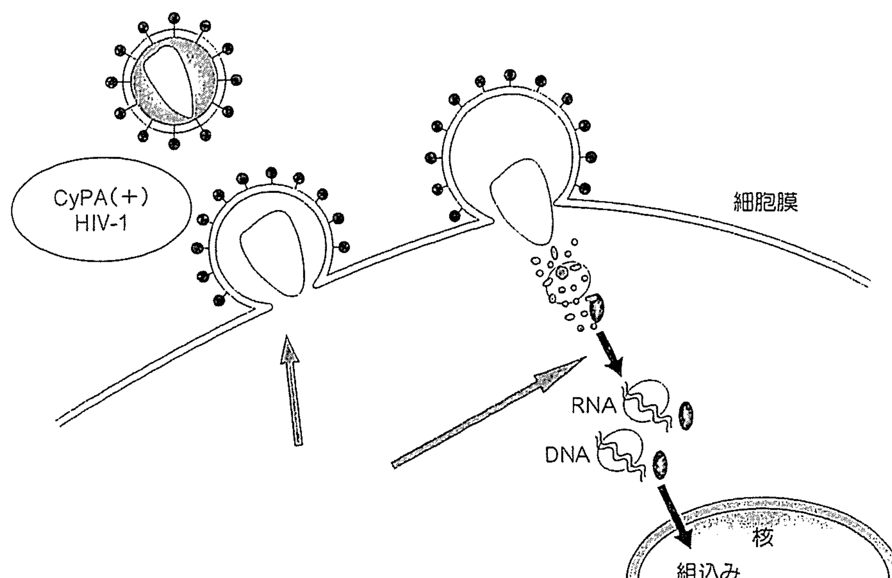


図4　シクロフィリンとHIV-1の複製
シクロフィリンAによるHIV-1複製の増強のメカニズムの概略を示した。シクロフィリンA(CyPA)存在下で産生されたHIV-1粒子は感染直後あるいは脱殻過程(灰色の矢印)が効率良く進行する。

期過程の効率を格段に増強させる（図4）。

現在，抗HIV-1治療の主体は多剤併用療法（HAART：highly active anti-retroviral therapy)である[1]。ウイルスの逆転写酵素やプロテアーゼに対する阻害剤を種々組合わせて用いることで顕著な治療効果が認められている。しかし，HAART法も，ウイルス増殖を完全には抑え込めないこと，抵抗性ウイルスの出現，さらには副作用など，克服すべき課題も多い。一方，シクロスポリンの感染個体における抗HIV-1効果は，免疫抑制作用を欠損させたアナログを含めて未だ明確になっていない。他の薬剤との併用など，今後抗HIV-1戦略の一つとして考慮する必要があると思われる。

3．HIV-1 DT

HIV-1の大きなウイルス学的特徴の一つにその宿主域の狭さがある。HIV-1はチンパンジーとヒトにしか感染せず，また，感染してもチンパンジーはエイズを発症しない。このため，ウイルスと感染細胞の分子生物学的解析が急速に進展しても，その成果がエイズ発症機構の解明や治療法の確立などの究極の目標達成に直接結びついていない。エイズの動物モデルを樹立するため様々なアイデアが試されたが，マウスなどの小動物を用いた実験系は目標にほど遠いも

のであった。筆者らは，HIV-1とサル免疫不全ウイルス（SIV：simian immunodeficiency virus）との間でキメラウイルス（SHIV：simian and human immunodeficiency）を作製し，カニクイザルやアカゲザルを用いた感染実験系の構築に成功した[8]。図5に示したように，実験用のサルで感染・増殖するSHIVを使用することにより，HIV-1遺伝子をゲノムに持つウイルスの動物実験が可能となった。しかし，このSHIVやその改変ウイルスはSIVの遺伝子やシークエンスを多く持つので，HIV-1とはかなり異なるウイルスであるといえる。

最近，サル細胞でのHIV-1複製阻害に関与する2つの細胞因子，TRIM5αおよびAPOBEC3G，が同定された（図6）。いずれのタンパク質もヒトとサル細胞に共通して存在するが，サルのものがより強力にHIV-1複製を阻害すると考えられている。TRIM5αによる複製阻害にはウイルスGag CA領域（図1および図2）が関与し，一方，APOBEC3Gを不活化するのはウイルスのVifである（図1）。筆者らもSHIV研究から，HIV-1のGag CA領域をSIVの対応する領域に置換することでサル細胞でのウイルス複製能を獲得するのではないかと考

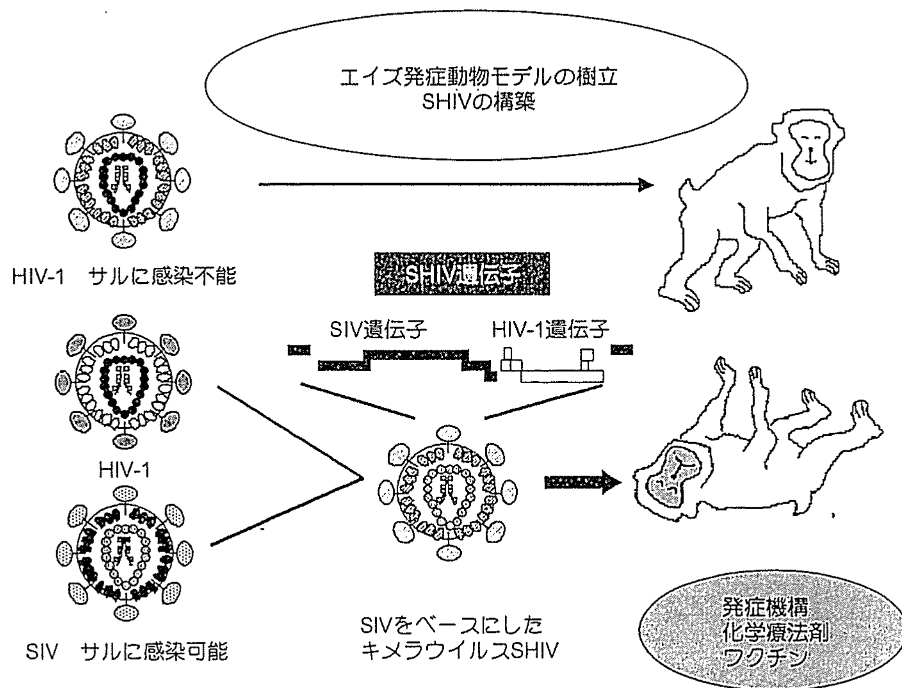


図5　SHIVに基づくHIV-1動物モデル

SIVとHIV-1との間で作製したキメラウイルスSHIVを用いたサル（アカゲザルあるいはカニクイザル）感染実験について，その概略および目標を示した。SHIVはSIVと同様にサルに感染・増殖してエイズを発症させる。

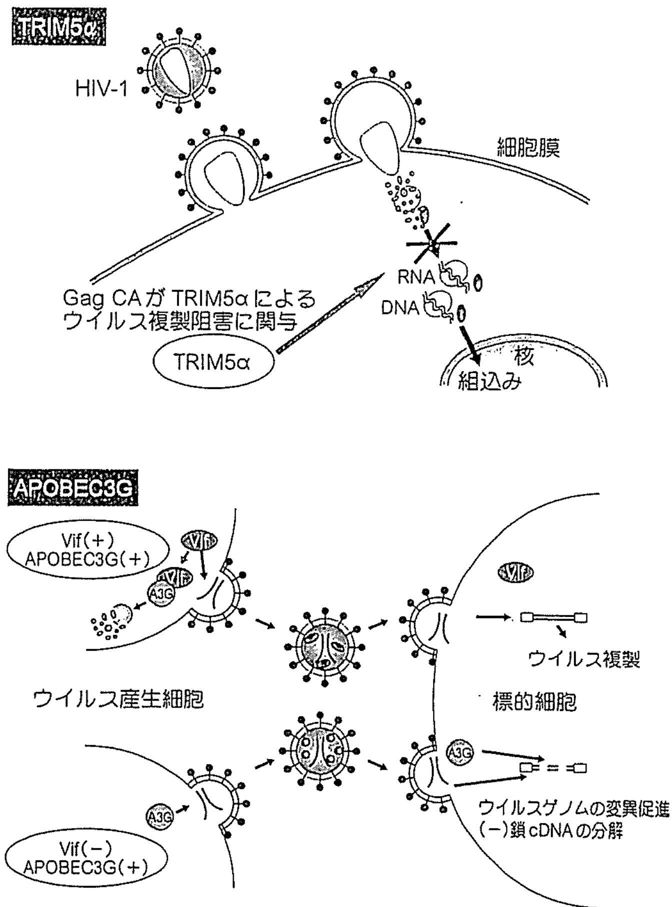
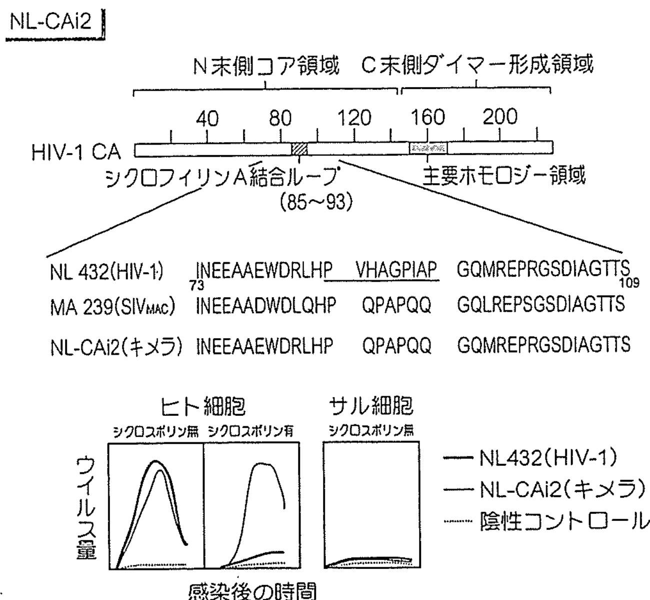


図6　サル細胞におけるHIV-1複製阻害因子
A3G：APOBEC3G
現在までに同定されているウイルス複製阻害因子であるTRIM5αおよびAPOBEC3Gについて，その作用機構の概略を示した。

え，キメラ部分がごく僅かの新しいキメラウイルス（図7）を作製した[9]。このウイルスはヒト細胞で効率良く増殖し，かつ，シクロスポリンに抵抗性となる点ではSIVと同じであったが，サル細胞では増殖できなかった。可能性として考えられたのは，HIV-1 VifがサルAPOBEC3Gを十分不活化できないことであった。筆者らは*gag*遺伝子の一部だけでなく*vif*遺伝子もSIV由来のウイルスクローンを構築した（図7）。HIV-1 dual-tropic（DT）と名付けたこのウイルスは，ヒトおよびサル細胞で良く増殖した。ゲノム長の約95%がHIV-1由来であり，今までに報告のあるサル細胞で感染・増殖可能なキメラウイルスのうち最もHIV-1に近い。今後，この



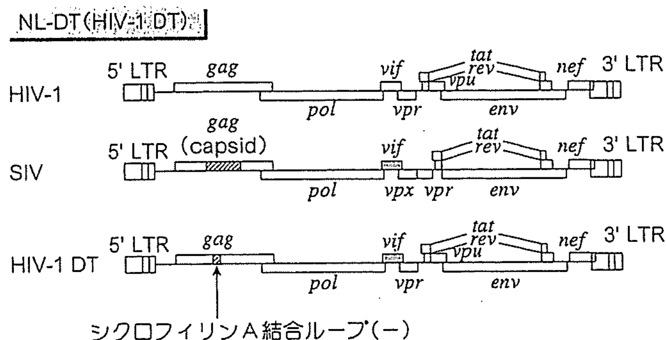


図7　新しいキメラウイルスの構築

HIV-1 をベースにした新しいキメラウイルス（NL-CAi2 および NL-DT）の構造を示した。NL-CAi2 は HIV-1 のシクロフィリン結合ループのみがアカゲザル由来 SIV（SIV_{MAC}）の配列に置換されており，シクロスポリン存在下で SIV_{MAC} と同様に増殖する。ただし，サル細胞には感染性がない。数字は NL432 CA のアミノ酸番号。NL-DT は NL-CAi2 の置換に加え *vif* 遺伝子も SIV_{MAC} のものと置換してあり，サル細胞およびヒト細胞で増殖する。

ウイルスを様々に改変したクローンを用いてサル感染実験を行うことで，ウイルスタンパク質と個体内ウイルス複製やウイルス病原性との関係解明，さらには，新しい化学療法剤やワクチンの開発に向けた研究を展開させていく予定である。

おわりに

シクロフィリンAとHIV-1 Gag CAの結合は，このウイルスの複製や種間トロピズムに大きな生物学的意義を持つ。霊長類免疫不全ウイルスの中でHIV-1のみがシクロフィリンA依存性に増殖するが，この現象のウイルス側決定領域CAがHIV-1の狭い宿主域の一因ともなっている。このテーマに関する研究は，エイズという世界レベルの感染症[10]に対抗する基礎戦略および個体モデルの確立に極めて重要である。日本を含むアジア・アフリカ地域でHIV-1感染者やエイズ患者が激増している現在，このような基礎研究に基づく基礎／臨床の有機的協力体制の構築が求められている。

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