Protein Folding

International Edition: DOI: 10.1002/anie.201701654
German Edition: DOI: 10.1002/ange.201701654

Preparation of Selenoinsulin as a Long-Lasting Insulin Analogue

Kenta Arai[+], Toshiki Takei[+], Masaki Okumura[+], Satoshi Watanabe[+], Yuta Amagai, Yuya Asahina, Luis Moroder, Hironobu Hojo, Kenji Inaba,* and Michio Iwaoka**

Abstract: *Synthetic insulin analogues with a long lifetime are current drug targets for the therapy of diabetic patients. The replacement of the interchain disulfide with a diselenide bridge, which is more resistant to reduction and internal bond rotation, can enhance the lifetime of insulin in the presence of the insulin-degrading enzyme (IDE) without impairing the hormonal function. The [C7U^A,C7U^B] variant of bovine pancreatic insulin (BPIns) was successfully prepared by using two selenocysteine peptides (i.e., the C7U analogues of A- and B-chains, respectively). In a buffer solution at pH 10 they spontaneously assembled under thermodynamic control to the correct insulin fold. The selenoinsulin (Se-Ins) exhibited a bioactivity comparable to that of BPIns. Interestingly, degradation of Se-Ins with IDE was significantly decelerated ($\tau_{1/2} \approx 8$ h vs. ≈ 1 h for BPIns). The lifetime enhancement could be due to both the intrinsic stability of the diselenide bond and local conformational changes induced by the substitution.*

Insulin, a small globular protein (5.8 kDa), comprises two peptide chains, the A-chain (Ins-A, 21 amino acid residues) and B-chain (Ins-B, 30 amino-acid residues). The native structure in a monomeric active state is stabilized by two interchain disulfide bridges, Cys^{A7}-Cys^{B7} and Cys^{A20}-Cys^{B19}, in addition to one intrachain disulfide linkage, Cys^{A6}-Cys^{A11}.[1] Considerable efforts have been directed toward development of various insulin analogues[2] which imitate either bolus secretion of insulin for expeditiously reducing postprandial blood glucose levels[3] or basal secretion of insulin to control the glucose level for an entire day.[4] The latter long-acting analogues have been designed so that insulin forms infusible precipitates or soluble oligomers (hexamer or dihexamer) under physiological conditions and slowly releases active insulin monomers.

In contrast, the insulin-degrading enzyme (IDE) is a possible alternative target for diabetes therapy. IDE, which is involved in clearance of insulin and amyloid β (Aβ),[5] is found in the liver and kidneys. Recent research has revealed that synthetic IDE inhibitors increase circulation of insulin by preventing its degradation in the liver, thus resulting in improvement of the postprandial glucose tolerance.[6] However, other research suggests that IDE inhibitors could induce accumulation of Aβ in the brain,[7] and would lead to Aβ-mediated cognitive impairment. Hence, the design of long-lasting insulin analogues resistant against IDE would be desirable.[8]

In this study, we have attempted a new approach to a long-lasting insulin analogue by exploiting the unique chemical properties of a diselenide bond. Namely, introduction of two juxtaposed selenium atoms to the insulin analogue could lead to a higher kinetic and thermodynamic stability than that of the wild-type without affecting the bioactivity. This new strategy is based primarily on the higher rotational barrier of a Se–Se bond (ca. 4 kcal mol^{-1}) than that of an S–S bond (ca. 3 kcal mol^{-1}),[9] and secondarily on the differing redox potentials of diselenides (E'_0 −381 mV) and disulfides (E'_0 −215 mV),[10] even though the structural features of a Se–Se and S–S bond are very similar to each other.

The lower redox potential of a Se–Se bond is also advantageous for preparation of a diselenide insulin analogue (selenoinsulin) because formation of a Se–Se bridge should occur independently of the presence of additional cysteine residues, and thus facilitate the correct oxidative folding of cysteine-rich peptides. This advantage was already fully confirmed by the quantitative oxidative production of the natural isomer of endothelin[11] and both the natural and non-natural isomers of apamin.[12] More recently, it was successfully used in the generation of the correct natural diselenide/disulfide isomers of many cysteine-rich peptides (for reviews see Ref. [13]). The obtained Sec-substituted peptides were found to retain the native structures with biological activities comparable to those of the wild-types, thus confirming the isomorphous structural character of the Se–Se versus the S–S bridges.[13a,c,14]

By employing bovine pancreatic insulin (BPIns; Figure 1 A) as a model protein, we designed a new selenoinsulin analogue in which Cys^{A7} and Cys^{B7} are replaced with two selenocysteine (Sec; U) residues. We chose this strategy not only because it would bring significant conformational stabilization around the solvent-exposed Cys^{A7}-Cys^{B7} bridge by substitution with a more robust Se–Se bridge, but also because it would inhibit undesired formation of the swap species having an incorrect Cys^{A6}-Cys^{B7} bridge.[15] By applying

[*] Dr. K. Arai,[+] T. Takei,[+] Prof. Dr. M. Iwaoka
Department of Chemistry, School of Science, Tokai University
Kitakaname, Hiratsuka-shi, Kanagawa 259-1292 (Japan)
E-mail: miwaoka@tokai.ac.jp
T. Takei,[+] Dr. Y. Asahina, Prof. Dr. H. Hojo
Institute for Protein Research, Osaka University
Yamadaoka, Suita-shi, Osaka 565-0871 (Japan)
E-mail: hojo@protein.osaka-u.ac.jp
Dr. M. Okumura,[+] Dr. S. Watanabe,[+] Dr. Y. Amagai, Prof. Dr. K. Inaba
Institute of Multidisciplinary Research for Advanced Materials,
Tohoku University
Aoba-ku, Sendai, 2-1-1 (Japan)
E-mail: kinaba@tagen.tohoku.ac.jp
Prof. Dr. L. Moroder
Max Planck Institute of Biochemistry
Am Klopferspitz 18, 82152 Martinsried (Germany)
[+] These authors contributed equally to this work.
Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: http://dx.doi.org/10.1002/anie.201701654.

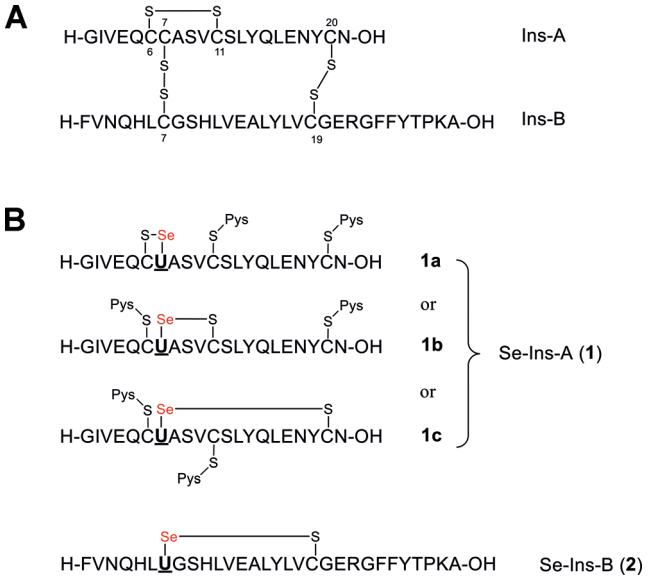


Figure 1. Primary amino-acid sequences and disulfide pattern. A) Wild-type BPIns. B) Synthetic selenoinsulin A-chain (Se-Ins-A; **1**) and B-chain (Se-Ins-B; **2**). U and Pys represent a selenocysteine residue and a 2-pyridylsulfanyl group, respectively.

Fmoc-based solid-phase peptide synthesis (SPPS), two selenopeptides, Se-Ins-A (**1**) and Se-Ins-B (**2**; Figure 1B), were prepared with yields of 9 and 6%, respectively. The Sec residue was introduced to a growing peptide chain on the resin by using Fmoc-Sec(MPM)-OH with DIC-HOBt activation. Since the fully reduced and unprotected form of the C7U^{A} analogue could not be obtained as a single product, probably because of facile formation of either the intrachain Se–S or S–S linkage at various positions, **1** was isolated as a 2-pyridylsulfanyl (Pys)-protected derivative with one Se–S linkage. According to reverse-phase (RP) HPLC analysis (see Figure S1D in the Suppporting Information), **1** contained a single component (either of **1a**, **1b**, or **1c**) but the exact position of the Se–S linkage could not be determined. The selenopeptide **2**, the C7U^{B} analogue, was obtained as the oxidized form with a Se–S linkage. Identities and purities of **1** and **2** were unambiguously confirmed by amino-acid analysis as well as by MALDI-TOF-MS and HPLC analyses (see the Supporting Information).

The oxidative chain assembly was initiated by treatment of the 1:1 mixture of **1** and **2** with DTT (four equivalents with respect to the sum of Sec and Cys residues), which according to its redox potential[10] should remove the Pys groups from the Cys residues as well as reduce the Se–S linkages between the Sec and Cys residues, at 4 °C in a sodium bicarbonate buffer solution at pH 10.0 containing 0.75 M urea to prevent aggregation of **1**. The reduced peptides **1** and **2**, with free thiol (SH) and selenol (SeH) functional groups, were subsequently allowed to assemble spontaneously under thermodynamic control by air oxygen. After 1 minute, an aliquot of the solution was taken, and the reaction was quenched with aqueous 2-aminoethyl methanethiosulfonate (AEMTS),[16] which rapidly converted the free SH and SeH groups into -SSCH$_2$CH$_2$NH$_3^+$ and -SeSCH$_2$CH$_2$NH$_3^+$, respectively. The folding intermediates present in the reaction solution were then analyzed by RP-HPLC (Figure 2) and MALDI-TOF-MS analysis. Various species, that is, 1SeS of **1** (peaks B, C, and D) and reduced forms of **1** and **2** (peaks A and G), were

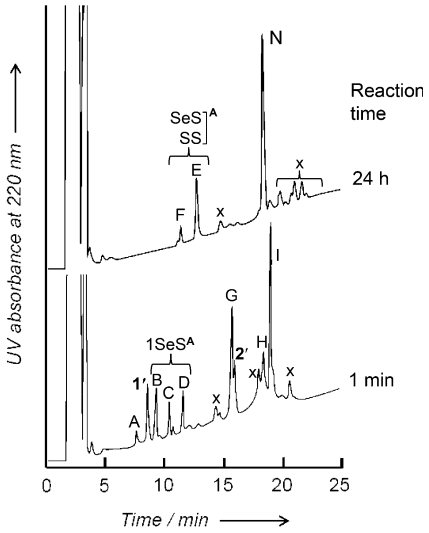


Figure 2. RP-HPLC chromatograms obtained from oxidative chain assembly experiments of Se-Ins in a 25 mM sodium bicarbonate buffer solution (pH 10.0). Folding of Se-Ins was initiated by addition of DTT under the conditions of [**1**] = [**2**] = 184 μM, [DTT] = 4 mM, and [urea] = 0.75 M at 4 °C. The symbols indicate. A = fully reduced **1**, B–D = 1SeS^{A}, E and F = fully oxidized **1** having one S–S and one Se–S linkage, G = reduced **2**, N = native Se-Ins, H = oxidized **2**, I = [Se-Ins-B]$_2$ = a homodimer of Se-Ins-B, *x* = not identified products, **1′** = Se-Ins-A-DTT$_2$ conjugate with two SH groups, and **2′** = Se-Ins-B-DTT$_2$ conjugate.

identified along with oxidized **2** (peak H). A homodimer of Se-Ins-B (peak I) was also observed in a significant amount.

After 24 hours, most of the species initially observed converged to the major product (peak N), concomitantly generating the fully oxidized **1** (peaks E and F) and some unidentified species (peaks *x*) as minor products (Figure 2). The major product was then isolated and characterized as correctly folded selenoinsulin (Se-Ins) by single-crystal X-ray analysis (see below). The yield of the isolated product was up to 21% as determined by amino-acid analysis. By optimizing the reaction conditions (see Table S1), the yield could be slightly increased to 24% by use of TCEP, instead of DTT, as a reductant to initiate the chain-assembly reaction. The yield was further increased to 27% when the chain assembly was carried out in the presence of less urea (0.5 M). It is worth noting that this preparation is the first example to show that the Cys-to-Sec substitution method[10–13] is effective for the oxidative folding of heterodimeric proteins.

The crystal structure of Se-Ins thus isolated was determined at 1.45 Å resolution by the single anomalous dispersion (SAD) method using anomalous scattering of the selenium atoms. The crystallographic data for Se-Ins are summarized in Table S2. Superposition of the X-ray crystal structures of Se-Ins and BPIns (PDB code: 2bn3)[17] demonstrates that Se-Ins has a nearly identical structure to that of BPins with an

RMSD of 0.985 Å over all identical atoms in both structures (Figure 3A) though small but significant changes have been induced by the diselenide bond substitution as described below. The two Sec residues form a diselenide bridge at the native position.

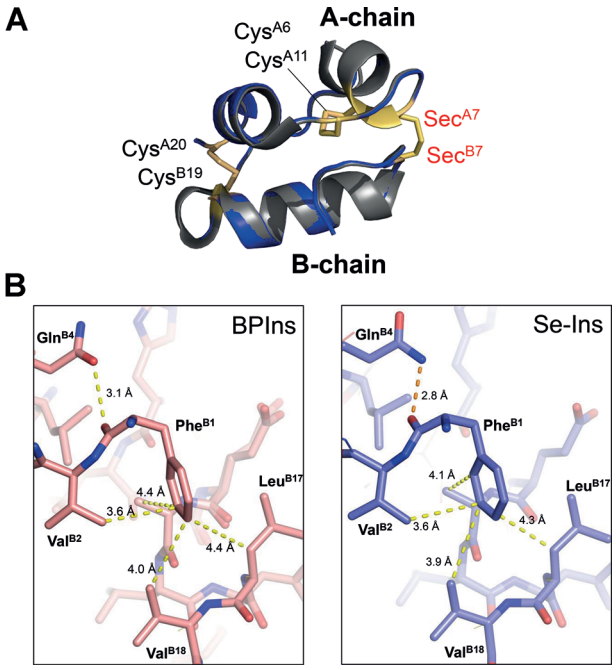


Figure 3. A) Superposition of X-ray crystal structures of Se-Ins (blue; PDB code: 5azz) and BPIns (gray; PDB code: 2bn3). B) Comparison of the N-terminal regions of B-chains between BPIns (left panel) and Se-Ins (right panel). Shorter distances between Phe1 and its adjacent residues in the B-chain of Se-Ins suggest that the N-terminal half of the B-chain is more tightly packed in Se-Ins.

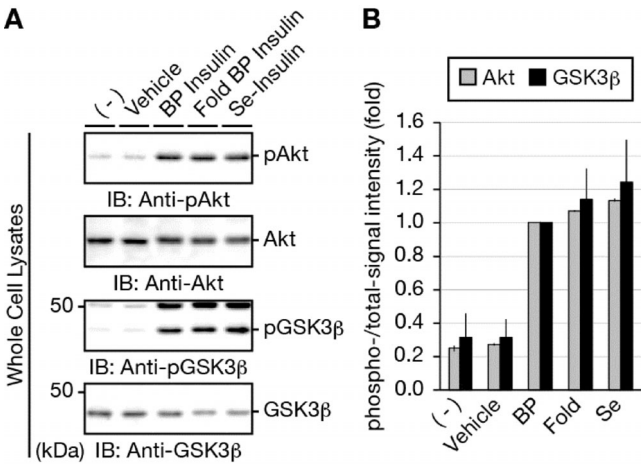


Figure 4. Se-Ins or refolded BPIns activates insulin signaling pathway. Serum-starved HeLa cells were stimulated with 1 μM of either BPIns, refolded BPIns, or Se-Ins for 2.5 min. A) Whole-cell lysates analyzed by immunoblotting. B) The quantitative data. Bars are shown as means ± SEM ($n=3$). The signal intensity of phosphorylated Akt and phosphorylated GSK3β was normalized against that of Akt and GSK3β, respectively.

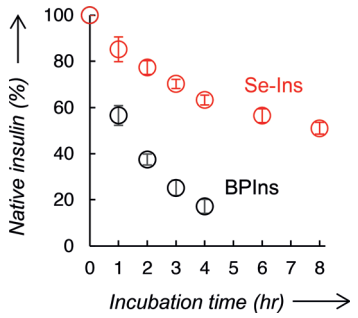


Figure 5. Degradation of BPIns and Se-Ins by insulin-degrading enzyme (IDE). Quantitative analyses of native BPIns (black) and Se-Ins (red). Values are the mean ± SD of three independent experiments.

The results of the X-ray analysis suggest that the function of Se-Ins should be comparable to that of native BPIns. This function was confirmed by experiments using cultured cells (Figure 4). Upon stimulation by Se-Ins, the phosphorylation levels of Akt and GSK3β were significantly increased in HeLa cells, as by stimulation with BPIns.[18] The data indicate that Se-Ins, as well as the synthetic folded BPIns, retain the physiological activity of insulin even after replacement of an interchain disulfide with a diselenide bridge.

The rate of degradation by IDE was subsequently compared for Se-Ins and BPIns (Figure 5). By treating the insulin samples with IDE at 30 °C in a Tris buffer solution at pH 8.0, a number of digested peptide fragments were produced as revealed by RP-HPLC analysis (see Figure S3). Of interest, the degradation rate of Se-Ins was about eight times slower than that of BPIns in terms of the half lifetime ($\tau_{1/2} \approx 8$ h for Se-Ins vs. ≈1 h for BPIns), thus strongly suggesting a long-lasting nature of Se-Ins in vivo. Though not fully evidenced, the following scenarios could account for this observation. Firstly, replacement of the solvent-exposed Cys^{A7}-Cys^{B7} bridge with a diselenide bond, which is more resistant to reduction and internal bond rotation, should confer extra structural robustness on the insulin fold, thus resulting in the enhanced resistance to IDE degradation. The second scenario could be drawn based on the local stabilization around the N- and C-terminal regions of Se-Ins. The crystal structure of the IDE-Ins complex showed that the N- and C-terminal regions of each chain are partially unfolded at the initial step of the degradation by IDE.[19] Local conformational changes induced by the Se–Se bond substitution enhances the interactions between the N-terminus (Phe^{B1}) and its neighboring residues in the Se-Ins B-chain (Figure 3B). We also note that water-mediated interactions involving Thr^{B27}, Ile^{A2}, and Tyr^{A19} are further stabilized at the interface of the N-terminal region of the A-chain and the C-terminal region of the B-chain in Se-Ins (see Figure S2). Such local stabilization found in the N- and C-terminal regions likely allows Se-Ins to prevent the partial unfolding by IDE to a significant extent.

In summary, we have applied the Cys-to-Sec substitution methodology to chain assembly/folding of BPIns and have succeeded in the synthesis of a new [C7U^{A},C7U^{B}] variant of insulin in reasonable yields upon isolation (up to 27%). The

molecular structure, determined by X-ray crystallography, reveals that the replacement of the Cys^{A7}-Cys^{B7} bridge with a diselenide bond exerts only a marginal effect on the native insulin structure. In accord with this, Se-Ins exhibited a bioactivity comparable to that of BPIns in the phosphorylation assays of Akt and GSK3β using HeLa cells. Meanwhile, the degradation rate by IDE was greatly decreased ($\tau_{1/2} \approx 8$ h vs. ≈ 1 h for BPIns), presumably because of the intrinsic stability of the diselenide bond as well as to the slight but significant changes in the local configuration induced by the replacement. Thus, Se-Ins could be a new class of long-acting insulin analogues for diabetes therapy.

Acknowledgments

K.A. and M.O. acknowledge Japan Society for the Promotion of Science (JSPS) for research fellowships for young scientists. We thank S. Shimodaira for the synthesis of the selenocysteine derivative and Dr. K. Mizuno (Tohoku University) for providing the antibodies. We also thank the beamline scientists at the Photon Factory for their help in the X-ray diffraction data collection. This work was supported by KAKENHI Grant Number 26888016 for Research Activity Start-up (to K.A.), in part by Research and Study Project of Tokai University, Educational System General Research Organization (to K.A. and M.I.), Cooperative Research Program of Institute for Protein Research, Osaka University CR-15-01 (to M.I. and H.H.), Cooperative Research Program of Network Joint Research Center for Materials and Devices (to M.I. and K.I.), CREST, JST [to K.I. (JPMJCR13M6)], Grant-in-Aids for Scientific Research on Innovative Areas from MEXT [to K.I. (26116005) and M.O. (15641922)], Takeda Science Foundation (to K.I.), Uehara Memorial Foundation (to M.O.), and the Platform Project for Supporting Drug Discovery and Life Science Research (Platform for Drug Discovery, Informatics, and Structural Life Science) from the Japan Agency for Medical Research and Development (AMED) [to K.I. (1017)].

Conflict of interest

The authors declare no conflict of interest.

Keywords: amino acids · drug discovery · protein folding · selenium · structure elucidation

[1] A. P. Ryle, F. Sanger, L. F. Smith, R. Kai, *Biochem. J.* **1955**, *60*, 541 – 556.
[2] J. M. Tibaldi, *Am. J. Med.* **2014**, *127*, S25 – S38.
[3] a) T. M. Chapman, S. Noble, K. L. Goa, *Drugs* **2002**, *62*, 1945 – 1981; b) N. A. Reynolds, A. J. Wagstaff, *Drugs* **2004**, *64*, 1957 – 1974; c) G. Dailey, *Expert Rev. Endocrinol. Metab.* **2011**, *6*, 527 – 542; d) K. Esposito, A. Capuano, D. Giugliano, *Expert Opin. Biol. Ther.* **2012**, *12*, 1541 – 1550.
[4] a) A. McElduff, *Diabetes Care* **2012**, *35*, 1968 – 1969; b) M. A. Tambascia, F. G. Eliaschewitz, *Diabetol. Metab. Syndr.* **2015**, *7*, 1 – 7; c) H. A. Blair, G. M. Keating, *Drugs* **2016**, *76*, 363 – 374.
[5] a) W. C. Duckworth, R. G. Bennett, F. G. Hamel, *Endocr. Rev.* **1998**, *19*, 608 – 624; b) R. G. Bennett, W. C. Duckworth, F. G. Hamel, *J. Biol. Chem.* **2000**, *275*, 36621 – 36625; c) I. V. Kurochkin, *Trends Biochem. Sci.* **2001**, *26*, 421 – 425.
[6] a) M. A. Leissring, E. Malito, S. Hedouin, L. Reinstatler, T. Sahara, S. O. Abdul-Hay, S. Choudhry, G. M. Maharvi, A. H. Fauq, M. Huzarska, P. S. May, S. Choi, T. P. Logan, B. E. Turk, L. C. Cantley, M. Manolopoulou, W.-J. Tang, R. L. Stein, G. D. Cuny, D. J. Selkoe, *PLoS ONE* **2010**, *5*, e10504; b) J. P. Maianti, A. McFedries, Z. H. Foda, R. E. Kleiner, X. Q. Du, M. A. Leissring, W.-J. Tang, M. J. Charron, M. A. Seeliger, A. Saghatelian, D. R. Liu, *Nature* **2014**, *511*, 94 – 98; c) J. Charton, M. Gauriot, Q. Guo, N. Hennuyer, X. Marechal, J. Dumont, M. Hamdane, V. Pottiez, V. Landry, O. Sperandio, M. Flipo, L. Buee, B. Staels, F. Leroux, W.-J. Tang, B. Deprez, R. Deprez-Poulain, *Eur. J. Med. Chem.* **2014**, *79*, 184 – 193; d) R. Deprez-Poulain, N. Hennuyer, D. Bosc, W. G. Liang, E. Enée, X. Marechal, J. Charton, J. Totobenazara, G. Berte, J. Jahklal, T. Verdelet, J. Dumont, S. Dassonneville, E. Woitrain, M. Gauriot, C. Paquet, I. Duplan, P. Hermant, F.-X. Cantrelle, E. Sevin, M. Culot, V. Landry, A. Herledan, C. Piveteau, G. Lippens, F. Leroux, W.-J. Tang, P. Endert, B. Staels, B. Deprez, *Nat. Commun.* **2015**, *6*, 8250; e) J. Charton, M. Gauriot, J. Totobenazara, N. Hennuyer, J. Dumont, D. Bosc, X. Marechal, J. Elbakali, A. Herledan, X. Wen, C. Ronco, H. Gras-Masse, A. Heninot, V. Pottiez, V. Landry, B. Staels, W. G. Liang, F. Leroux, W.-J. Tang, B. Deprez, R. Deprez-Poulain, *Eur. J. Med. Chem.* **2015**, *90*, 547 – 567; f) T. B. Durham, J. L. Toth, V. J. Klimkowski, J. X. C. Cao, A. M. Siesky, J. Alexander-Chacko, G. Y. Wu, J. T. Dixon, J. E. McGee, Y. Wang, S. Y. Guo, R. N. Cavitt, J. Schindler, S. J. Thibodeaux, N. A. Calvert, M. J. Coghlan, D. K. Sindelar, M. Christe, V. V. Kiselyov, M. D. Michael, K. W. Sloop, *J. Biol. Chem.* **2015**, *290*, 20044 – 20059; g) W.-J. Tang, *Cell Press* **2016**, *27*, 24 – 34.
[7] a) R. B. DeMattos, K. R. Bales, D. J. Cummins, J.-C. Dodart, S. M. Paul, D. M. Holtzman, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 8850 – 8855; b) R. E. Tanzi, L. Bertram, *Cell* **2005**, *120*, 545 – 555; c) Y. Zhang, D. H. S. Lee, *Neuroscientist* **2011**, *17*, 163 – 173; d) S. Costes, P. C. Butler, *Cell Metab.* **2014**, *20*, 201 – 203.
[8] Very recently, a similar approach to an insulin analogue with enhanced thermodynamic stability by replacing the Cys^{A6}-Cys^{A11} SS linkage with a chemically unreactive SC bond was reported: J. A. Karas, N. A. Patil, J. Tailhades, M.-A. Sani, D. B. Scanlon, B. E. Forbes, J. Gardiner, F. Separovic, J. D. Wade, M. A. Hossain, *Angew. Chem. Int. Ed.* **2016**, *55*, 14743 – 14747; *Angew. Chem.* **2016**, *128*, 14963 – 14967.
[9] J. J. BelBruno, *Heteroat. Chem.* **1996**, *7*, 39 – 43.
[10] The redox potentials of diselenides (E'_0 −381 mV) and selenosulfides (E'_0 −326 mV) were measured against DTT (E'_0 −323 mV), and that of disulfides (E'_0 −215 mV) was measured against glutathione (E'_0 −215 mV) as determined in a model octapeptide by Moroder and associates: D. Besse, F. Siedler, T. Dierks, H. Kessler, L. Moroder, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 883 – 885; *Angew. Chem.* **1997**, *109*, 915 – 917.
[11] S. Pegoraro, S. Fiori, S. Rudolph-Böhner, T. X. Watanabe, L. Moroder, *J. Mol. Biol.* **1998**, *284*, 779 – 792.
[12] a) S. Pegoraro, S. Fiori, J. Cramer, S. Rudolph-Böhner, L. Moroder, *Protein Sci.* **1999**, *8*, 1605 – 1613; b) S. Fiori, S. Pegoraro, S. Rudolph-Böhner, J. Cramer, L. Moroder, *Biopolymers* **2000**, *53*, 550 – 564.
[13] a) L. Moroder, *J. Pept. Sci.* **2005**, *11*, 187 – 214; b) L. Moroder, H.-J. Musiol, M. Götz, C. Renner, *Biopolymers* **2005**, *80*, 85 – 97; c) M. Muttenthaler, P. F. Alewood, *J. Pept. Sci.* **2008**, *14*, 1223 – 1239; d) N. Metanis, J. Beld, D. Hilvert, *Patai's Chemistry of Functional Groups* (Ed.: Z. Rappoport), Wiley, **2011**; e) H.-J.

Musiol, L. Moroder, *Chimica Oggi/Chem.Today* **2012**, *30*, 14 – 19.
[14] M. Muttenthaler, S. T. Nevin, A. A. Grishin, S. T. Ngo, P. T. Choy, N. L. Daly, S.-H. Hu, C. J. Armishaw, C.-I. A. Wang, R. J. Lewis, J. L. Martin, P. G. Noakes, D. J. Craig, D. J. Adams, P. F. Alewood, *J. Am. Chem. Soc.* **2010**, *132*, 3514 – 3522.
[15] a) Q. Hua, S. N. Gozani, R. E. Chance, J. A. Hoffmann, B. H. Frank, M. A. Weiss, *Nat. Struct. Biol.* **1995**, *2*, 129 – 138; b) Q. Hua, W. Jia, B. H. Frank, N. F. B. Phillips, M. A. Weiss, *Biochemistry* **2002**, *41*, 14700 – 14715.
[16] T. W. Bruice, G. L. Kenyon, *J. Protein Chem.* **1982**, *1*, 47 – 58.
[17] M. H. Nanao, G. M. Sheldrick, R. B. Ravelli, *Acta Crystallogr. Sect. D* **2005**, *61*, 1227 – 1237.
[18] C. M. Taniguchi, B. Emanuelli, C. R. Kahn, *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 85 – 98.
[19] M. Manolopoulou, Q. Guo, E. Malito, A. B. Schilling, W.-J. Tang, *J. Biol. Chem.* **2009**, *284*, 14177 – 14188.

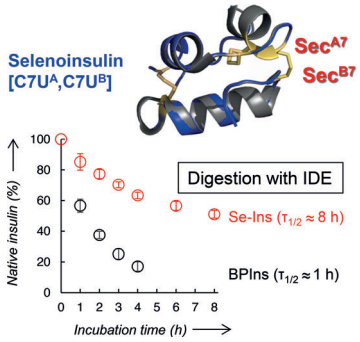
Manuscript received: February 14, 2017
Revised: March 21, 2017
Final Article published: ■■ ■■, ■■■■

Communications

Protein Folding

K. Arai, T. Takei, M. Okumura, S. Watanabe, Y. Amagai, Y. Asahina, L. Moroder, H. Hojo,* K. Inaba,* M. Iwaoka* ——— ■■■■–■■■■

Preparation of Selenoinsulin as a Long-Lasting Insulin Analogue



Long-lasting insulin, the selenium analogue [selenoinsulin (Se-Ins)] of bovine pancreatic insulin (BPIns), is a [C7U^A,C7U^B] variant and has a structure and a bioactivity comparable to that of BPIns. Degradation of Se-Ins with insulin-degrading enzyme (IDE) was significantly decelerated ($\tau_{1/2} \approx 8$ h vs. ≈ 1 h for BPIns). The lifetime enhancement is not only a result of the intrinsic stability of a Se—Se bond but also to the local conformational changes induced by the substitution.

