Research Article

Journal of PeptideScience

Received: 7 January 2009 Revised: 13 February 2009 Accepted: 18 February 2009 Published online in Wiley Interscience: 20 April 2009

(www.interscience.com) DOI 10.1002/psc.1132

Modulation of protein–ligand interactions by photocleavage of a cyclic peptide using phosphatidylinositol 3-kinase SH3 domain as model system

Isao Takahashi,[a‡] Shigeki Kuroiwa,[b‡] Hanna E. Lindfors,[c] Lionel A. Ndamba,[c] Yoshitaka Hiruma,[c] Tatsuo Yajima,[b] Nobuyuki Okishio,[d] Marcellus Ubbink[c] and Shun Hirota[a,b*]**

To photomodulate the interaction of the phosphatidylinositol 3-kinase SH3 domain with a peptide ligand, a cyclic peptide (*cyclic*-1) with a photolabile side chain-to-side chain linker was synthesized. The conformation of *cyclic*-1 differs from that of the parent linear peptide, but becomes identical by UV-irradiation. Accordingly, the binding affinity of *cyclic*-1 to the SH3 domain increased upon conversion of the cyclic to a linear flexible structure by irradiation (K_d: 3.4 ± 1.7 and 0.9 ± 0.3 mM, respectively). These results confirm the usefulness of a photocleavable peptide for photocontrol of peptide–protein interactions. Copyright © 2009 European Peptide Society and John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article

Keywords: protein–peptide interaction; phosphatidylinositol 3-kinase SH3 domain; RLP1 peptide; photocleavage; cyclic peptide; photomodulation

Introduction

Photoactive molecules have attracted much interest, since they can be used with high space and time selectivity [1–21]. For example, photocleavable molecules have been introduced into proteins to study the folding mechanism, whereby the native protein structure is produced instantly by irradiation of the modified protein with light [9–11]. A photoisomerizable intramolecular cross-linker has been used to optically control not only the peptide conformation [12–17] but also its complex formation with biomolecules [18–21]. To optically modify the protein–peptide interaction, we chemically modified a protein-interacting peptide with a photolabile linker and produced a photocleavable cyclic peptide (Figure 1a). Cyclization of the peptide restricts its conformation and reduces its interaction with the protein. By light irradiation, the cyclic peptide was converted to a linear flexible peptide, which increased the binding affinity to the protein (Figure 1b).

As a model of the protein–peptide interaction, we utilized the Src homology 3 (SH3) domain of phosphatidylinositol 3-kinase (PI3K) and its peptide ligand, RKLPPRPSK (RLP1) [22]. PI3K is a heterodimeric enzyme composed of a noncatalytic 85 kDa (p85) subunit, which has an SH3 domain, and a catalytic 110 kDa (p110) subunit, which is essential for the kinase activity. It is well known that the SH3 domain is important in signal transduction and cytoskeletal architecture. The SH3 domain interacts with proline-rich sequences to mediate specific protein–protein interactions [23–25], and the association process between the SH3 domain and the proline-rich peptide has been widely studied, in which long-range electrostatic attraction and hydrophobic interaction play key roles [22,26–29]. We introduced a cysteine to both *N*- and *C*-termini of the acetylated RLP1 peptide (Ac-C-*RKLPPRPSK*-C; ***linear*-1**) and converted the linear peptide to a photocleavable cyclic peptide (***cyclic*-1**) by modification with a photolabile linker, 2,5-bis(bromomethyl)nitrobenzene (Figure 1a). The binding affinity of ***cyclic*-1** to the PI3K SH3 domain was weaker compared to that of ***linear*-1**. However, irradiation of ***cyclic*-1** with light of appropriate wavelength cleaved the peptide at the linker position and produced a ***linear*-1**-like peptide, which showed an increased binding affinity to the SH3 domain.

* *Correspondence to: Shun Hirota, Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan. E-mail: hirota@ms.naist.jp*

** *Marcellus Ubbink, Leiden Institute of Chemistry, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands. E-mail: m.ubbink@chem.leidenuniv.nl*

a *Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan*

b *Department of Physical Chemistry, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan*

c *Leiden Institute of Chemistry, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands*

d *Faculty of Medicine, Kanazawa University, Kanazawa, Ishikawa 920-8640, Japan*

‡ *Both authors contributed equally to the work.*

Copyright © 2009 European Peptide Society and John Wiley & Sons, Ltd.

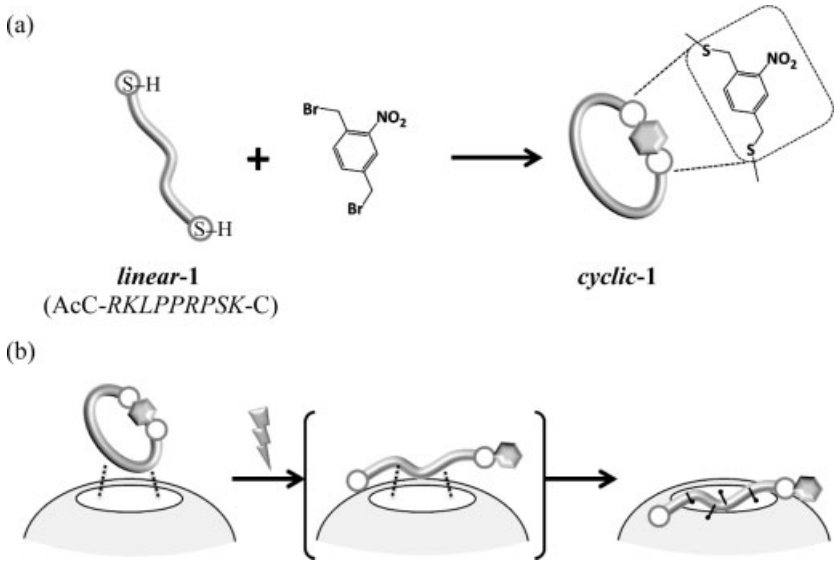

Figure 1. Schematic views of (a) production of a photocleavable cyclic peptide and (b) photocontrol of protein–peptide interaction.

Materials and Methods

Synthesis of 2,5-bis(bromomethyl)nitrobenzene

Synthesis of 2,5-bis(bromomethyl)nitrobenzene was performed by stirring the mixture of 2,5-bis(hydroxymethyl)nitrobenzene (0.9 g, 5 mmol), triphenylphosphine (2.6 g, 10 mmol) and carbon tetrabromide (3.3 g, 10 mmol) in anhydrous diethyl ether (20 ml) at 25 °C for 12 h under N_2 atmosphere. The resulting solution was evaporated and purified by column chromatography (silica gel, hexane). After evaporation of the solution, the resulting yellow oil was purified by column chromatography (silica gel, ethyl acetate/hexane, 1 : 5). Fractions containing the purified product were evaporated and the residue recrystalized from ethyl acetate/hexane. The colorless crystals were collected and dried *in vacuo*. Yield: 470 mg (30%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.08 (d, 1H), 7.64 (dd, 1H), 7.57 (d, 1H), 4.82 (s, 2H), 4.50 (s, 2H); elemental analysis: calcd. (%) for $C_8H_7NBr_2O_2$: C 31.10, H 2.28, N 4.53; found: C 31.23, H 2.30, N 4.48.

Synthesis of Cyclic-1

The linear peptide (Ac-CRKLPPRPSKC, ***linear*-1**) was synthesized by the solid phase method (Shimadzu). ***Linear*-1** in 10 mM potassium phosphate buffer (pH 7.4) was mixed with an equimolar amount of 2,5-bis(bromomethyl)nitrobenzene in DMF (buffer/DMF = 9/1 (v/v)). The mixed solution was stirred at 50 °C for 40 min under N_2 atmosphere in the dark to avoid disulfide formation and photocleavage. The crude compound was purified by HPLC using a Shim-pack PREP-ODS(H) column (2 cmϕ × 25 cm, Shimadzu). The peptide was eluted at 32.9 min with a linear gradient from 100% solution A/0% solution B (0 min) to 80% A/20% B (30 min) and finally 70% A/30% B (60 min) (flow rate, 1 ml/min; solution A, H_2O with 5% MeCN and 0.1% TFA; solution B, 100% MeCN with 0.1% TFA). The absorbance of the eluted solution was monitored at 230, 280 and 315 nm. Fractions containing the desired product were lyophilized and the product was stored in a deep freezer until used. Yield: *ca.* 30%.

Photocleavage of Cyclic-1

Photo-irradiation of ***cyclic*-1** in the presence and absence of the SH3 domain was performed in aqueous solution at 4 °C for 20 min by 355-nm pulses obtained from the third harmonic of a Nd : YAG laser (7 mJ, 10 Hz).

Expression and Purification of PI3K SH3 Domain

Unlabelled PI3K SH3 domain was expressed and purified by using the expression vector pLM1 according to the reported method [22]. The ^{15}*N*-labelled PI3K SH3 domain was expressed and purified as described below.

The purchased recombinant DNA encoding the His-tagged PI3K SH3 domain (GENE ART) was ligated into the vector pET28a and verified by DNA sequencing as follows:
agatctcgatcccgcgaaattaatacgactcactataggggaattgtgagcggataac-aattcccctctagaaataattttgtttaactttaagaaggagatataccatggctgaagg-ctatcagtatcgtgcgctgtatgattacaaaaaagaacgcgaagaagatattgatctgc-atctgggcgatattctgaccgtgaacaaaggcagcctggtggcgctgggctttagcg-atggccaggaagcgcgtccggaagaaattggctggctgaacggctataacgaaacc-accggcgaacgtggcgattttccgggcacctatgtggaatatatcggccgcaaaaaa-ctcgagcaccaccaccaccaccactgagatccggctgctaacaaagcccgaaaggaa-gctgagttggctgctgccaccgctgagcaataactagcataacccctttggggcctcta-aacgggtcttgaggggttttttg

^{15}*N*-labelled recombinant PI3K SH3 domain was prepared by culturing freshly transformed *Escherichia coli* BL21 (DE3) cells in M9 minimal medium containing $^{15}NH_4Cl$ as the sole nitrogen source. Cells were incubated at 37 °C to $OD_{600} \approx 0.8$, induced with IPTG (0.5 mM) and allowed to grow for a further 20 h at 22 °C.

The harvested cells were resuspended in 20 mM sodium phosphate buffer (pH 7.0; 500 mM NaCl/1 mM PMSF/50 μg ml^{-1} DNase) and disrupted by sonication. After centrifugation for 1 h at 15 000 rpm, the crude extract was loaded onto a Ni affinity chromatography column equilibrated with 5 mM imidazole buffer (pH 7.6, 5 mM imidazole/500 mM NaCl/20 mM Tris), washed with 60 mM imidazole buffer and eluted with 500 mM imidazole buffer. Finally, gel filtration chromatography on a Superose12 column equilibrated with 50 mM sodium phosphate buffer (pH 7.0, including 100 mM NaCl) was performed. Protein concentrations were determined from the absorbance at 280 nm (ε = 15.93 mM^{-1} cm^{-1}).

 Copyright © 2009 European Peptide Society and John Wiley & Sons, Ltd.

Journal of PeptideScience

Absorption, CD and Mass Measurements

UV-vis and CD spectra were measured with a UV-2450 spectrophotometer (Shimadzu) and a J-720WI spectropolarimeter (Jasco) with 1 cm and 2 mm path-length quartz cells, respectively. Sample conditions are described in each figure caption. MALDI-TOF mass spectra were recorded on an AXIMA-CFR (Shimadzu) in the positive ion reflection mode using α-cyano-4-hydroxycinnamic acid as a matrix. ESI-TOF mass spectra were obtained with a JMS-T100LC AccuTOF (JEOL) in the positive ion mode.

NMR Measurements of the ^{15}N-labelled SH3 Domain

All NMR experiments were recorded at 298 K on a Bruker DMX600 spectrometer equipped with a TCI-Z-GRAD (^{1}H, ^{13}C, ^{15}N) cryoprobe. NMR samples contained 0.12 mM ^{15}N-labeled SH3 domain in 50 mM sodium phosphate buffer (pH 7.0, 100 mM NaCl/6% D_2O for lock). ***Cyclic*-1** and photo-irradiated ***cyclic*-1** were dissolved in 50 mM sodium phosphate buffer (pH 7.0, 100 mM NaCl) to a concentration of 5 mM. For the assignment of amide backbone resonances of the ^{15}*N*-labelled SH3 domain, 2D [^{15}N,^{1}H] HSQC, 3D [^{15}N,^{1}H] NOESY-HSQC and 3D [^{15}N,^{1}H] TOCSY-HSQC were recorded. The data were processed in AZARA (http://www.bio.cam.ac.uk/azara).

A series of 2D [^{15}N,^{1}H] HSQC spectra were recorded upon the addition of microliter aliquots of the ***cyclic*-1** or photo-irradiated ***cyclic*-1** solution to the SH3 domain sample. Before addition of the peptides, reference 2D [^{15}N,^{1}H] HSQC spectra were recorded with the free ^{15}*N*-labelled SH3 domain. Chemical shift changes of amide resonances for the ^{15}*N*-labeled SH3 domain in the presence of the unlabeled peptides were analyzed by overlaying the spectra of the bound form with the free SH3 domain in ANSIG [30]. After NMR titrations, the sample containing the ^{15}*N*-labelled SH3 domain and ***cyclic*-1** was transferred into an eppendorf tube and irradiated with 365 nm light for 20 min on ice, using a Spectroline E-series lamp(EF-260C). The irradiated sample was immediately transferred back to an NMR tube and 2D [^{15}N,^{1}H] HSQC spectrum was recorded.

Chemical shift perturbations of amide resonances ($\Delta\delta_{binding}$) were plotted against the molar ratio of peptide over protein (*R*). A single-site binding model that corrects for dilution was used and a two-parameter nonlinear least-squares fit was performed in the program Origin version 7.5 (Microcal Software, Northampton, MA), using equations 1 and 2:

$$\Delta\delta_{binding} = \frac{1}{2}\Delta\delta_{max}\left(A - \sqrt{A^2 - 4R}\right) \quad (1)$$

$$A = 1 + R + \frac{PR + C}{PCK_a} \quad (2)$$

in which $\Delta\delta_{binding}$ is the chemical shift perturbation at a given [peptide]/[protein] ratio, $\Delta\delta_{max}$ is the chemical shift perturbation at 100% bound SH3 domain, *P* is the initial concentration of the ^{15}*N*-labelled SH3 domain (0.12 mM), *C* is the concentration of the peptide stock solution (5 mM) and K_a is the association constant, respectively.

The average chemical shift perturbations ($\Delta\delta_{avg}$) of an amide resonance was calculated from

$$\Delta\delta_{avg} = \sqrt{\frac{\left(\Delta\delta^{N}_{binding}/5\right)^2 + \Delta\delta^{H}_{binding}{}^2}{2}} \quad (3)$$

in which $\Delta\delta^{N}_{binding}$ and $\Delta\delta^{H}_{binding}$ represent the chemical shift perturbations of the amide ^{15}N and ^{1}H, respectively.

The observed chemical shift changes at a molecular ratio of [peptide]/[protein] = 8.4 ($\Delta\delta^{N}_{binding}$, $\Delta\delta^{H}_{binding}$) were extrapolated to 100% bound protein using the K_a values from the titrations, and the average values ($\Delta\delta_{avg,max}$) were calculated from Eqn (3).

Results and Discussion

***Cyclic*-1** was synthesized by reacting ***linear*-1** with 2,5-bis(bromomethyl)nitrobenzene and purifying the reaction mixture by reversed phase HPLC. The MALDI-TOF mass spectrum of ***cyclic*-1** showed a mass peak at 1473.79 (1+), as well as the ESI-TOF mass spectrum at 1473.75 (1+), 737.38 (2+) and 491.92 (3+). These mass peaks were consistent with the calculated mass numbers and isotopic patterns ($[M + H^+]^+ = 1473.75$, $[M + 2H^+]^{2+} = 737.38$ and $[M + 3H^+]^{3+} = 491.92$; Supporting Information, Figure S1). Ellman's test and Edman degradation of ***cyclic*-1** indicated that there was no free thiol group and no modified amino acid except the cysteine. These results confirm the cyclic structure of the purified peptide. In addition, the CD spectrum of ***cyclic*-1** was clearly different from that of ***linear*-1** (Figure 2a). This difference results from a decrease in the amount of type II polyproline helix making the conformation of ***cyclic*-1** less favorable for binding to the SH3 domain compared to ***linear* -1**.

Since ***cyclic*-1** exhibited a broad absorption band around 320 nm (Supporting Information, Figure S2), the peptide was irradiated with 355-nm pulses obtained from the third harmonic of a Nd : YAG laser to cleave the photoresponsive linker of ***cyclic*-1**. Upon UV-irradiation of ***cyclic*-1** in the presence of dithiothreitol (DTT), the absorption spectrum immediately showed bands at 272 and 375 nm and then exhibited a band at 309 nm after 2.5 h of irradiation. These absorption changes were similar to the photolytic behavior of caged ATP in the presence of DTT, in which the conversion from a 4-formyl-3-nitrosobenzyl to an anthranyl structure by DTT has been reported [31]. The expected mass peaks of the peptide attached to an anthranyl group were also observed in the ESI-TOF mass spectrum of photo-irradiated ***cyclic*-1** (observed mass numbers: 1457.76 (1+), 729.38 (2+) and 486.58 (3+), calculated ones: $[M+H^+]^+ = 1457.75$, $[M+2H^+]^{2+} = 729.38$ and $[M + 3H^+]^{3+} = 486.59$, Supporting Information, Figure S3a). The CD spectrum of photo-irradiated ***cyclic*-1** compares well to that of ***linear*-1** (Figure 2a). In the absence of DTT, the ESI-TOF mass spectrum of photo-irradiated ***cyclic*-1** gave the expected mass peaks of the peptide attached to a hydrolytic derivative of the 4-formyl-3-nitrosobenzyl group (observed mass numbers: 1491.74 (1+), 746.38 (2+) and 497.93 (3+), calculated ones: $[M + H^+]^+ = 1491.76$, $[M + 2H^+]^{2+} = 746.38$ and $[M + 3H^+]^{3+} = 497.92$, Supporting Information, Figure S3b). All of these data suggest that ***cyclic*-1** was converted to a ***linear*-1**-like structure by UV-irradiation both in the presence and absence of DTT.

The CD spectra of the SH3 domain and ***cyclic*-1** were recorded in 100 mM potassium phosphate buffer (pH 7.0) to determine their interactions. The difference CD spectrum of the SH3 domain complexed with and in the absence of ***linear*-1** showed relatively strong bands around 221, 279, 285 and 291 nm and a weaker broad band at 250–300 nm (Figure 2b). This spectral behavior was similar to that reported for the SH3 domain–RLP1 peptide system, and results from a decrease in the helical content (around 221 nm) and the environmental and/or structural changes of the aromatic residues Tyr12, Tyr14, Tyr73 and Trp55 of the SH3 domain (around 279, 285, 291 and 250–300 nm) induced by association with the peptide [26–28]. As clearly seen in Figure 2b, ***cyclic*-1** did not

 Copyright © 2009 European Peptide Society and John Wiley & Sons, Ltd.

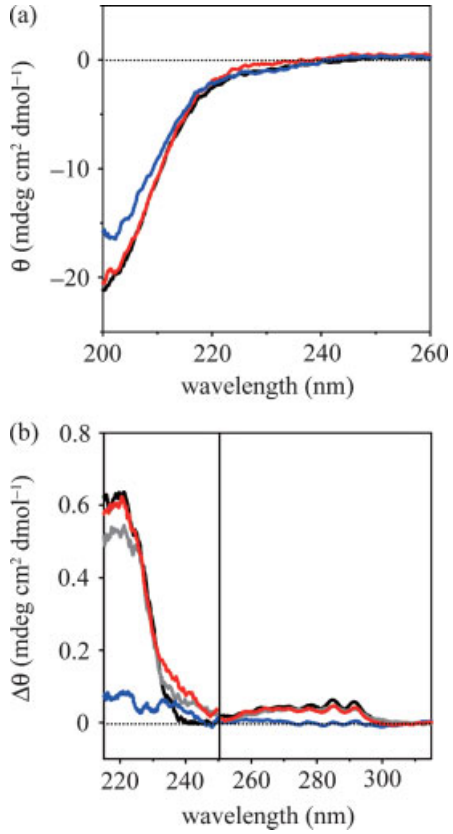

Figure 2. (a) CD spectra of the ***linear*-1** (*black line*), ***cyclic*-1** (*dark grey line; shown in blue online*) and photo-irradiated ***cyclic*-1** (*grey line which is overlapped with the black line; shown in red online*) peptide. Sample conditions were 40 μM in an aqueous solution containing 1 mM DTT. (b) Difference CD spectra of the PI3K SH3 domain. The spectra were calculated between with and without ***linear*-1** (with – without, *black line*), between with and without ***cyclic*-1** (with – without, *dark grey line; shown in blue online*) and between before and after irradiation in the presence of ***cyclic*-1** (after – before, *gray line*). The sum of *blue* and *gray lines* is shown as a *grey line which is overlapped with the black line; shown in red online*, which corresponds to the difference spectrum between with and without photo-irradiated ***cyclic*-1**. Sample conditions were 10 μM (215–250 nm) and 100 μM SH3 domain (250–315 nm) with and without four equivalents of peptide in 100 mM potassium phosphate buffer (pH 7.0) containing 1 mM DTT. Pulse irradiation (7 mJ, 10 Hz) was performed at 4 °C for 20 min for both (a) and (b). This figure is available in colour online at www.interscience.wiley.com/journal/jpepsci.

induce these bands in the corresponding difference CD spectrum, whereas photo-irradiated ***cyclic*-1** did this in a similar manner as ***linear*-1**. These observations imply that ***cyclic*-1** binds stronger with the SH3 domain upon photo-irradiation.

Simulation studies suggest that the interaction between the SH3 domain and a proline-rich motif is initiated by long-range electrostatic attraction between negatively charged residues of the protein and the positively charged arginine side chains of the peptide, followed by hydrophobic interactions between ligand and protein together with an interfacial dewetting [29]. When the CD spectra were measured under low ionic strength conditions (10 mM potassium phosphate buffer, Supporting Information, Figure S4), photo-irradiated ***cyclic*-1** showed a similar difference spectrum to that of ***linear*-1**, indicating strong binding with the SH3 domain. However, under low ionic strength even ***cyclic*-1** gave similar bands (221 (weak), 279, 285 and 291 nm) except for the broad band (250–300 nm). These results allow for three important conclusions: (i) Intermolecular electrostatic interaction plays an important role in binding of both cyclic and linear peptides to the SH3 domain, (ii) Although ***cyclic*-1** and the linear peptide bind at the same site on the SH3 domain, the interactions with the aromatic residues are not entirely the same, since the broad CD band (250–300 nm) is not detected in the ***cyclic*-1** difference spectrum, (iii) The binding constants with the SH3 domain are different between the cyclic and linear peptides (*vide infra*).

To evaluate the binding constants and the binding sites between the SH3 domain and the peptides, ***cyclic*-1** and photo-irradiated ***cyclic*-1** were titrated into the ^{15}N-labeled SH3 domain, and a series of 2D [^{15}N, ^{1}H] HSQC spectra were recorded under 170 mM ionic strength. In both cases, significant chemical shift perturbations of the amide resonances of the SH3 domain were observed (Figure 3). The HSQC spectrum of photo-irradiated ***cyclic*-1** with the SH3 domain was essentially the same as that obtained by UV-irradiation of the mixture of ***cyclic*-1** and the SH3 domain (Figure 3). These results indicate that addition of the SH3 domain to ***cyclic*-1** during light irradiation does not alter the binding effect of the photo-irradiated peptide. The chemical shift perturbation values for several amide nitrogens of the SH3 domain were plotted against the peptide-to-SH3 domain molar ratio, and fitted globally to a 1 : 1 binding model (Supporting Information, Figure S5). The estimated dissociation constants (K_d) were 3.4 (±1.7) and 0.9 (±0.3) mM for ***cyclic*-1** and photo-irradiated ***cyclic*-1**, respectively. Therefore, UV-irradiation of ***cyclic*-1** results in a fourfold stronger binding of the peptide to the SH3 domain. From these dissociation constants, 23% and 46% of ***cyclic*-1** and photo-irradiated ***cyclic*-1**, respectively, were estimated to bind to the SH3 domain at the peptide-to-SH3 domain molar ratio of 8.4. The observed amide chemical shift changes at the molar ratio of 8.4 were extrapolated to reflect the estimated changes when 100% of the protein is complexed (Supporting Information, Figures S6 and S7), and their average values ($\Delta\delta_{avg,max}$) were used to color-code the SH3 residues (Figure 4). From these binding maps, the majority of the affected amides are located at the reported peptide binding site of the SH3 domain for both ***cyclic*-1** and photo-irradiated ***cyclic*-1** [22]. Although the yellow areas in the binding map of photo-irradiated ***cyclic*-1** seemed to be expanded, the $\Delta\delta_{avg,max}$ values were similar to those of ***cyclic*-1**. In the case of photo-irradiated ***cyclic*-1**, the R18 residue was affected significantly by the binding of the peptide, but its signal was not observed at the ratio of [peptide]/[protein] = 8.4 due to exchange broadening. Accordingly, the peptides interact with similar sites of the SH3 domain, although the precise binding mode is slightly different as evidenced by the differences in the CD spectra.

Since the charges of the two peptides, ***cyclic*-1** and photo-irradiated ***cyclic*-1**, are presumably the same at neutral pH, the difference in the dissociation constants should be attributed to the change in the hydrophobic rather than electrostatic interactions. Conformational restriction may also reduce the structural adjustment ability of the peptide for complexation with the SH3 domain, although the conformation stabilized by cyclization may increase the binding ability [32–34]. The photo-irradiated ***cyclic*-1**, which was converted to the linear flexible structure by light irradiation, should be able to adopt a conformation appropriate for protein binding. However, the calculated dissociation constant of photo-irradiated ***cyclic*-1** (0.9 mM) were much higher than that of the RLP1 peptide (9.1 μM) [22]. The steric repulsion between the polypeptide chain of the protein and the two cysteine residues and/or the attached Bzl derivatives of the peptide may have caused a weaker binding. This study shows the possibility

 Copyright © 2009 European Peptide Society and John Wiley & Sons, Ltd.

Journal of PeptideScience

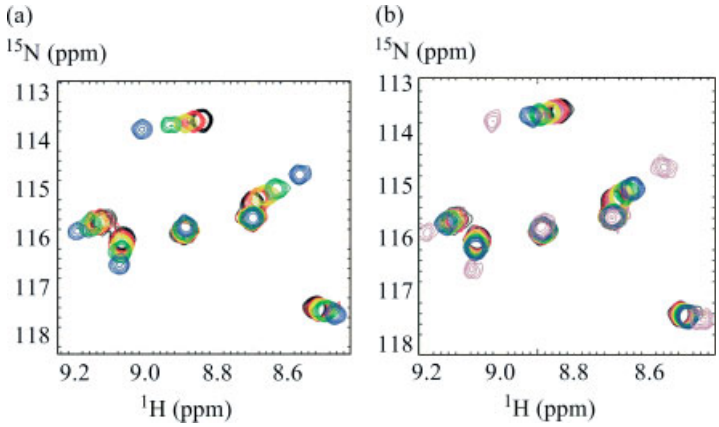

Figure 3. Region of overlaid 2D [^{15}N, ^{1}H] HSQC spectra: Titration of (a) photo-irradiated ***cyclic*-1** and (b) ***cyclic*-1** with the SH3 domain. Free ^{15}*N*-labelled SH3 domain is represented in *black*, and peak shifts upon titration of the SH3 domain with increasing amounts of each peptide (*red, yellow, green* and finally *blue*). Peak shifts upon irradiation of the solution of the SH3 domain titrated with ***cyclic*-1** are shown in *purple* in (b).

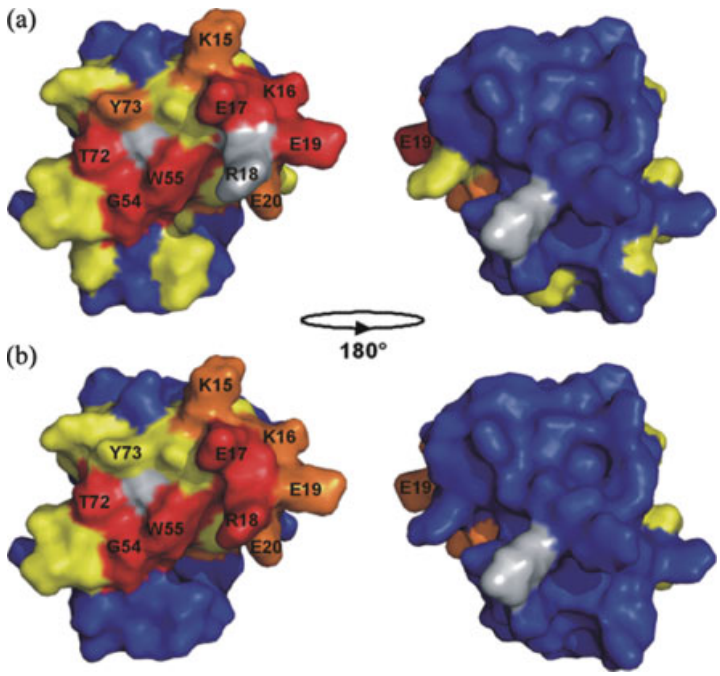

Figure 4. Perturbed residue maps of the SH3 domain upon interaction with (a) ***cyclic*-1** and (b) photo-irradiated ***cyclic*-1**. The averaged chemical shift changes ($\Delta\delta_{avg,max}$) are calculated from the values extrapolated to 100% bound proteins and are color-coded according to the size of shift: $\Delta\delta_{avg,max} \geq 0.35$ ppm represented by *red*, $0.35 > \Delta\delta_{avg,max} \geq 0.20$ ppm by *orange*, $0.20 > \Delta\delta_{avg,max} \geq 0.10$ ppm by *yellow* and $0.10 \text{ ppm} > \Delta\delta_{avg,max}$ by *blue*. Gray-colored residues were not assignable.

to modulate the interaction of a photocleavable peptide with a protein by modifying the peptide structure.

Supporting information

Supporting information may be found in the online version of this article.

Acknowledgements

The authors acknowledge Professor Stuart L. Schreiber (Harvard University) for kindly providing us with the pLM1-PI3K SH3 expression vector and Shimadzu Corporation (Kyoto, Japan) for the synthesis of ***linear*-1**. This work was partially supported to S. H. by Grant-in-Aid for Scientific Research from MEXT (Priority Areas, Water and Biomolecules, No. 18031038), JSPS (Category C, No. 19550169, and the 21st COE Program), JST, The Mochida Memorial Foundation for Medical and Pharmaceutical Research, and Kato Memorial Bioscience Foundation. M. U. was supported financially by the Netherlands Organisation for Scientific Research, VIDI grant 700.52.425, H. E. L. was supported by the Cyttron Consortium, BSIK 3036 and L. A. N. by the Volkswagenstiftung, grant I/80 854. I. T. is financially supported as an Inoue Fellow from the Inoue Foundation for Science.

References

1 McCray JA, Trentham DR. Properties and uses of photoreactive caged compounds. *Annu. Rev. Biophys. Biophys. Chem.* 1989; **18**: 239–270.

 Copyright © 2009 European Peptide Society and John Wiley & Sons, Ltd.

2 Hess GP, Grewer C. Development and application of caged ligands for neurotransmitter receptors in transient kinetic and neuronal circuit mapping studies. *Methods Enzymol.* 1998; **291**: 443–473.
3 Gorostiza P, Isacoff E. Optical switches and triggers for the manipulation of ion channels and pores. *Mol. Biosyst.* 2007; **3**: 686–704.
4 Lu HSM, Volk M, Kholodenko Y, Gooding E, Hochstrasser RM, DeGrado WF. Aminothiotyrosine disulfide, an optical trigger for initiation of protein folding. *J. Am. Chem. Soc.* 1997; **119**: 7173–7180.
5 Hansen KC, Rock RS, Larsen RW, Chan SI. A method for photoinitiating protein folding in a nondenaturing environment. *J. Am. Chem. Soc.* 2000; **122**: 11567–11568.
6 Taniguchi A, Sohma Y, Kimura M, Okada T, Ikeda K, Hayashi Y, Kimura T, Hirota S, Matsuzaki K, Kiso Y. "Click peptide" based on the "*O*-acyl isopeptide method": Control of A β1–42 production from a photo-triggered A β1–42 analogue. *J. Am. Chem. Soc.* 2006; **128**: 696–697.
7 Kostiainen MA, Smith DK, Ikkala O. Optically triggered release of DNA from multivalent dendrons by degrading and charge-switching multivalency. *Angew. Chem. Int. Ed. Engl.* 2007; **46**: 7600–7604.
8 Li H, Hah J-M, Lawrence DS. Light-mediated liberation of enzymatic activity: "Small molecule" caged protein equivalents. *J. Am. Chem. Soc.* 2008; **130**: 10474–10475.
9 Okuno T, Hirota S, Yamauchi O. Folding properties of cytochrome *c* studied by photocleavable *o*-nitrobenzyl modification of methionine 65 and 80. *Chem. Lett.* 2000; **29**: 290–291.
10 Okuno T, Hirota S, Yamauchi O. Folding character of cytochrome *c* studied by *o*-nitrobenzyl modification of methionine 65 and subsequent ultraviolet light irradiation. *Biochemistry* 2000; **39**: 7538–7545.
11 Hirota S, Fujimoto Y, Choi J, Baden N, Katagiri N, Akimiya M, Hulsker R, Ubbink M, Okajima T, Takabe T, Funasaki N, Watanabe Y, Terazima M. Conformational changes during apoplastocyanin folding observed by photocleavable modification and transient grating. *J. Am. Chem. Soc.* 2006; **128**: 7551–7558.
12 Behrendt R, Schenk M, Musiol H-J, Moroder L. Photomodulation of conformational states. Synthesis of cyclic peptides with backbone-azobenzene moieties. *J. Pept. Sci.* 1999; **5**: 519–529.
13 Behrendt R, Renner C, Schenk M, Wang F, Wachtveitl J, Oesterhelt D, Moroder L. Photomodulation of the conformation of cyclic peptides with azobenzene moieties in the peptide backbone. *Angew. Chem. Int. Ed. Engl.* 1999; **38**: 2771–2774.
14 Renner C, Kusebauch U, Löweneck M, Milbradt AG, Moroder L. Azobenzene as photoresponsive conformational switch in cyclic peptides. *J. Pept. Res.* 2005; **65**: 4–14.
15 Renner C, Moroder L. Azobenzene as conformational switch in model peptides. *ChemBioChem* 2006; **7**: 868–878.
16 Woolley GA. Photocontrolling peptide α helices. *Acc. Chem. Res.* 2005; **38**: 486–493.
17 Ulysse L, Cubillos J, Chmielewski J. Photoregulation of cyclic peptide conformation. *J. Am. Chem. Soc.* 1995; **117**: 8466–8467.
18 Guerrero L, Smart OS, Weston CJ, Burns DC, Woolley GA, Allemann RK. Photochemical regulation of DNA-binding specificity of MyoD. *Angew. Chem. Int. Ed. Engl.* 2005; **44**: 7778–7782.
19 Guerrero L, Smart OS, Woolley GA, Allemann RK. Photocontrol of DNA binding specificity of a miniature engrailed homeodomain. *J. Am. Chem. Soc.* 2005; **127**: 15624–15629.
20 Schütt M, Krupka SS, Milbradt AG, Deindl S, Sinner E-K, Oesterhelt D, Renner C, Moroder L. Photocontrol of cell adhesion processes: model studies with cyclic azobenzene-RGD peptides. *Chem. Biol.* 2003; **10**: 487–490.
21 Caamaño AM, Vázquez ME, Martínez-Costas J, Castedo L, Mascareñas JL. A light-modulated sequence-specific DNA-binding peptide. *Angew. Chem. Int. Ed. Engl.* 2000; **39**: 3104–3107.
22 Yu H, Chen JK, Feng S, Dalgarno DC, Brauer AW, Schreiber SL. Structural basis for the binding of proline-rich peptides to SH3 domain. *Cell* 1994; **76**: 933–945.
23 Pawson T. Protein modules and signaling networks. *Nature* 1995; **373**: 573–580.
24 Kapeller R, Cantley LC. Phosphatidylinositol 3-kinase. *BioEssays* 1994; **16**: 565–576.
25 Vanhaesebroeck B, Waterfield MD. Signaling by distinct classes of phosphoinositide 3-kinases. *Exp. Cell Res.* 1999; **253**: 239–254.
26 Okishio N, Nagai M, Fukuda R, Nagatomo S, Kitagawa T. Interactions of phosphatidylinositol 3-kinase Src homology 3 domain with its ligand peptide studied by adsorption, circular dichroism, and UV resonance Raman spectroscopies. *Biopolymers* 2000; **57**: 208–217.
27 Okishio N, Tanaka T, Fukuda R, Nagai M. Role of the conserved acidic residue Asp21 in the structure of phosphatidylinositol 3-kinase Src homology 3 domain: circular dichroism and nuclear magnetic resonance studies. *Biochemistry* 2001; **40**: 119–129.
28 Okishio N, Tanaka T, Nagai M, Fukuda R, Nagatomo S, Kitagawa T. Identification of tyrosine residues involved in ligand recognition by the phosphatidylinositol 3-kinase Src homology 3 domain: circular dichroism and UV resonance Raman studies. *Biochemistry* 2001; **40**: 15797–15804.
29 Ahmad M, Gu W, Helms V. Mechanism of fast peptide recognition by SH3 domains. *Angew. Chem. Int. Ed. Engl.* 2008; **47**: 7626–7630.
30 Helgstrand M, Kraulis P, Allard P, Härd T. Ansig for windows: An interactive computer program for semiautomatic assignment of protein NMR spectra. *J. Biomol. NMR* 2000; **18**: 329–336.
31 Barth A, Corrie JET, Gradwell MJ, Maeda Y, Mäntele W, Meier T, Trentham DR. Time-resolved infrared spectroscopy of intermediates and products from photolysis of 1-(2-nitrophenyl)ethyl phosphates: reaction of the 2-nitrosoacetophenone byproduct with thiols. *J. Am. Chem. Soc.* 1997; **119**: 4149–4159.
32 Friedler A, Friedler D, Luedtke NW, Tor Y, Loyter A, Gilon C. Development of a functional backbone cyclic mimetic of the HIV-1 Tat arginine-rich motif. *J. Biol. Chem.* 2000; **275**: 23783–23789.
33 Mills NL, Daugherty MD, Frankel AD, Guy RK. An α-helical peptidomimetic inhibitor of the HIV-1 Rev–RRE interaction. *J. Am. Chem. Soc.* 2006; **128**: 3496–3497.
34 Chaloin L, Smagulova F, Hariton-Gazal E, Briant L, Loyter A, Devaux C. Potent inhibition of HIV-1 replication by backbone cyclic peptides bearing the Rev arginine rich motif. *J. Biomed. Sci.* 2007; **14**: 565–584.

 Copyright © 2009 European Peptide Society and John Wiley & Sons, Ltd.