Caged cysteine and thiophosphoryl peptides

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Abstract Photoreleasable molecules are important in studies of various biological phenomena, especially cell signaling. Here we report a generally applicable approach for 'caging' unprotected cysteine-containing or thiophosphorylated peptides in aqueous solution with 2-nitrobenzyl bromides. Photolysis of the caged peptides was achieved with near UV light with product quantum efficiencies of 0.06–0.62 under conditions that produced no damage to attendant biological macromolecules. Yields of uncaged peptides were 55–70%. Selective reaction of the side-chain of thiophosphoryl serine with 2-nitrobenzyl bromide in the presence of a cysteinyl residue was also demonstrated, establishing a means for functional caging of various signal transduction proteins without prior modification or mutagenesis.

Key words: Caged reagent; Phosphoserine; Photoactivation; Photorelease; Signal transduction

1. Introduction

The photorelease ('uncaging') of reagents by chemical bond cleavage is widely applied in experimental biology [1–3]. The ability to control the time, dose and site of activation of these reagents by photolysis is a tremendous advantage. Abundant work on caged small molecules has appeared, e.g. caged Ca^{2+} [4], NO [5], CO [6], nucleotides [7], neurotransmitters [8] and amino acids [9]. Caged fluorophores attached to biomolecules have been applied to measurements of the active or diffusional movement of molecules in biological systems [10]. Photocleavable crosslinking reagents and caged biotins have also been devised [11–13]. By contrast, relatively little work has been done on caged peptides and proteins.

One approach to caged proteins is random chemical modification to introduce photocleavable protecting groups [14–18]. Random modification can also be used to introduce photoisomerizable groups, which can provide a degree of reversible control of enzyme activity [19]. Targeted modification is likely to be more predictable and reproducible, and will be essential for caging single domains in multifunctional proteins. Caged proteins, such as lysozyme, have been made by the incorporation of unnatural amino acids at specific sites during in vitro translation [20]. This technique has recently been used to initiate protein splicing from a caged precursor [21]. A second rational approach to the photoregulation of enzymes involves derivatization with an active-site directed reagent. For example, the protease thrombin can be acylated on the active site serine with an *o*-hydroxycinnamoyl group that is stable until irradiated, when it undergoes *trans-cis* isomerization. Thermal deacylation follows by lactonization of the leaving group [22,23]. A third approach is targeted chemical modification of single cysteine residues [24,25], which can be introduced by genetic engineering [24]. We have reported the use of 2-bromo-2-(2-nitrophenyl)acetic acid (BNPA) to cage an engineered staphylococcal α-hemolysin and produce a photoactivatable pore-forming protein [24]. Caged heavy meromyosin has been produced by selective derivatization of one of two natural cysteines with 4,5-dimethoxy-2-nitrobenzyl bromide (DMNBB) [25].

Less work still has been done on caged peptides. *S*-Nitrobenzyl cysteine [26] and many similarly blocked amino acids [27] have been produced for peptide synthesis and might be used to make caged peptides. Reversible alterations in peptide conformation can be obtained by incorporating photoisomerizable groups [28]. Here, we report a general approach to caging peptides by reacting various 2-nitrobenzyl bromides at the sulfur atoms of cysteine or thiophosphoryl serine (Fig. 1). The caged peptides are efficiently photolyzed by UV light to regenerate the uncaged form. Because the caging chemistry is performed in aqueous solution, the procedures should be applicable to proteins, including phosphorylatable components of signal transduction pathways.

2. Materials and methods

2.1. Materials

Kemptide (LRRASLG) was purchased from Peninsula Laboratories. C-Kemptide (LRRACLG) was synthesized at the WFBR Protein Chemistry Facility. Kemptide CS-dimer (LRRACLGLRRASLG) was synthesized by Quality Controlled Biochemicals and further purified by HPLC. Recombinant murine protein kinase A (PKA) catalytic subunit was kindly provided by Dr. Susan Taylor (University of California, San Diego). Adenosine 5′-*O*-(3-thiotriphosphate) (ATP(γ)S) was purchased from Calbiochem-Novabiochem Corp. and phage λ protein phosphatase (λ-PPase) from New England BioLabs. 2-Bromo-2-(2-nitrophenyl)acetic acid (BNPA) was synthesized previously [24] and is now available from Molecular Probes. 2-Nitrobenzyl bromide (NBB) was purchased from Fluka and 4,5-dimethoxy-2-nitrobenzyl bromide (DMNBB) from Aldrich. 1-(2-Nitrophenyl)ethyl phosphate was purchased from Molecular Probes as the diammonium salt.

2.2. Mass spectrometry

Mass spectrometry was performed with a PerSeptive Voyager matrix-assisted laser desorption/ionization mass spectrometer (MALDI-MS) equipped with a 337 nm nitrogen laser. The analyte samples in ~25% acetonitrile/0.1% TFA or in a low salt buffer solution (0.6 μl) were spotted onto the sample plate, prior to the addition of a saturated matrix solution: α-cyano-4-hydroxycinnamic acid in 25% acetonitrile/0.1% TFA (0.6 μl). The sample was dried at room temperature before analysis at a scan rate of 5 s^{-1}. Electrospray ionization

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Abbreviations: ATP(γ)S, adenosine 5′-*O*-(3-thiotriphosphate); BNPA, 2-bromo-2-(2-nitrophenyl)acetic acid; DMNBB, 4,5-dimethoxy-2-nitrobenzyl bromide; ESI-MS, electrospray ionization mass spectrometry; IP_3, 1-D-*myo*-inositol 1,4,5-trisphosphate; λ-PPase, phage λ protein phosphatase; MALDI-MS, matrix-assisted laser desorption/ionization mass spectrometry; NBB, 2-nitrobenzyl bromide; PKA, cAMP-dependent protein kinase; TFA, trifluoroacetic acid

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mass spectrometry (ESI-MS) was carried out by the Harvard Microchemistry Facility.

2.3. Thiophosphorylation of peptides

Kemptide or kemptide CS-dimer (1 mg) was dissolved in 100 mM MOPS, pH 7.0, containing 100 mM KCl and 10 mM $MgCl_2$ (300 μl). ATP(γ)S in H_2O (300 μl) was added in 5-fold molar excess over peptide. Thiophosphorylation was initiated by the addition of PKA catalytic subunit (2 μl; final concentration of 1.0 μg/ml). Thiophosphorylated peptides were purified by HPLC with a Vydac C_{18} column (buffer A: 0.1% TFA in water; buffer B: 0.08% TFA in acetonitrile/water (7: 3)) and then analyzed by MALDI-MS. Ions were observed at *m/z* 869 and 1638, corresponding to the $[M+1H]^+$ protonation states of monomeric thiophosphoryl kemptide and kemptide CS-dimer, respectively. Purified thiophosphorylated peptides were lyophilized and stored at −20°C.

2.4. Synthesis of caged peptides

The reactions of C-kemptide (0.5 mM) with caging reagents, NBB, DMNBB, and BNPA, were carried out in 100 mM Tris-HCl, pH 7.2, at 25°C in the dark for 1 h. Stock solutions (50 mM) of NBB, DMNBB, and BNPA were prepared in, respectively, 95% ethanol, dimethylformamide, and 100 mM Tris-HCl, pH 7.2. Final concentration of 2 mM NBB and BNPA, and 1 mM DMNBB were present in the reaction mixture. The caged C-kemptides were purified by HPLC (see above). Caged thiophosphoryl kemptides were synthesized by reaction with NBB and DMNBB under the same conditions used for caging C-kemptide. Reaction of thiophosphoryl kemptide with BNPA was also carried out in 100 mM sodium acetate buffer, pH 4.0, at 37°C in the dark for 4 h. Reactions of kemptide CS-dimer (0.5 mM) or thiophosphoryl kemptide CS-dimer (0.5 mM) with NBB (2 mM) were carried out in 100 mM Tris-HCl, pH 7.2, at 25°C in the dark for 100 min. This yielded kemptide CS-dimer caged on the cysteinyl residue (MALDI-MS, *m/z* 1677) and thiophosphoryl kemptide CS-dimer caged on both the cysteinyl residue and thiophosphoryl serine (MALDI-MS, *m/z* 1909). Selective caging of thiophosphoryl kemptide CS-dimer (0.5 mM) with NBB (2 mM) was achieved in 100 mM sodium acetate buffer, pH 4.0, at 25¡C in the dark for 100 min. The product was purified by HPLC (MALDI-MS, *m/z* 1774).

2.5. Photolysis in solution

Caged peptide (600 μl, 1.6–1.8 mM) in 100 mM sodium acetate buffer, pH 5.8, was placed in two wells of a 96-well microplate on ice. Irradiation was carried out with a 30 W UV lamp, which was set 1.5 cm above the plate (2200 μW cm^{-2}, peak emission 312 nm, Cole-Parmer H-09815-22). A glass filter, providing a window at 290–380 nm (Oriel #51124), was used in all experiments. Irradiated material was removed at various time points and subjected to analysis by HPLC. The relative product quantum efficiencies (ϕ) for caged C-kemptides and thiophosphoryl kemptides were determined by comparison to the caged phosphate 1-(2-nitrophenyl)ethyl phosphate, ϕ = 0.54 [29,30]. Free phosphate was measured with a kit from Sigma Diagnostics. Quantum efficiencies were calculated by using $v_1/v_2 = \phi_1/\phi_2$ where v is the rate of photolysis. This equation is valid for solutions of high absorbance.

3. Results

3.1. Caging C-kemptide with NBB, BNPA, and DMNBB

Caged C-kemptide was obtained in 95% yield by reaction with 2 mM NBB in 100 mM Tris-HCl, pH 7.2, at 25°C for 1 h. The caged peptide was purified by HPLC and analyzed by MALDI-MS. A peak at *m/z* 923 corresponded to the +1 charge state of caged C-kemptide. A second peak at *m/z* 907 was presumably due to conversion of the nitro group to a nitroso group [31] in the laser beam of the mass spectrometer. A third peak at *m/z* 788 was consistent with uncaged C-kemptide, produced by laser-induced photolysis of the nitrobenzyl group. Similarly, C-kemptide could be caged by reaction with DMNBB and BNPA with yields of more than 95% (Table 1). C-kemptide caged by BNPA appeared as two peaks (both *m/z* 966) upon HPLC, corresponding to the diastereomers of α-carboxy-2-nitrobenzyl C-kemptide. Laser-induced photolysis (both $-NO_2 \rightarrow -NO$ and uncaging) was also observed in these cases.

3.2. Caging thiophosphoryl kemptide with NBB, BNPA, and DMNBB

Thiophosphoryl kemptide was reacted with NBB under the same conditions used for C-kemptide. Caged thiophosphoryl kemptide was formed in 95% yield, purified by HPLC and analyzed by MALDI-MS. There were two sets of peaks in the mass spectrum. One set comprised the major signal at *m/z* 1004, corresponding to the +1 charge state of caged thiophosphoryl kemptide, and a peak at *m/z* 988, which again was presumably due to the loss of an O atom from the nitro group [31]. The second set of peaks at *m/z* 869 and 853, represented the formation of uncaged thiophosphoryl kemptide (*m/z* 869), and, presumably, phosphokemptide in which the sulfur of the thiophosphoryl group had been exchanged for an oxygen atom (*m/z* 853). When the caged thiophosphoryl kemptide was analyzed by ESI-MS, the *m/z* 1004 peak was prominent and the *m/z* 988, 869 and 853 peaks were absent (data not shown), consistent with the view that the latter three peaks originated by laser photolysis in the MALDI mass spectrometer. Sulfur-oxygen exchange did not occur during photolysis in solution (see below). Thiophosphoryl kemptide can also be caged by DMNBB with a yield of 95% under the same reaction conditions (Table 1). All three peaks corresponding to those attributed to photolysis in the case of NB-thiophosphoryl kemptide were seen upon MALDI-MS. No reaction

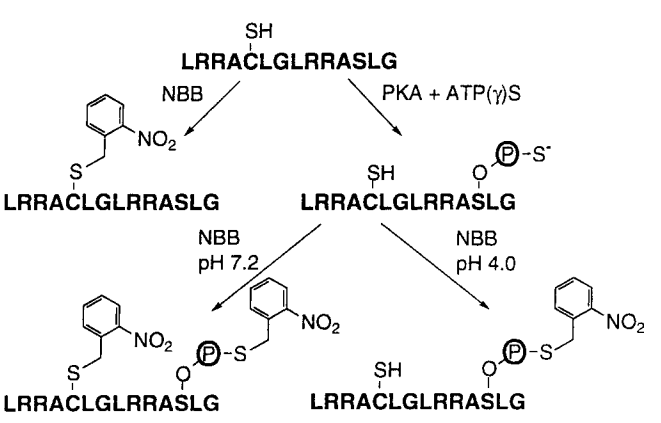

Fig. 1. Strategy for caging of cysteinyl and thiophosphoryl peptides, including selective caging of thiophosphoryl peptides in the presence of free cysteinyl residues.

Table 1
Yields for caging of peptides, and yields and quantum efficiencies for photolysis of caged peptides

Peptide	Reagent	(%) Yield of		ϕ
		Caging	Photolysis	
C-kemptide	NBB	95	70	0.62
C-kemptide	DMNBB	95	67	0.15
C-kemptide	BNPA	95	62	0.21
Thiophosphoryl kemptide	NBB	95	70	0.23
Thiophosphoryl kemptide	DMNBB	95	55	0.06
Thiophosphoryl kemptide	BNPA	10	–	–

occurred between thiophosphoryl kemptide and BNPA at neutral or basic pH. Incubation of thiophosphoryl kemptide with 2 mM BNPA at pH 4.0, 37°C, for 4 h gave ~10% yield of the diastereomers of the caged thiophosphoryl kemptide. These derivatives were not investigated further due to the low yields and the relatively harsh reaction conditions.

3.3. Selective reaction of thiophosphate in a cysteine-containing peptide

To examine the selective caging of serine thiophosphate in the presence of a cysteinyl residue, kemptide CS-dimer (LRRACLGLRRASLG), a 14-mer containing one unit of C-kemptide and one unit of kemptide, was synthesized and thiophosphorylated with ATP(γ)S and PKA catalytic subunit. When thiophosphoryl CS-dimer was reacted with NBB at pH 7.2, both the thiophosphate and Cys residue were modified as determined by MALDI-MS (*m/z* 1909) of the HPLC purified product. Selective caging of the thiophosphate group was achieved in 80% yield by treatment with 2 mM NBB in 100 mM sodium acetate buffer at pH 4.0, 25°C, for 100 min. MALDI-MS (*m/z* 1774) of the HPLC purified product was consistent with only one nitrobenzyl group per peptide. Thiophosphoryl CS-dimer, caged with NBB at pH 4.0, was digested with trypsin and the fragments were analyzed directly by MALDI-MS (Fig. 2). The peak at *m/z* 734 is consistent with the caged thiophosphoryl fragment RAS^{p*}LG, and the peak at *m/z* 600 with its uncaged product. This experiment clearly demonstrates that, at pH 4.0, thiophosphorylated serine was selectively caged in the presence of a free cysteinyl residue.

3.4. Photolysis of caged peptides

The caged peptides were photolyzed in aqueous solution with UV light (λ_{max} = 312 nm). At pH 5.8, both NB-C-kemptide and NB-thiophosphoryl kemptide afforded the uncaged peptides in ~70% yield (Fig. 3). The half-times for release of C-kemptide and thiophosphoryl kemptide under our irradiation conditions were 8 and 20 min, respectively. Photolysis efficiencies for both NB-C-kemptide and NB-thiophosphoryl kemptide were decreased at pH 7.2 (Fig. 3). Photolysis at pH 5.8 of C-kemptide caged by BNPA and DMNBB released uncaged peptide in yields of 62 and 67%, respectively. Thiophosphoryl kemptide caged with DMNBB could be photolytically converted to the uncaged peptide in ~55% yield (Table 1). The relative quantum efficiencies for the photolytic release of peptides from caged C-kemptide and thiophosphoryl kemptide were determined by comparison of the rates of photolysis of the caged peptides with the rate of photolysis of 1-(2-nitrophenyl)ethyl phosphate for which ϕ has been determined to be 0.54 by the ferrioxalate actinometer method [29,30]. The values ranged from ϕ = 0.06 to 0.62 (Table 1). Product quantum efficiencies for the caged thiophosphates 1-(2-nitrophenyl)ethyl ATP(γ)S (ϕ = 0.49, pH 7.1) and 1-(2-nitrophenyl)ethyl 5-thio-IP$_3$ (ϕ = 0.57, pH 7.0) are higher than that for NB-caged thiophosphoryl kemptide. Nevertheless, the latter can be uncaged in the presence of protein (see below). No literature values for thioethers are available for comparison with NB-caged C-kemptide.

3.5. Effect of protein phosphatase on caged thiophosphoryl kemptide

When thiophosphoryl kemptide was incubated with phage λ protein phosphatase (λ-PPase) at 30°C for 3 h, 90% dethiophosphorylation occurred as determined by HPLC and MALDI-MS. By contrast, the thiophosphate group of NB-thiophosphoryl kemptide was fully protected against λ-PPase (Fig. 4). However, when the caged thiophosphoryl kemptide was irradiated by UV light for 40 min and then incubated with λ-PPase at 30°C for 3 h, ~70% dethiophosphorylated peptide was found upon HPLC analysis. About 10% of the uncaged thiophosphoryl kemptide was not yet dethiophosphorylated and about 5% of the caged thiophosphoryl peptide was not yet photolyzed. If NB-thiophosphoryl kemptide was

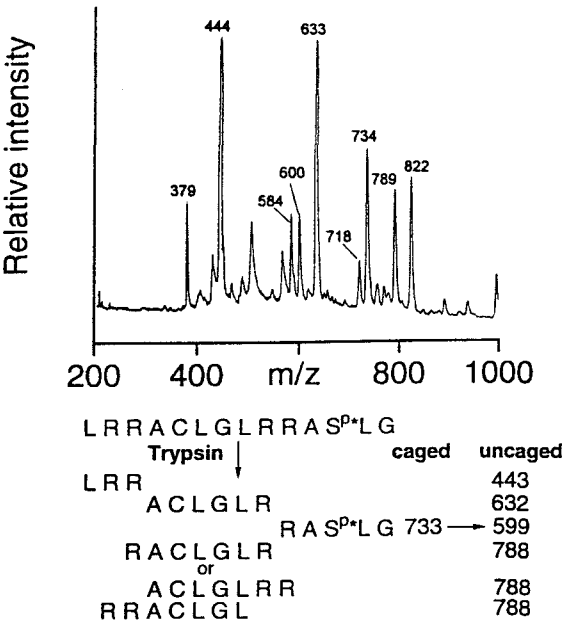

Fig. 2. MALDI-MS peptide map of the reaction product between thiophosphoryl CS-dimer and NBB at pH 4.0. The derivatized peptide was digested with trypsin (1 μg/ml) in 25 mM NH_4HCO_3 at pH 8.9 for 10 min. Identified tryptic fragments are tabulated, including those uncaged by the laser.

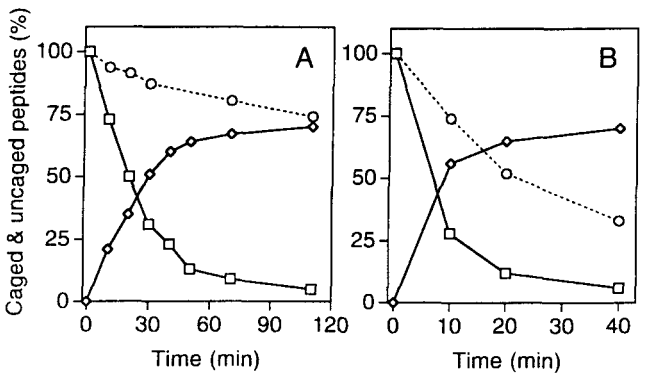

Fig. 3. Photolysis of NB-caged peptides: (A) NB-caged thiophosphoryl kemptide; (B) NB-caged C-kemptide. The caged peptides (1.6–1.8 mM) in 100 mM sodium acetate, pH 5.8, or in 100 mM Tris-HCl, pH 7.2, were irradiated with UV light. Samples were removed for HPLC analysis at the time points shown: (□) caged peptide, pH 5.8; (◇) uncaged peptide, pH 5.8; (○) caged peptide, pH 7.2.

mixed with λ-PPase and then photolyzed under the same conditions, a ~65% yield of dethiophosphorylated kemptide was obtained, showing that the enzyme is stable to the irradiation conditions (Fig. 4).

4. Discussion

We have demonstrated a general approach for caging unprotected cysteine and thiophosphoryl peptides in aqueous solution with 2-nitrobenzyl protecting groups. Further, we have shown that thiophosphoryl serine residues can be selectively caged in peptides that contain free cysteine (Fig. 1). Both the caged cysteine and thiophosphoryl peptides are effectively uncaged by near UV light, under conditions that do not damage a test protein.

Two major considerations dictate the choice of sulfhydryl-directed 2-nitrobenzyl reagents for derivatizing peptides and proteins. First is the ability to obtain a high yield of caged product. The possibility of carrying out the caging reaction in aqueous solution is especially important for proteins. Second, the caged reagent should be efficiently photolyzed at wavelengths that do not damage biological molecules. With the first consideration in mind, we previously synthesized the highly water-soluble BNPA [24]. However, while BNPA reacts efficiently with cysteine peptides, the yield of caged product was poor when a thiophosphorylated peptide was used (Table 1). Therefore, we tested NBB and DMNBB, which can be dissolved in 95% ethanol or dimethylformamide, for reaction with thiophosphoryl peptides. The final concentration of organic solvent can be as low as 2%, which is tolerated by peptides and most proteins. Thiophosphoryl peptides were indeed efficiently caged by NBB and DMNBB under these conditions. While this work was in progress, DMNBB was used to cage heavy meromyosin by selective reaction at Cys-707 in buffer containing 2% dimethylformamide [25].

The second consideration of efficient photolysis at benign wavelengths was addressed by comparing the simple 2-nitrobenzyl chromophore with 4,5-dimethoxy-2-nitrobenzyl. This substitution increases the extinction coefficient at longer wavelengths [3,7]. However, the quantum efficiencies of photolysis of the 4,5-dimethoxy-2-nitrobenzyl peptides were ~4-fold lower than the 2-nitrobenzyl peptides (Table 1). Therefore, the beneficial effect of increased absorption (~3.2-fold with our lamp and filter system, emission maximum 312 nm) is canceled by a lower ϕ, which has been the experience in several related cases [7]. Nevertheless, we show that a caged 2-nitrobenzyl thiophosphoryl peptide can be uncaged by photolysis in the presence of λ-PPase. The released peptide then acts as a substrate for the enzyme, showing that neither the enzyme nor the peptide are damaged by the UV light (Fig. 4). The 4,5-dimethoxy-2-nitrobenzyl peptides could be useful where yet longer wavelengths (≥360 nm) are required to prevent tissue damage and 2-nitrobenzyl absorption is still weaker.

The reactivities of cysteinyl and thiophosphoryl groups towards NBB differ at low pH and allow the caging of thiophosphoryl serine in the presence of free cysteine in the same peptide (Fig. 2). Presumably, the cysteinyl residue ($pK_a \approx 9.5$) is protonated under these conditions, while the thiophosphoryl group is a reactive monoanion. We are extending this approach to thiophosphoryl proteins. In previous, and continuing, attempts to cage proteins at specific sites we have focused on single cysteine mutants [24]. Very often this requires considerable knowledge of the structure and function of the protein and the availability of numerous single cysteine candidates [24]. The activities of many proteins are regulated by phosphorylation, which can often be mimicked by thiophosphorylation. Further, thiophosphorylated proteins are often relatively resistant to cellular phosphatases. Because, the requirements for phosphorylation are quite specific, we can be certain that in most cases caging will block the functional effects of thiophosphorylation and uncaging will restore them. In a previous study, a fluorescent probe was attached to a thiophosphorylated protein, but under conditions that required blocking of cysteines [32]. The present approach

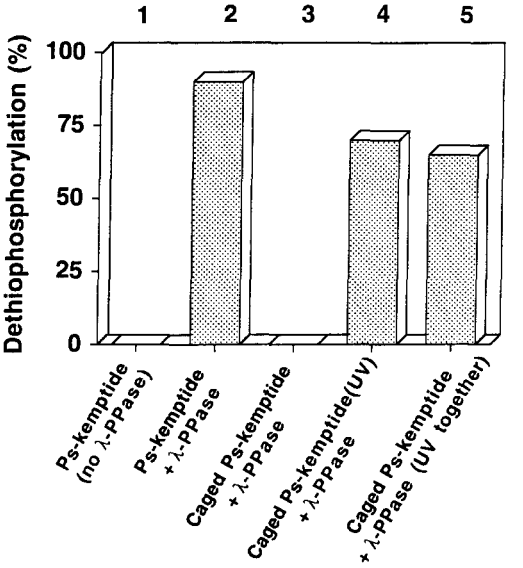

Fig. 4. Treatment of NB-caged thiophosphoryl kemptide by λ-PPase before and after photolysis. The peptide (8 μM) was incubated for 3 h at 30°C with λ-PPase (2000 units) in 55 μl of 50 mM Tris-HCl, pH 7.8, containing 5 mM DTT, 2 mM $MnCl_2$ and 100 μg/ml bovine serum albumin. (1) Thiophosphoryl kemptide (P_s-kemptide), no enzyme; (2) thiophosphoryl kemptide with λ-PPase; (3) NB-caged thiophosphoryl kemptide, no UV, with λ-PPase; (4) NB-caged thiophosphoryl kemptide, first UV treated, then treated with λ-PPase; (5) NB-caged thiophosphoryl kemptide and λ-PPase UV treated together before the incubation at 30°C.

should be applicable to native proteins provided they are not irreversibly denatured or precipitated at the low pH required for selective modification. Shifts in the pK_a values of cysteinyl and thiophosphoryl groups induced by local environments on proteins may facilitate or complicate selective modification and the reaction conditions will have to be adjusted for each case.

Recognizing that the activities of many proteins are modulated by phosphorylation, we envisage several applications for caged thiophosphoryl peptides and proteins, especially in signal transduction research. Signal transduction proteins that are reversibly phosphorylated in vivo include protein kinases and phosphatases themselves, transcription factors, GTP-binding proteins, and proteins that bind to adaptor modules such as SH2 and 14-3-3 domains. Where protein-protein interactions are modulated by phosphorylation, caged peptides (rather than proteins) may provide a means to intervene in cell signaling. In most cases, the peptide or protein reagent will be microinjected into a single cell or several cells in a collection of cells. Subsequent light activation will bring about activation (or in certain cases inactivation) in defined doses with spatial and temporal control [33]. For example, in studies of early development, signaling molecules might be uncaged in one or a few cells in an embryo. Localized activation within a cell will be particularly useful. In studies of neuronal modulation, presynaptic or postsynaptic events might be distinguished from events mediated by the cell nucleus. In addition to cellular studies, the caging technology will be applicable in biophysics. For example, protein-ligand interactions or conformational changes of proteins might be rapidly initiated and then monitored by various means to obtain time-resolved structural information [34]. In biotherapeutics, caged peptides and proteins might be released in specific tissues in controlled doses, at defined times [35].

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References

[1] Gurney, A.M. and Lester, H.A. (1987) Physiol. Rev. 67, 583–617.
[2] Morrison, H. (1993) Biological Applications of Photochemical Switches, Wiley, New York.
[3] Adams, S.R. and Tsien, R.Y. (1993) Annu. Rev. Physiol. 55, 755–784.
[4] Kaplan, J.H. (1990) Annu. Rev. Physiol. 52, 897–914.
[5] Makings, L.R. and Tsien, R.Y. (1994) J. Biol. Chem. 269, 6282–6285.
[6] Lev-Ram, V., Makings, L.R., Keitz, P.F., Kao, J.P.Y. and Tsien, R.Y. (1995) Neuron 15, 407–415.
[7] Corrie, J.E.T. and Trentham, D.R. (1993) in: Biological Applications of Photochemical Switches (Morrison, H. ed.) pp. 243–299, Wiley, New York.
[8] Hess, G.P. (1993) Biochemistry 32, 989–1000.
[9] Fodor, S.P.A., Read, J.L., Pirrung, M.C., Stryer, L., Lu, A.T. and Solas, D. (1991) Science 251, 767–773.
[10] Reinsch, S.S., Mitchison, T.J. and Kirschner, M. (1991) J. Cell Biol. 115, 365–379.
[11] Goldmacher, V.S., Senter, P.D., Lambert, J.M. and Blättler, W.A. (1992) Bioconj. Chem. 3, 104–107.
[12] Olejnik, J., Sonar, S., Krzymańska-Olejnik, E. and Rothschild, K.J. (1995) Proc. Natl. Acad. Sci. USA 92, 7590–7594.
[13] Sundberg, S.A., Barrett, R.W., Pirrung, M., Lu, A.L., Kiangsoontra, B. and Holmes, C.P. (1995) J. Am. Chem. Soc. 117, 12050–12057.
[14] Marriott, G., Miyata, H. and Kinosita, K. (1992) Biochem. Int. 26, 943–951.
[15] Marriott, G. (1994) Biochemistry 33, 9092–9097.
[16] Thompson, S., Spoors, J.A., Fawcett, M.-C. and Self, C.H. (1994) Biochem. Biophys. Res. Commun. 201, 1213–1219.
[17] Golan, R., Zehavi, U., Naim, M., Patchornik, A. and Smirnoff, P. (1996) Biochim. Biophys. Acta 1293, 238–242.
[18] Self, C.H. and Thompson, S. (1996) Nature Medicine 2, 817–820.
[19] Willner, I. and Shai, R. (1996) Angew. Chem. Int. Ed. Engl. 35, 367–385.
[20] Mendel, D., Ellman, J.A. and Schultz, P.G. (1991) J. Am. Chem. Soc. 113, 2758–2760.
[21] Cook, S.N., Jack, W.E., Xiong, X., Danley, L.E., Ellman, J.A., Schultz, P.G. and Noren, C.J. (1995) Angew. Chem. Int. Ed. Engl. 34, 1629–1630.
[22] Stoddard, B.L., Bruhnke, J., Porter, N., Ringe, D. and Petsko, G.A. (1990) Biochemistry 29, 4871–4879.
[23] Stoddard, B.L., Bruhnke, J., Koenigs, P., Porter, N., Ringe, D. and Petsko, G.A. (1990) Biochemistry 29, 8042–8051.
[24] Chang, C.-Y., Niblack, B., Walker, B. and Bayley, H. (1995) Chem. Biol. 2, 391–400.
[25] Marriott, G. and Heidecker, M. (1996) Biochemistry 35, 3170–3174.
[26] Hazum, E., Gottlieb, P., Amit, B., Patchornik, A. and Fridkin, M. (1981) in: Peptides 1980. Proceedings of the Sixteenth European Peptide Symposium, Helsingør, Denmark (Brunfeldt, K. ed.) pp. 105–110, Scriptor, Copenhagen.
[27] Haugland, R.P. (1996) Handbook of Fluorescent Probes and Research Chemicals, 6th edn., Molecular Probes, Eugene, OR.
[28] Ulysse, L., Cubillos, J. and Chmielewski, J. (1995) J. Am. Chem. Soc. 117, 8466–8467.
[29] Kaplan, J.H., Forbush, B. and Hoffman, J.F. (1978) Biochemistry 17, 1929–1935.
[30] Walker, J.W., Reid, G.P., McCray, J.A. and Trentham, D.R. (1988) J. Am. Chem. Soc. 110, 7170–7177.
[31] Morrison, H.A. (1969) in: The Chemistry of the Nitro and Nitroso Groups (Feuer, H. ed.) pp. 165–213, Interscience, New York.
[32] Facemyer, K.C. and Cremo, C.R. (1992) Bioconj. Chem. 3, 408–413.
[33] Wang, S.-H. and Augustine, G.J. (1995) Neuron 15, 755–760.
[34] Stoddard, B.L., Koenigs, P., Porter, N., Petratos, K., Petsko, G.A. and Ringe, D. (1991) Proc. Natl. Acad. Sci. USA 88, 5503–5507.
[35] Bayley, H., Gasparro, F. and Edelson, R. (1987) Trends Pharmacol. Sci. 8, 138–143.