

EPQY_p(HP)EEIPILG and EPQY_p(NB)EEIPILG were uncaged by photolysis at 312 nm.^[38] EPQY_pEEIPILG was released from EPQY_p(HP)EEIPILG in a yield of 50 to 70% at both pH 5.8 and pH 7.3. The $t_{1/2}$ values were 0.55 min at pH 5.8 and 0.52 min at pH 7.3, which correspond to product quantum yield (ϕ_p) values of 0.65 and 0.56 at the respective pH values. At pH 8.2, the yield was greatly reduced and several by-products were formed that could not be identified by MALDI-MS. Under the same conditions, EPQY_pEEIPILG was released from EPQY_p(NB)EEIPILG in 50 to 60% yield. The $t_{1/2}$ values were 5.5 min at pH 5.8 and 8.0 min at pH 7.3, which correspond to ϕ_p values of 0.37 and 0.25.

Finally, the caged peptides and uncaged products were tested in a biochemical assay, which measured the ability of the peptides to bind to an immobilized SH2 domain^[39] (Figure 1). When ³⁵S-EPQY_pEEIPILG was incubated with excess Src SH2-GST attached to agarose,^[40, 41] 27–31% of the radiolabel bound. When the caged ³⁵S-labeled peptides EPQY_p(HP)EEIPILG and EPQY_p(NB)EEIPILG were tested only 1–5% of the radioactivity was bound, which largely represents nonspecific binding, as determined with competing unlabeled peptide or by using an irrelevant peptide. After photolysis of EPQY_p(HP)EEIPILG and EPQY_p(NB)EEIPILG at 312 nm, in both cases, 25–31% of the radioactivity in the uncaged product, repurified by HPLC, was bound to the immobilized SH2 domains. When the uncaged product was not subjected to repurification, the extent of release as judged by Src SH2-agarose binding (corrected for binding efficiency) was consistent with the yield of 50–70% obtained by HPLC.

Here, we have shown that an SH2-binding peptide can be enzymatically thiophosphorylated on tyrosine and then caged with either the NB or HP protecting group. The caged peptides are no longer able to bind to an SH2 domain, but this activity is restored upon photolysis. The NB and HP groups serve their purpose equally well when caging efficiency, the stability of the caged peptide, and the yield of functional peptide after uncaging are considered. However, the HP-protected peptide is about 10 times more sensitive to near-UV radiation, which can be attributed largely to higher ϵ values in the wavelength range used here and to a lesser extent to higher ϕ_p values. While the

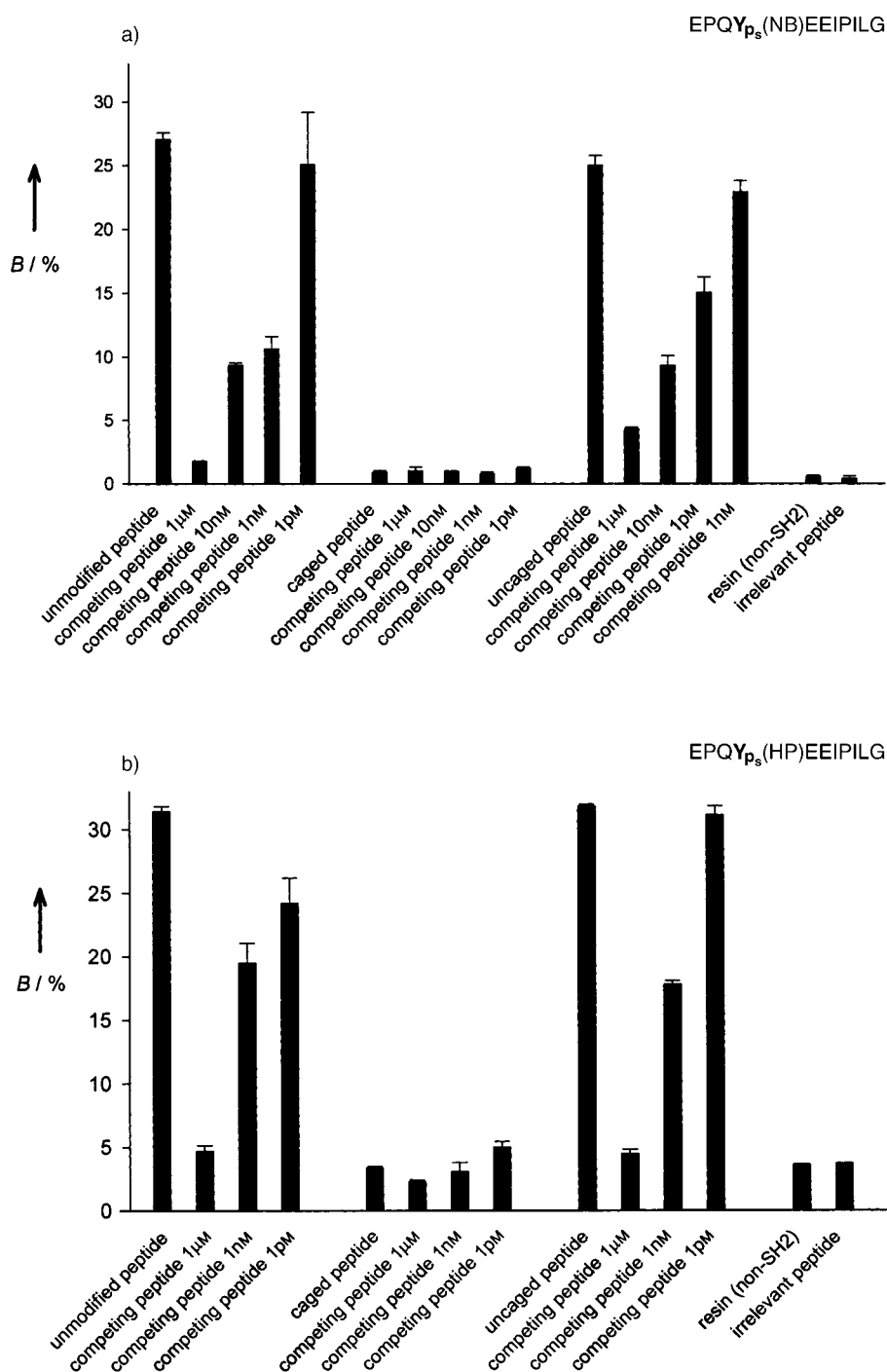


Figure 1. Binding of caged and uncaged peptides to a recombinant SH2 domain. a) EPQY_pEEIPILG caged with NBB; b) EPQY_pEEIPILG caged with HPB. ³⁵S-labeled caged or uncaged peptides were incubated with a Src SH2-SH3-GST fusion protein immobilized on glutathione-agarose beads. *B* is the percentage of the radioactivity retained by the beads. The bound peptides were eluted from the washed beads and assayed by scintillation counting. Competing peptide: EPQY_pEEIPILG; irrelevant peptide: ³⁵S-labeled thiophosphoryl kemptide (LRRASLG).

photosensitivity of a caged reagent ($\phi_{p\epsilon}$) is most important for experiments in cell biology, where photochemical damage to living cells and tissues must be avoided, ϕ_p itself can be of primary importance in certain biophysical experiments, where processes must be triggered with a single flash of light. The values of ϕ_p determined here were in the same range as those measured for *p*-hydroxyphenacyl carboxylate and phosphate

esters under various conditions in the neutral pH range (all $\phi_p > 0.2$).^[27, 29, 30] The HP group generally has the additional advantage of much faster departure after absorption of a photon^[27] compared with the NB group^[42] and we expect that this finding will apply to thiophosphate esters caged on sulfur. At pH values above 8.0, the photolysis of **EPQY_p(HP)EEI-PILG** produced *p*-products as well as the uncaged peptide. The pK_a of the *p*-hydroxyphenacyl group in water is ~ 8 . Zhang et al. found that the ϕ_p of *p*-hydroxyphenyl-2-oxoethyl acetate dropped to zero when the phenolic group was deprotonated in the ground state.^[29] It might be possible to adjust the pK_a of hydroxyphenacyl reagents, and thereby ϕ_p , by judicious substitution of the aromatic ring. That work is in progress. In any event, with ϕ_{pE} values of ~ 5000 , *p*-hydroxyphenacyl reagents are of indisputable utility and we are now extending our studies to signaling proteins thiophosphorylated on serine and tyrosine and to applications in cells.

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- [35] The **EPQYEEIPILG** peptide (2 mM final) was thiophosphorylated with Hck kinase in buffer (100 μ L) containing 50 mM Tris·HCl, pH 7.3, 0.015% Brij 35, 50 μ M *N,N'*-(1,2-ethanediyl)bis[(*N*-carboxymethyl)glycine] (EDTA), 10 mM CoCl₂, ATP(γ)S (Sigma, in 100 μ L of water) was added in five-fold molar excess with respect to the peptide. Hck kinase (0.25–0.5 μ g in 1–2 μ L of storage buffer) was added and the mixture was incubated at 30 °C for 30–60 min. The thiophosphorylated peptide was purified by reverse-phase HPLC with a Vydac C18 column (linear gradient of 10–40% CH₃CN; buffer A: 0.1% CF₃COOH in water; buffer B: 0.075% CF₃COOH in CH₃CN) and identified by MALDI-MS. To prepare ³⁵S-labeled peptide, 100 μ Ci ³⁵S-ATP(γ)S (Amersham) was used, diluted to a specific activity of 177 Ci mmol⁻¹ with ATP(γ)S.
- [36] The thiophosphorylated peptide **EPQY_pEEIPILG** was chemically modified with HPB or NBB in 100 mM sodium acetate buffer (pH 5.8), or 100 mM Tris·HCl (pH 7.3 or 8.0), at 25 °C for 30 min in the dark. Both reagents were prepared as 50 mM stock solutions in 95% EtOH. The final concentration of the reagents was 1 mM, a five-fold molar excess with respect to the peptide. The caged products were purified by HPLC by using the reverse-phase column. The products were identified by MALDI-MS. **EPQY_pEEIPILG** could not be modified cleanly with 2-bromo-2-(2-nitrophenyl)acetic acid (BNPA) or 4,5-dimethoxy-2-nitrobenzyl bromide (DMNBB) (data not given).
- [37] The radiolabeled caged peptides were stored at pH 5.8 (100 mM sodium acetate), pH 7.3 (100 mM Tris·HCl), and pH 8.2 (100 mM Tris·HCl) at both 4 °C and 37 °C for 24 h. No decomposition was detected by HPLC.
- [38] Photolysis of HPLC-purified ³⁵S-labeled caged peptides at 4 nm concentration was carried out in volumes of 200 μ L at pH 5.8 (100 mM sodium acetate), pH 7.3 (100 mM Tris·HCl), and pH 8.2 (100 mM Tris·HCl) in the wells of a 96-well plate (well size of 360 μ L: 0.36 cm² $\times$ 1 cm) by irradiation with a 30 W UV lamp (2200 μ W cm⁻², peak emission 312 nm, Cole-Parmer E-09815-22). The lamp was set 1.5 cm above the sample, which was shielded by a glass band-pass filter to provide a window at 280–370 nm (Oriel 59154). The light intensity was measured by the irradiation of 6 mM K₃Fe(C₂O₄)₃ as described previously.^[43] The photolysis products were removed at various time points during irradiation and subjected to HPLC analysis. The peptides were identified by MALDI-MS and quantified by scintillation counting. The half-time of photolysis ($t_{1/2}$) and product quantum yields (ϕ_p) were estimated by using $t_{1/2} = 0.3/\phi_p I_0$.^[3] Corrections were made for the spectral distribution of the lamp and absorption by the filter.
- [39] A Src SH2-SH3-GST fusion protein was expressed in *E. coli* and purified.^[44] The protein was immobilized on glutathione-Sepharose 4B beads (Pharmacia, No. 17–0756–01) as described previously.^[40] ³⁵S-labeled peptides (10 μ L, 50 nM), photolyzed where indicated, were incubated with SH2-SH3-GST agarose (20 μ L slurry, 1.6 μ M bound protein) in a total volume of 250 μ L containing 50 mM Tris·HCl, pH 7.3, 200 mM NaCl, 10 mM 1,4-dithio-threitol (DTT), 0.05% (w/v) bovine serum albumin for 5 h at room temperature and cooled to 4 °C. After collecting the flow-through, the beads were washed twice, within 1 min, at 4 °C with the incubation buffer (400 μ L). Bound peptide was then eluted with CF₃COOH (0.1%, 100 μ L) and assayed by scintillation counting.
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