

# Synthesis of a photoaffinity labeling analogue of the inactivating peptide of the *Shaker* B potassium channel

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**Abstract** A photoactivatable derivative of the inactivating peptide of the *Shaker* B potassium channel (ShB peptide) has been synthesized from ShB peptide containing an added cysteine residue at the peptide carboxy-terminus and 1-(*p*-azidosalicylamido)-4-(iodoacetamido)butane. The peptide derivative restores rapid inactivation in the deletion mutant *Shaker* BΔ6–46 potassium channel in a manner indistinguishable from that of the wild-type ShB peptide. Also, both peptides display similar conformational behavior when challenged *in vitro* by an artificial model target that partly imitates the properties of the putative receptor site for the inactivating peptide in the *Shaker* B potassium channel. Therefore, we conclude that both functionally and conformationally the photoreactive peptide derivative is an adequate analogue of the wild-type ShB peptide, suitable for photoaffinity labeling of its binding site in the *Shaker* B potassium channel. Moreover, because the ShB peptide also serves as an efficient inactivating peptide for a large variety of other potassium channels, it appears that the photoreactive analogue may be useful to explore homologous sites in many different channel proteins.

**Key words:** Ion channel inactivation; Inactivating peptide; conformation; Photoactivatable peptide analog; Photoaffinity labeling

## 1. Introduction

Voltage-dependent ion channels, such as those specific for Na<sup>+</sup> or K<sup>+</sup>, are integral membrane proteins formed by the assembly of four subunits or domains arranged with a 4-fold symmetry around a central, transmembrane aqueous pore. At resting membrane potentials these channels are usually closed, whereas at sufficiently positive membrane potentials the channels open or activate, to allow a flow of ions down electrochemical gradients established across the membrane [1–3]. In many cases, however, the ion current is transient because very shortly after activation the channels enter into a non-conductive state, the inactivated state. Since the pioneering studies by Armstrong and Bezanilla [4], rapid inactivation has been envisioned as resulting from the occlusion of the channel internal mouth by a flexible cytoplasmic domain of the channel protein itself, which acts as an open channel blocker (the ‘ball and chain’ model of channel inactivation). Nowadays, a number of studies on the inactivation of different voltage-gated channels [1–3] have provided strong experimental support for this hypothesis and, indeed, the identification at the mo-

lecular level of the current-inactivating ‘ball’, its connecting ‘chain’, the receptor site for the ball on the channel protein and the mechanisms determining their mutual interactions, are being pursued.

Much of our present knowledge on such molecular components derives from studies on the *Shaker* B (ShB) K<sup>+</sup> channel, one of the spliced variants of the voltage-dependent K<sup>+</sup> channels coded in the *Shaker* locus of *Drosophila* [5–7]. In this channel, the inactivating ball peptide (ShB peptide) corresponds to the first 20 amino acids in the N-terminal region of each of the ~70 kDa channel subunits (H<sub>2</sub>N-MAAVA-GLYGLGEDRQHRKKQ) [8,9]. A remarkable finding in those studies was that the addition of a synthetic ShB peptide, even when it is not covalently attached to the rest of the protein, restores rapid (N-type) inactivation in mutant channels that did not inactivate because of deletions in their N-terminal region [9,10]. This observation demonstrates that a synthetic peptide with an appropriate sequence contains all the necessary molecular determinants to recognize the channel protein and to induce inactivation. Moreover, the availability of the synthetic peptides in relatively large quantities opens the possibility to use them as specific probes in channel inactivation studies.

As to the receptor site for the ball peptide, studies of inactivation of the ShB K<sup>+</sup> and other channels by inactivating peptides of different origins (reviewed in [3]) have led to the conclusion that it must consist of a hydrophobic protein vestibule able to interact with the hydrophobic part of the inactivating peptide, separated from the cytoplasm by a region of the protein with a negative surface potential. In an attempt to identify such a site with precise portions of the channel, Isacoff et al. [11] found that mutation of five critical residues, spaced as if they were located on the same face of a short  $\alpha$ -helix connecting transmembrane segments S4 and S5 of the channel protein (the S4–S5 loop), altered channel inactivation in the wild-type ShB K<sup>+</sup> as well as in the drk1 channel and thus, the S4–S5 loop is thought to be part of the receptor site for the ball peptide. These important residues of the S4–S5 loop are highly conserved in most K<sup>+</sup> channels of known sequence and include a glutamate and a particularly critical leucine (L385 in the ShB K<sup>+</sup> channel). These findings, as well as the possibility of a conformational rearrangement of the S4–S5 loop coupled to channel opening, have been included in building structural models of voltage-gated K<sup>+</sup> channels [12]. Nonetheless, whenever an independent experimental confirmation might be possible, the assignment of sites in complex proteins based exclusively on mutation data should not be taken as unequivocal, since there is the possibility that the mutation could also cause structural alteration at topologi-

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cally unrelated regions of the protein, distant to the mutated site. Ideally, one would like to have a specific, high affinity probe to covalently label the desired region of the protein as unequivocally as possible. In this respect, photoaffinity labeling probes may offer such a possibility by (i) having an affinity for the target site comparable to that of the natural effector, and (ii) being able to produce covalent adducts with the target site on photolysis of preformed probe/target complexes [13,14]. We report here the synthesis of an analogue of the inactivating ShB peptide designed for covalent photoaffinity labeling of its receptor site in the channel protein.

## 2. Materials and methods

The wild-type ShB peptide (MAAVAGLYGLGEDRQHRKKQ) and the non-inactivating mutant peptide ShB-L7E (MAAVAGEYGLGEDRQHRKKQ) [8,9] were synthesized as C-terminal amides on an automatic multiple synthesizer (AMS 422, Abimed) using a solid-phase procedure and standard Fmoc chemistry [15]. The peptides were purified by reverse-phase HPLC to better than 95% purity, submitted to several lyophilization-solubilization cycles in 10 mM HCl [16] and their composition and molecular mass were confirmed by amino acid analysis and mass spectrometry, respectively [15].

The cysteine-containing peptides ShB-21C (MAAVAGLYGLGEDRQHRKKQC) and ShB-L7E-21C (MAAVAGEYGLGEDRQHRKKQC) were also synthesized as carboxy-terminal amides as described above for the ShB and ShB-L7E peptides. The sulphhydryl group at the carboxy-terminal cysteine of both of these peptides was alkylated by reaction with 1-(*p*-azidosalicylamido)-4-(iodoacetamido)butane (ASIB) (Pierce), a photoreactive, iodinated, sulphhydryl-specific cross linker. Briefly, lyophilized aliquots containing 1–3 mg of either cysteine-peptide were dissolved in 0.6 ml of 0.5 M Tris buffer, pH 8.5, reacted for 1 h and in the dark with a 4-fold molar excess of the reducing agent DTT and alkylated by the addition of an excess of ASIB (2.5-fold molar excess over the DTT used in each experiment) previously dissolved in dimethylsulfoxide (DMSO). The reaction mixtures were incubated for 1 h in the dark under an inert atmosphere, then subjected to chromatography in a Biogel P-2 (Fine) column with 0.2 M ammonium acetate as eluant. The labeled peptides were eluted in the void volume and subsequently lyophilized, rechromatographed, divided into aliquots, lyophilized again and kept frozen. Peptide concentration in these samples was determined by amino acid analysis.

Photolysis of the samples was carried out in 1 × 1 cm quartz cuvettes by irradiation with a UVGL-25 lamp (Ultraviolet Products Inc., San Gabriel, CA) on the long-wavelength setting at room temperature. The distance between the lamp and the cuvette was 1 cm.

For infrared amide I band recordings, lyophilized aliquots of the synthetic peptides and the phospholipid (phosphatidylglycerol (PG) prepared from egg yolk phosphatidylcholine, Avanti Polar Lipids) were separately hydrated in 25 µl of D<sub>2</sub>O buffer (made from D<sub>2</sub>O, 99.9% by atom, Sigma Chemical Co.) to avoid interference by H<sub>2</sub>O infrared absorbance (1645 cm<sup>-1</sup> [17]). The solutions were mixed by placing them into a liquid demountable cell (Harrick, Ossining, NY) equipped with CaF<sub>2</sub> windows and 50 µm thick mylar spacers and maintained at room temperature for 30 min to ensure that the isotopic H-D amide proton exchange reached equilibrium. FT-IR spectra were taken in a Nicolet 520 instrument equipped with a DTGS detector, as previously described [18].

ShBΔ6-46 cDNA [8,19] was transiently expressed in CHO cells using the p513 vector (Stratagene) [20]. The cells were electroporated in 272 mM sucrose buffer with 3 µg of the construction DNA, using a gene pulser apparatus (Biorad). The electroporation pulses were performed with the 125 µF capacitor and at 350 V, resulting in time constants of 24–26 ms. After electroporation the cells were maintained at 37°C and 5% CO<sub>2</sub>. Ionic currents were recorded 24–48 h after transfection, using the whole-cell configuration of the patch-clamp technique [21] and a List EPC7 amplifier. Voltage-clamp pulses were generated, and currents analyzed, using PulseFit software (Heka) in a Macintosh Quadra 650 computer. Capacity compensation and subtraction of linear leakage current were performed. Electrodes

made from soft glass had a resistance of 1–3 MΩ and no series resistance compensation was performed. Transfected cells were plated on slivers of glass coverslips. The external solution (pH 7.4) contained (in mM): 140 NaCl, 2.7 KCl, 2.5 CaCl<sub>2</sub>, 4 MgCl<sub>2</sub> and 10 HEPES. The pipette solution (pH 7.2) contained (in mM): 80 KCl, 30 potassium glutamate, 20 KF, 4 MgCl<sub>2</sub>, 4 ATP, 10 EGTA and 10 HEPES. Peptides were dissolved in the pipette solution at a concentration of 500 µM. In previous experiments, we estimated that complete dialysis of the cytoplasm with the pipette solution occurs in less than 2–3 min.

## 3. Results

### 3.1. Synthesis of the ASIB-labeled peptides

The reaction between ASIB and the cysteine-containing peptides ShB-21C and ShB-L7E-21C was carried out under conditions identical to those previously used to prepare fluorescence peptide derivatives from iodoacetamide forms of several fluorophores [22]. Briefly, this includes DTT reduction of the carboxy-terminal cysteine to the free sulphhydryl and subsequent alkylation by an excess of ASIB at pH 8.5, to produce the corresponding sulfide and HI (Fig. 1A). Separation of each of the resulting labeled peptides from the excess free probe by exclusion chromatography allows simple, quantita-

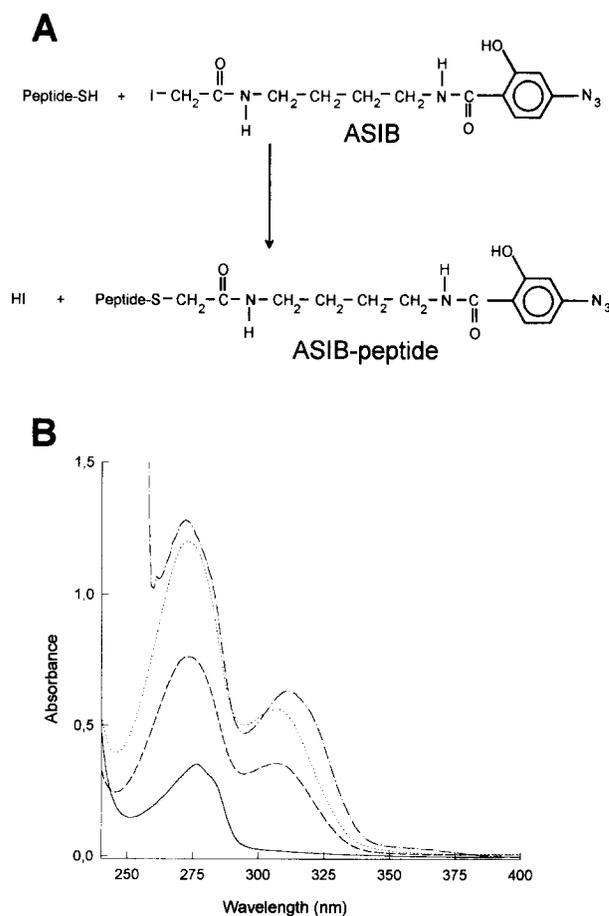


Fig. 1. (A) Scheme of the reaction between 1-(*p*-azidosalicylamido)-4-(iodoacetamido)butane (ASIB) and a cysteine-containing peptide to yield the corresponding ASIB-peptide. (B) Absorbance spectra of free ASIB (67 µM in DMSO) (---), ASIB-labeled ShB-21C (38 µM in 0.05% acetic acid) (---) and ASIB-labeled ShB-L7E-21C (61 µM in 0.05% acetic acid) (···). The spectrum of unlabeled ShB peptide (250 µM in 0.05% acetic acid) (—) is also included to illustrate the contribution of peptide absorbance to the 272 nm maximum.

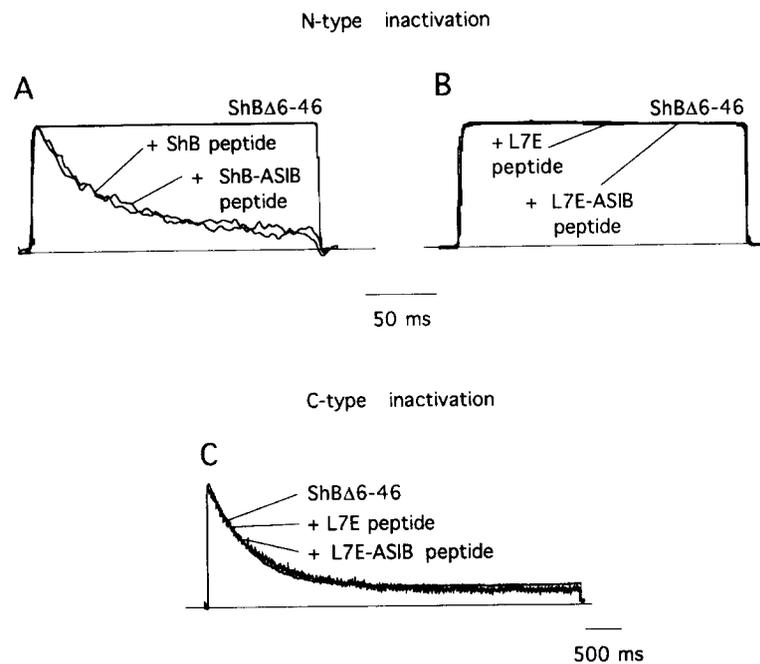


Fig. 2. Effects of ShB peptides and derivatives on ShB $\Delta$ 6-46 whole cell potassium currents. Peptides were dissolved in the pipette solution at a concentration of 500  $\mu$ M in all cases. (A) Comparison of the effects of wild-type ShB peptide and ASIB-labeled, ShB-21C peptide derivative on ShB $\Delta$ 6-46 K<sup>+</sup> current recorded during depolarization to +20 mV from a holding potential of –80 mV. Pulse duration, 200 ms. Note the almost identical inactivating effects of the two peptides. (B) The mutant ShB-L7E peptide and its ASIB-labeled ShB-L7E-21C derivative have no effects on the whole cell ShB $\Delta$ 6-46 current under the same experimental protocol. (C) The latter two peptides also have no influence on the slow, C-type inactivation time course of the ShB $\Delta$ 6-46 current recorded during depolarization to +20 mV from a holding potential of –80 mV. Pulse duration, 5 s.

tive recovery of peptide material. Furthermore, the extent of peptide derivatization, in the range 94–95% in all cases, can easily be established based on the carboxymethylcysteine/peptide molar ratios determined by quantitative amino acid analysis of labeled peptide samples.

Fig. 1B shows the absorbance spectra of ASIB-labeled ShB-21C and ShB-L7E-21C peptides in an aqueous buffer. Absorbance maxima are seen in both samples at 272 and 310 nm, which are similar to the absorbance maxima exhibited by free ASIB dissolved in DMSO. The molar extinction coefficients for the labeled peptides (approx. 9000 M<sup>-1</sup> cm<sup>-1</sup> for either one) were calculated at the 310 nm absorbance maximum to avoid possible interference from peptide absorbance in the 272 nm maximum (the unlabeled ShB and ShB-L7E peptides have a molar extinction coefficient at 280 nm of approx. 1300 M<sup>-1</sup> cm<sup>-1</sup> [15]). Also, the infrared spectrum of purified ASIB-labeled peptides exhibits a band at approx. 2115 cm<sup>-1</sup> characteristic of aromatic azides (not shown), thus further substantiating the presence of an intact azide moiety in the peptide derivatives.

### 3.2 Studies of K<sup>+</sup> channel inactivation by the inactivating peptide derivative

The ability of the ASIB-labeled peptides to induce K<sup>+</sup> channel inactivation was assessed by studying the effects of unlabeled and ASIB-labeled peptides on restoring rapid inactivation in an ShB K<sup>+</sup> channel mutant, ShB $\Delta$ 6-46, that cannot inactivate because the large deletion near its N-terminus causes it to lack the constitutive inactivating peptide moiety present in the wild-type ShB K<sup>+</sup> channel [8,19]. Fig. 2 shows that the macroscopic currents elicited in response to depolarizing pulses in CHO cells expressing ShB $\Delta$ 6-46 channels in-

deed do not inactivate in 200 ms (Fig. 2A). Addition of either unlabeled ShB or ASIB-labeled ShB-21C peptides restores rapid (N-type) channel inactivation in a practically indistinguishable manner (Fig. 2A), with inactivation time constants ( $\tau$ ) values ranging from 137  $\pm$  11 (mean  $\pm$  S.D.,  $n=9$ ) ms to 130  $\pm$  9 ( $n=10$ ) ms, for unlabeled ShB and ASIB-labeled ShB-21C peptides, respectively. These results strongly suggest that the labeled peptide behaves as a functional analogue of the wild-type ShB peptide and that the presence of either the cysteine residue added at position 21 or the covalently bound ASIB moiety in the labeled peptide does not prevent it from restoring rapid channel inactivation. Similarly, comparison of the effects of the unlabeled, non-inactivating ShB-L7E mutant peptide [8,9] with those of the ASIB-labeled ShB-L7E-21C derivative shows that neither of these peptides has any effects on (i) restoring rapid inactivation (Fig. 2B) or (ii) changing the slow, C-type inactivation exhibited naturally by the expressed ShB $\Delta$ 6-46 channels (Fig. 2C) [19]. Moreover, some of the cells used in the above experiments expressing the ShB $\Delta$ 6-46 channels at moderate levels (peak current amplitude at +20 mV ranging from 0.8 to 1.2 nA) were used to determine the voltage-dependent rates of channel activation. At membrane potentials ranging from –20 to +40 mV, none of the peptides used in these experiments (the unlabeled ShB or ShB-L7E peptides as well as their corresponding ASIB-labeled derivatives) were found to alter the activation rates exhibited by the ShB $\Delta$ 6-46 channel in the absence of any peptide (not shown).

### 3.3 Conformational properties of the inactivating peptide derivative

The conformation adopted by the inactivating ShB peptide

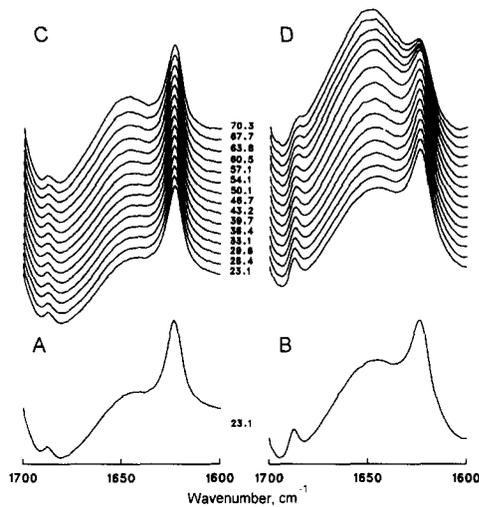


Fig. 3. Infrared amide I band ( $1600\text{--}1700\text{ cm}^{-1}$ ) of the original spectra of the ShB peptide (A,C) and the ASIB-labeled ShB-21C peptide derivative (B,D) in the presence of PG vesicles. (A,B) Spectra taken at room temperature. The stacks of spectra in (C,D) illustrate the temperature-induced changes in the infrared amide I bands of both peptides. The spectra were taken at the indicated temperatures ( $^{\circ}\text{C}$ ) during a 'heating cycle' in which the samples are heated at progressively higher temperatures in the spectrometer cell. The samples were in a  $\text{D}_2\text{O}$  medium prepared from 10 mM HEPES buffer, pH 7.0, containing 130 mM KCl and 20 mM NaCl. The final concentration of either peptide in the samples was approx. 4.5 mM, while the phospholipid concentrations was 32.5 mM. In each case, the spectrum of the buffer alone, taken under identical experimental conditions, was subtracted from that of the peptide-containing samples.

when complexed to the channel protein in the inactivated state remains unknown. However, it has been reported that the ShB peptide undergoes a characteristic transition from a non-ordered conformation exhibited in aqueous solution, to a strongly hydrogen-bonded, intramolecular  $\beta$  structure when challenged *in vitro* by anionic phospholipid vesicles, a model target that attempts to mimic the presumed major components of the receptor site for the inactivating peptide in the ShB  $\text{K}^+$  channel by providing a hydrophobic domain (the vesicle bilayer) separated from the aqueous media by a region with a negative surface potential (the anionic vesicle surface) [15,22]. Fig. 3 shows that both the wild-type ShB peptide and the ASIB-labeled ShB-21C derivative display similar conformational behavior when challenged by anionic PG vesicles, as both of the peptides exhibit a prominent spectral component at  $1623\text{ cm}^{-1}$  in their infrared amide I band (A,B), characteristic of the adoption of the above mentioned  $\beta$  structure. Nevertheless, differences in the relative importance of the  $1623\text{ cm}^{-1}$  absorbance in the spectra of the two samples, as well as in the thermal denaturation profiles obtained by heating the peptide/vesicle mixtures at increasing temperatures (Fig. 3C,D), reveal that the structure adopted by the ASIB-labeled derivative is less stable than in the wild-type ShB peptide, as the characteristic  $1623\text{ cm}^{-1}$  component is lost more extensively and at lower temperatures in the former.

#### 3.4. Photolysis of ASIB-labeled peptides in solution

Photolysis of the ASIB-labeled peptides in aqueous solution by irradiation with long-wavelength ultraviolet light results in spectral changes similar to those occurring under similar conditions during the irradiation of free ASIB dissolved in

DMSO (Fig. 4). A decrease at both 272 and 310 nm maxima is observed as the photolysis progresses. Also, two isosbestic points at approx. 250 and 325 nm are maintained during the initial phase of photolysis (up to  $\sim 300$  s of irradiation for the labeled peptides or up to 100 s of irradiation for the free ASIB), suggesting that photolysis leads initially to the production of a single major product, but becomes more complex as the irradiation time is increased. IR monitoring of the photolyzed ASIB-labeled peptides indicates that the infrared band at  $2115\text{ cm}^{-1}$  characteristic of aromatic azides disappears completely as a consequence of irradiation. On the other hand, irradiation under similar conditions of the unlabeled peptides does not result in any appreciable changes in their characteristic UV-visible spectra due to peptide absorbance, suggesting that irradiation by itself does not damage the peptide moiety in the derivatives. Fig. 4 also shows the time course of photolysis under the irradiation conditions used in these experiments (see the insets in each panel). The  $t_{1/2}$  of photolysis for either of the labeled peptides was approx. 110 s, while that for free ASIB in DMSO solution was 64 s.

#### 4. Discussion

In designing an analogue of the inactivating ShB peptide suitable for photoaffinity labeling of the corresponding receptor site in the ShB channel protein, we considered firstly that peptide derivatization should (i) be easy to carry out experimentally by anyone without extensive chemical training, (ii) occur efficiently with high yields and (iii) involve specific residues in the peptide primary structure. The synthesis and purification of the ASIB-labeled peptide derivatives described here comply fully with those requirements as the alkylation reaction is straightforward and extremely efficient, involves only the free sulphhydryl at the peptide's carboxy-terminal cysteine and allows virtually quantitative recovery of the reaction product.

Secondly, a photoaffinity labeling probe should by definition retain as much as possible the structural features and functional properties of the parent molecule. From the structural viewpoint, the reported FTIR studies indicate that the wild-type ShB peptide and its ASIB-labeled derivative adopt a similar, although not identical, secondary structure when challenged by a model target that partly mimics the presumed major components of the receptor site for the inactivating peptide in the ShB  $\text{K}^+$  channel [15,22]. On the other hand, the electrophysiological measurements described above have shown that the ASIB-labeled ShB-21C is functionally indistinguishable from the wild-type ShB peptide in restoring rapid (N-type) inactivation in the ShBA6-46 channel. In addition, ASIB-labeled peptides do not influence other channel properties such as the activation rates or the C-type inactivation time courses. From those observations, one could reasonably assume that the affinity of the ASIB-labeled ShB peptide analogue towards the ShBA6-46 channel must be comparable to that exhibited by the parental wild-type ShB peptide. A quantitative estimation of the  $K_D$  for the labeled peptide analog, however, was not attempted under our experimental conditions because we used the whole-cell recording configuration in which, because of potential problems arising from limited intracellular diffusion or proteolytic degradation of the peptide, the 'effective' peptide concentration is not known. Nevertheless, a 'true'  $K_D$  of approx.  $3\text{ }\mu\text{M}$  has been reported for the

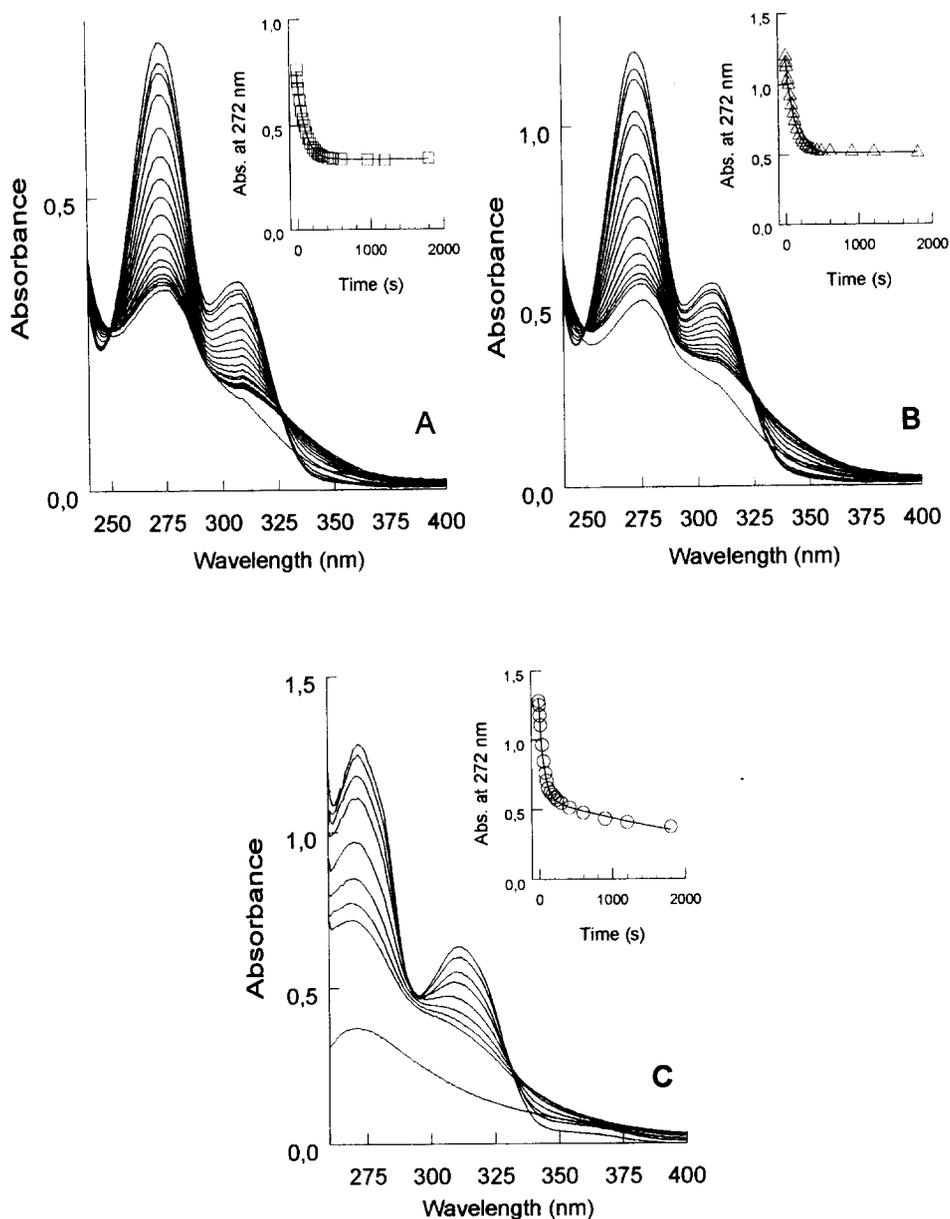


Fig. 4. Changes in the absorbance spectra of ASIB as a function of the irradiation time. The photolysis of ASIB-labeled ShB-21C (A) and ShB-L7E-21C (B) peptides dissolved at 38 and 61  $\mu\text{M}$ , respectively, in 0.05% acetic acid, is shown. (C) Photolysis of free ASIB (67  $\mu\text{M}$ ) dissolved in DMSO. The spectra of the unphotolyzed compounds correspond to those with the highest absorbance, while the rest of the spectra correspond to samples irradiated for increasingly longer periods of time (from 5 s up to 30 min). The spectrum with the lower absorbance in each of the three panels corresponds to samples irradiated for 30 min. The insets are plots of the absorbance at the 272 nm maximum versus the irradiation time.

inactivation of ShB $\Delta$ 6-46 channels by the wild-type ShB peptide in excised membrane patches from transfected *Xenopus* oocytes [10], where peptide diffusion or proteolysis should no longer be a problem.

Thirdly, photolysis of the photoactivatable moiety in an adequate photoaffinity labeling probe must produce a reactive intermediate with the shortest possible lifetime to ensure that covalent binding of the photogenerated species to the 'specific' site in the target protein will occur rapidly and efficiently upon photolysis of pre-formed, non-covalent probe/target complexes. Either singlet or triplet nitrenes produced from photolysis of aromatic azides, such as that in ASIB, are among the photochemical intermediates known to have the shortest lifetimes. In particular, the lifetime of singlet state nitrenes, which

would be the predominant reacting species when confronted with the target channel protein, is probably less than 30  $\mu\text{s}$  [23]. In our case, the above considerations on the reactivity of the excited state nitrene intermediates, and the fact that the affinity of the peptide analogue towards the channel must be similar to that of the wild-type ShB peptide (see above), lead to the conclusion that photolysis of the non-covalent peptide analogue/channel protein 'inactivated' complex in future photoaffinity labeling experiments should mostly result in the specific labeling of the appropriate site in the channel protein, likely by reaction with C-H or N-H bonds ('insertion' reactions), as well as with side-chain nucleophile groups. Under those conditions, other time-requiring processes such as the diffusion of the photogenerated species out of their non-

covalent binding pocket, their association with extraneous sites in the channel protein and the undesirable, 'non-specific' photolabeling of such unrelated sites, become much less likely. Likewise, nitrenes resulting from photolysis of free (unbound) probe should rapidly react with solvent or buffer components or self-react, without having time to diffuse sufficiently near to the protein to react with it [13,14]. In any case, since the ASIB derivative of the non-inactivating ShB-L7E mutant peptide is also available, it may provide the means to correct for any possible non-specific labeling of channel components.

It should be noted here that, although the nitrene intermediates are rapidly reacting species, relatively long irradiation times are required for their formation. Obviously, this could be optimized (irradiation time shortened) by increasing the intensity of the long-wavelength UV source, by decreasing the distance between the lamp and the sample or by choosing an adequate protocol of irradiation pulses. Nevertheless, such optimization should only increase the extent (efficiency) of photolabeling of the target protein, and should not change the labeling specificity, which is mainly determined by the affinity of the unphotolyzed peptide analogue for the target binding site and by the lifetime of the nitrene intermediates.

Lastly, it is well known that the inactivating peptide of the ShB K<sup>+</sup> channel also serves as an efficient inactivating ball for a variety of other voltage-dependent K<sup>+</sup> channels [9,11,24], high conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels [25,26] or cyclic nucleotide-gated channels [27], some of which do not normally inactivate. Therefore, it seems quite likely that the ASIB-labeled ShB peptide analogue described here could be used as a suitable tool to explore the nature of the receptor site for the inactivating peptide in a large variety of different channels. Moreover, because ASIB is radioiodinatable, the use of a peptide derivative made from ASIB labeled with radioactive iodine should facilitate the identification of the photoaffinity-labeled receptor site.

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