Fluorescent labeling of a peptide toxin retains the specificity and affinity required of a research tool

Review Article

Peptide toxins from animals are potent inhibitors of ion channels and show great promise both as drugs to treat a range of disorders and as valuable tools in drug research. A research team based in France has explored ways of fluorescently labeling a scorpion peptide, BeKm-1, that inhibits the hERG ion channel. This channel is involved in cardiac activity and blocking it must be avoided when developing new drugs. The research team's approach included protein-protein docking studies that enabled them to identify solvent-exposed residues in the peptide that were suitable for labeling. This enabled them to design and test analogs that maintained both specificity and mode of action of the native peptide.

Peptide toxins – a goldmine for drug leads and R&D tools

Peptide toxins from terrestrial and marine animals are invaluable tools in research into the role ion channels play in physiological and pathological conditions. Scorpion venom, alone, has been shown to contain as many as 320 peptide toxins that act on 41 different ion channels (1). Several toxin-derived peptides have also become drugs for the management of diabetes, hypertension, chronic pain, and other medical conditions, while dozens are in pre-clinical development or undergoing clinical trials (2). One example of an FDA-approved peptide drug is Prialt® or Ziconotide, a synthetic version of a 25-amino acid peptide found in the venom of a marine snail that blocks N-type voltage-gated calcium channels. Ziconotide is intended for the treatment of chronic severe pain in patients that are intolerant or refractory to systemic analgesics or intrathecal morphine (3). Hundreds of thousands of peptides remain to be investigated as possible pharmacological tools or drug leads (4).

Designer toxins to target hERG ion channels

Key drivers in this field are the availability of crystal structures of target channels and insights into the binding of toxins, which open up the possibility of designing toxin analogues. One example is the hERG channel that, together with the EAG-1 channel, belongs to the eag (ether-a-go-go) family of voltage-gated K^+ channels.

Mutations in KCNH2, the gene encoding the hERG channel, cause the long QT syndrome (LQT), which involves prolonged ventricular repolarization that can lead to sudden cardiac arrest. LQT can also be caused by drugs that block hERG, including antibiotics, antipsychotics, and antihistamines. Such interactions are a major challenge in pharma development and must be studied early in the discovery process.

One powerful tool in the evaluation of hERG is a potent blocker in the form of the toxin peptide, BeKm-1 (IC_{50} =3.3 nM), isolated from the venom of the scorpion, *Buthus eupeus*. Collaboration between Institut des Molécules Max Mousseron, Pole d'expertise Biotechnologie and Smartox Biotechnology in France has led to the design of fluorescent analogs of BeKm-1 that specifically bind hERG channels with the aim of developing fluorescent analogs that preserve correct binding and inhibition of the channel (5).

A model to support residue selection

The team developed a model using the tetrameric hERG channel structure to predict BeKm-1 binding to the outer part of hERG to help in identifying the residues on the toxin that are exposed to solvent and therefore accessible for attachment of chemical groups. The molecular modeling study showed that the α-helix of the toxin contacts a hydrophobic surface at the bottom of the binding site of hERG and the side-chains of N-terminal Arg-1 and Arg-27 are exposed to solvent in all binding modes, resulting in the decision to select these residues for chemical labeling.

Design and synthesis of BeKm-1 analogs

The team designed and synthesized four fluorescent BeKm-1 analogs (Fig. 1). The N-terminal of BeKm-1 was used to attach linkers to three of the analogs: 4-pentynoic acid (four additional carbon atoms; linker4), 6-azido-hexanoic acid (+6 carbons atoms; linker6), or 10-undecynoic acid (+ 10 carbons; linker10). All these linkers are compatible with click chemistry (alkyne function for linker4 and linker10, and azide function for linker6). The fourth BeKm-1 analog was based on the solvent accessibility of Arg-27, but since Arg-27 is not involved in hERG channel recognition, the residue was replaced by a click chemistry-compatible Lys residue that contains an alkyne function. Cy5-based dyes with additional spacers were then grafted onto the peptides.

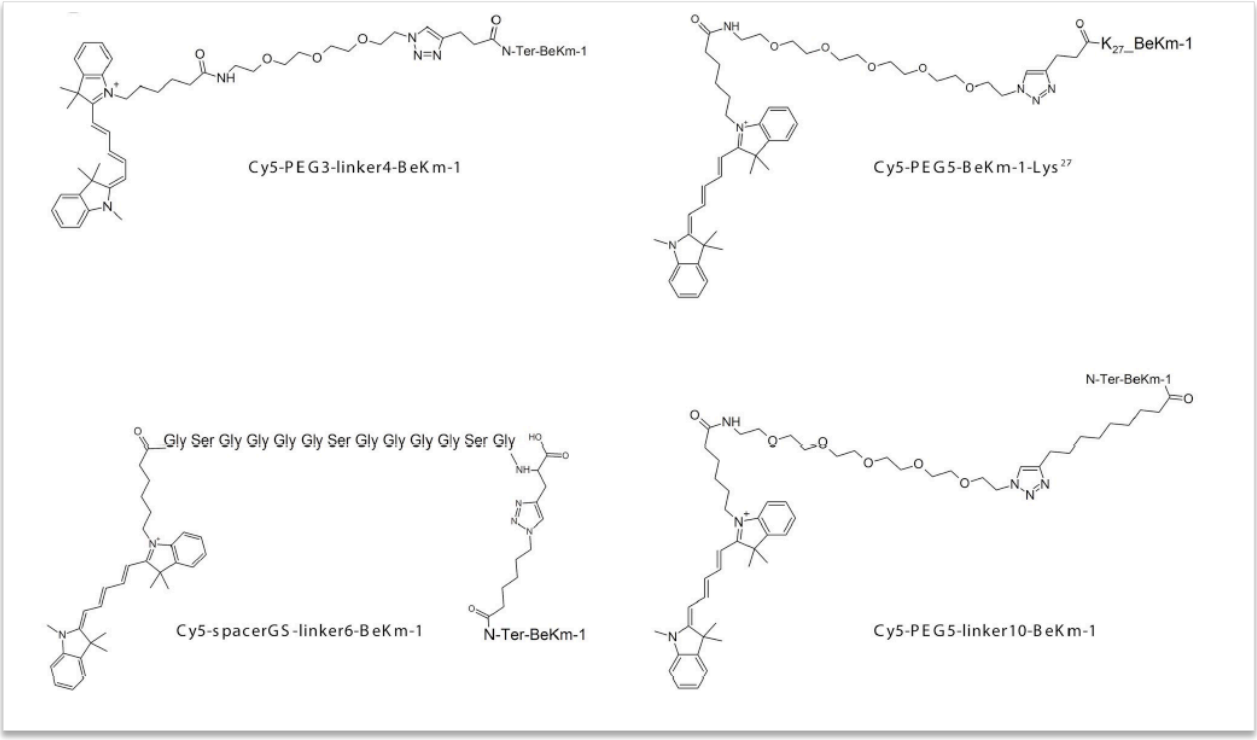

Figure 1. *Fluorescent BeKm-1 analogues were designed with different linkers grafted onto the N-terminal of Arg-1 or the side chain of Arg-27 mutated for a Lysine. From Figure 1B, Vasseur et al, 2019.*

The peptides were synthesized stepwise using fmoc solid-phase chemistry on a Symphony® X peptide synthesizer before linkers were coupled to the N-terminus of the 36-mer BeKm-1 sequence. In the case of BeKm-1 Lys-27, the wild-type L-Arg-27 residue was replaced by L-Lys(pentynoyl)-OH. The spacer GS included L-propargyl glycine (Pra) at the C-terminus with an alkyne function on the side chain, and Cy5 was coupled through a peptide bond at the N-terminus.

All peptides were assembled using a 2-chlorotrityl chloride resin. After resin cleavage and deprotection, crude toxin analogues were folded/oxidized and purified by C18 reversed phase chromatography (RP-HPLC). Click chemistry was used to couple Cy5 plus linker to give Cy5-PEG3-linker4-BeKm-1, Cy5-PEG5-linker10-BeKm-1, Cy5-spacer-GS-linker6-BeKm-1, and Cy5-PEG5-BeKm-1 Lys275. All products were purified to homogeneity by RP-HPLC.

Analogs retain specificity and mode of action but with reduced affinity

The effects of labeling BeKm-1 were evaluated using hERG channel expressed in *Xenopus laevis* oocytes. All analogs showed dose-dependent inhibition of the outward current, but the IC_{50} values were higher (60–80 nM) compared to the IC_{50} of the native toxin peptide (12 nM; Fig. 2). BeKm-1 and the four analogs all had a Hill coefficient close to 1, indicating that they had the same mode of action.

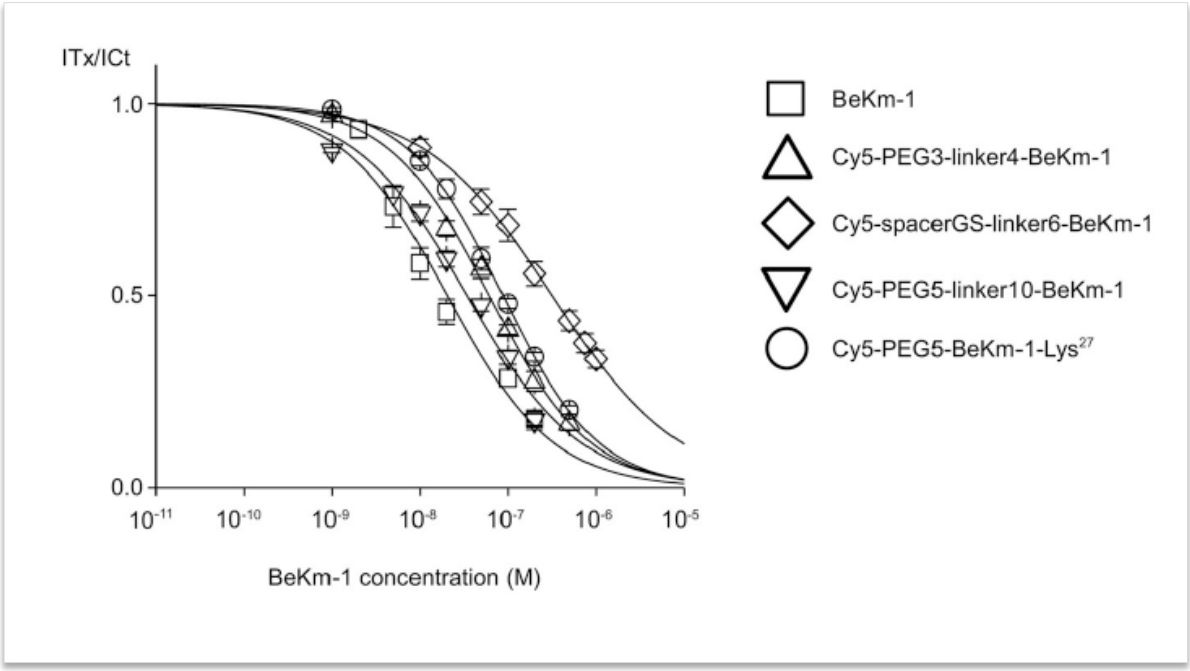

Figure 2. *Inhibition curves of native BeKm-1 and its four analogs. The peak current amplitude in the presence of toxin (ITx) was normalized to the current recorded in the absence of toxin (ICt). From Figure 2B, Vasseur et al, 2019.*

The specificity of native BeKm-1 and its analogs to hERG could be confirmed by a lack of effect on the closely-related K^+ channel, hEAG1, while experiments on a mutated form of hERG, which is resistant to inhibition by BeKm-1, gave similar results for the native peptide and the analogs. The overall conclusion was that the analogs had the same specificity and mode of action as the native peptide but had a lower affinity for the hERG ion channel.

A promising approach to developing designer toxin peptides

This study illustrates how protein-protein docking tools can be used to facilitate the design of modified toxins that can be used as reporter molecules in ion channel imaging or in biochemical purification/characterization. Approaches like this could also provide valuable tools to study unwanted side effects of new drug candidates on ion channels, characterize the mode of action of peptide toxins, and open up new possibilities for drug development.

As Dr. Michel De Waard, CSO Smartox Biotechnology and INSERM senior research director, pointed out, Symphony X peptide synthesizer played a key role in this work. *"Symphony X is a valuable addition to our synthetic capabilities here at Smartox. As a long-time user of GPT products we not only rely on the instrument's robust construction and reliability, but also the increased flexibility this model offers when producing peptides with non-standard amino acids and different chemistries. Being able to synthesize multiple peptide analogs simultaneously is a huge advantage, allowing us to increase efficiency and productivity for our peptide laboratory."*

References

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