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Review

Contribution of the functional dyad of animal toxins acting on voltage-gated Kv1-type channels

STEPHANIE MOUHAT,[a,b] MICHEL DE WAARD[c] and JEAN-MARC SABATIER[a,b]*

[a] Laboratoire Cellpep S.A., 13-15 Rue Ledru-Rollin, 13015 Marseille, France
[b] Laboratoire d'Ingénierie des Protéines, CNRS FRE 2738, Bd Pierre Dramard, 13916 Marseille, France
[c] INSERM Unité 607, Canaux Calciques, Fonctions et Pathologies, CEA, DRDC, 17 Rue des Martyrs, 38380 Grenoble, France

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Abstract: The 'functional dyad', a well-defined pair of amino acid residues (basic and hydrophobic residues), is a key molecular determinant present in most animal toxins acting on voltage-gated Kv1 channels. It is increasingly used as a working concept to explain how toxins are able to recognize and block their specific ion channel targets. However, other crucial toxin determinants are emerging and the actual role of this 'functional dyad' ought to be clarified, which is the object of the present mini-review.

Keywords: functional dyad; animal toxins; voltage-gated K^+ channels; toxin determinants; ion channel recognition

CURRENT VIEW ON THE TOXIN 'FUNCTIONAL DYAD'

In recent years, the 'functional dyad' of animal toxins has been proposed as a key molecular determinant for their binding to voltage-gated Kv1-type channels [1–5]. In addition, it is also used as a working concept to describe the pharmacological properties of a number of toxins [6–12]. In view of this ever-increasing trend, the present opinion is intended to provide a more balanced view of the probable contribution of the toxin 'functional dyad' with regard to Kv1 channel recognition, affinity and K^+ ion efflux blocking potency. The 'functional dyad' of toxins has first been described by Ménez and collaborators [1]. According to these authors, it is composed of a pair of key amino acid residues that would be present in Kv1 channel-acting toxins. A 'functional dyad' typically consists of a lysine residue and a hydrophobic residue, generally Tyr, Phe or Leu, separated by a distance of 6–7 Å [1]. It has been identified in toxins from various animal venom sources, and is generally present regardless of the type of peptide fold and disulfide bridging pattern [13]. Dyads of toxins active on voltage-gated K^+ channels are spatially superimposed [1,3]. Of note, toxins containing structurally different dyads (i.e. nature of the residues, distance between both residues, etc...), such as those active on voltage-gated Ca^{2+} channels, are inactive on K^+ channels [10,14]. Toxins that contain the 'functional dyad' are thought to act by an ion channel pore-blocking mechanism. According to the current view, the side-chain of the Lys residue positions itself into the channel pore, in the centre of a ring composed of the carbonyl groups of four equivalent acidic residues (Asp or Glu) [9,15,16], each belonging to one of the four α-subunits that form a functional tetrameric K^+ channel. The Lys residue of the functional dyad must be in contact with permeant ions inside the pore of K^+ channels as toxin binding is destabilized in a voltage-dependent manner by outward K^+ efflux, as evidenced first with charybdotoxin [17]. The interaction between the Lys residue of the dyad and the selectivity filter was also demonstrated by Ranganathan and collaborators [18] using agitoxin 2 and Kv1-type *Shaker* B channels. It is also thought that the critical hydrophobic residue (aromatic or aliphatic) of the 'functional dyad' interacts with a cluster of ion channel aromatic residues.

CONTRIBUTION OF OTHER TOXIN RESIDUES: THE MULTIPOINT INTERACTION MODEL

There is a general agreement in the field to assume that other toxin determinants, besides the 'functional dyad', are required for a high affinity interaction with Kv1 channels, thereby shaping a multipoint interaction model between the toxin and its target [19]. In precept reports, Miller and collaborators showed that, besides the concerned Lys residue of charybdotoxin, a number of other toxin residues were required for interaction with *Shaker* B [20,21]. For instance, Met^{29} of charybdotoxin was shown to interact with Thr^{449} of *Shaker* B [22], whereas its Lys^{11} senses channel

*Correspondence to: Dr Jean-Marc Sabatier, Laboratoire d'Ingénierie des Protéines IFR Jean Roche, CNRS FRE 2738, Faculté de Médecine Nord, Bd Pierre Dramard, 13916, Marseille Cédex 20, France;
e-mail: sabatier.jm@jean-roche.univ-mrs.fr

Lys^{427} through space electrostatic forces [23]. Other functionally important toxin residues, not belonging to the dyad, were also identified by the group of Chandy [24]. Among other toxin determinants, the existence of a ring of basic residues was highlighted recently within Pi1 that would interact with acidic residues of the Kv1.2 channel turrets above the K^+ channel outer vestibule [9]. In a way, some functionally important basic residues of this ring had been discovered in the past, such as Arg^{24} and Arg^{31} of kaliotoxin, or Arg^{25} and Arg^{31} of charybdotoxin [24]. What appears to be more controversial, however, is the real contribution of the 'functional dyad' to the overall process of ion channel recognition (including selectivity, affinity and K^+ ion efflux blockage potency). According to one proposed scheme, the 'functional dyad' might constitute a minimal functional core for the toxins to bind to Kv1 channels [3,5]. The role of additional toxin determinants would be to increase both the affinity and/or the selectivity towards particular Kv1 channel subtypes. Due to the central positioning of the critical Lys residue side chain into the ionic pore, it is also thought that the toxin 'functional dyad' is pivotal for K^+ current blockage. According to this model, the binding of the toxin 'functional dyad' onto the channel is the key step for Kv1 channel interaction and resulting K^+ current blockage. Other toxin residues would intervene secondarily to strengthen toxin binding to a (or several) specific Kv1 channel subtype(s), thereby guiding the specificity of the interaction. It was found that point mutation changes in toxins alter their pharmacological profiles. This was clearly documented for various toxins, including BgK [25] and ShK [26,27], in which point mutation increases their selectivity for a given Kv1 channel type. In a model of multipoint interaction, the influence of secondary interactions on the efficacy of current block remains, however, an open question. Formerly considering the 'functional dyad' as the minimal functional core for Kv1 channel recognition by a toxin has several drawbacks that should be noted. First, what was considered as a signature for the recognition of Kv1 channels should now also be considered as its Achilles' heel. Indeed, if the 'functional dyad' were central to the recognition of Kv1 channels, one would expect that all toxins harbouring this dyad, with the adequate characteristics (nature and relative positioning of the pair of key residues), would be able to recognize the variety of Kv1 channel types, at least with a low affinity. We have identified a series of K^+ channel-acting toxins containing the 'functional dyad' but inactive at high concentrations on one (or several) Kv1 channel subtype(s). Representative examples include Pi1 [28], Pi4 [15], κ-conotoxin PVIIA [29] and tityustoxin-Kα [30]. Second, specific animal toxins (e.g. Tc32 [31], parabutoxin 1 [8], parabutoxin 3 [7]) have now been characterized that lack a 'functional dyad' but still reportedly block K^+ currents of some Kv1 channel subtypes, albeit with reduced potencies. However, these toxins still act in the nanomolar to micromolar concentration range. Additionally, a similar blocking activity of Kv1 channels was observed for a Pi1 analogue in which the functional dyad was selectively mutated [9]. Altogether, these data strongly suggest that the 'functional dyad' cannot represent by itself the minimal functional core that binds onto Kv1 channels [1–5]. This implicates that other toxin residues (e.g. ring of basic residues), which were initially proposed to play a role in defining Kv1 channel selectivity and affinity, are involved in the very first steps of ion channel recognition [9]. This contrasts with the initial scheme and confers a somewhat more secondary role of the toxin 'functional dyad' in channel recognition. It should, however, be emphasized that the contribution of the 'functional dyad' is far from negligible. Obviously, it does confer much greater toxin affinities. This was powerfully demonstrated in recent studies in which the 'functional dyad' was restored in toxins that naturally lacked it [7,8]. Owing to the central position of the Lys residue side chain into the ion channel pore, one would have expected that removal of the 'functional dyad' would dramatically alter toxin-blocking potencies. Interestingly, this does not appear to be the case since, in spite of presenting reduced affinity, toxins lacking the 'functional dyad' are still able efficiently to block K^+ efflux (i.e. up to a maximal current block of nearby 100%) [31,8,7]. This suggests that, besides its lack of precise role in channel recognition, the 'functional dyad' may also not be so critical for the extent of current blockage. It raises interesting research avenues on the mechanisms whereby toxins block K^+ currents and, in particular, on the functional role of additional toxin residues which do not belong to the 'functional dyad' but are obviously required for channel recognition [9,32]. Of note, it should be mentioned that in the absence of a 'functional dyad', toxin affinity towards its ion channel target might apparently be weaker than actual probably because K^+ efflux destabilizes toxin binding through electrostatic repulsive effects.

BINDING OF TOXINS TO Kv1-TYPE CHANNELS: AN EMERGING SCHEME

Based on these considerations, an alternative scheme is proposed for the contribution of toxin residues to Kv1 channel recognition. In this pictorial view, toxins first recognize the various Kv1 channel subtypes through various sets of residues (such as a ring of basic residues in the case of Pi1 [9]) distinct from those of the 'functional dyad'. These interactions presumably occur with specific residues of the ion channel turrets [9,15,16]. This set of molecular contacts defines the selectivity of the toxin towards specific Kv1 channel subtypes, and is sufficient *per se* to

provide a consistent block of K^+ efflux, albeit with reduced affinity. The toxin 'functional dyad', though not mandatory, potentially serves two purposes. First, it provides a supplementary anchoring point, deeper into the channel pore structure, thereby contributing to a greater toxin affinity, but without altering the ion channel selectivity profile. Second, the crucial positioning of both residues constitutes a first efficient physical barrier, mainly played by the side chain of the Lys residue, which is opposed to the K^+ ion efflux. The extent to which the 'functional dyad' contributes to toxin affinity and blocking potency may vary from toxin to toxin, depending on the number and nature of the additional residues that interact with the K^+ channel under consideration. Such a view is consistent with several docking simulation experiments of toxins onto Kv1-type channels [9,15,16], but will require further experimental validation.

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