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Interaction of the C-terminal domain of Bcl-2 family proteins with model membranes

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Abstract

Bcl-2 family proteins are involved in the cell homeostasis by regulating programmed cell death. Some of these proteins promote apoptosis, while others inhibit the same process. The C-terminal hydrophobic domain of some of these proteins is predicted to be involved in anchoring them to a variety of cell membranes, such as mitochondrial, endoplasmic reticulum and nuclear membranes. We have used five synthetic peptides imitating the C-terminal domain from both anti-apoptotic (Bcl-2) and pro-apoptotic members (Bak, Bax, and two mutants of this last protein) of this family to study their interaction with model membranes. Some differences were detected in the interaction with these peptides. The addition of all the peptides to large unilamellar vesicles destabilized them and released encapsulated carboxyfluorescein to different degrees, so that fluidity and the increase in negative curvature favoured the extent in the release of carboxyfluorescein. Bcl-2-C and Bax-C peptides produced the highest release levels in most cases, while BaxS184K-C was the least efficient in this respect. These results indicate that these C-terminal domains are able to insert themselves in the membranes, each in a different way that is probably related with their different way which can be related to their differing locations within the cell and their different roles in regulating apoptosis.

Keywords: Bcl-2; Bax; Bak; Apoptosis; Protein–lipid interaction; Fluorescence

1. Introduction

Programmed cell death (apoptosis) controls normal tissue homeostasis by balancing cell proliferation and removing damaged, unwanted, aged and infected cells [1–3]. Alteration of the normal apoptotic process is symptomatic of several human diseases [4,5]. Due to its vital importance, apoptosis is a highly regulated mechanism, the proteins of the Bcl-2 family being one of the most important factors involved in such regulation since they are responsible for integrating diverse survival and death signals that are generated outside and inside the cell [6,7]. These proteins can form homo- and heterodimers [8–10].

The multi-BH domain family members are either anti- or pro-apoptotic proteins. In general, the anti-apoptotic members (e.g., Bcl-2, Bcl-x_L, Mcl-1, Bcl-w from mammals and Ced-9 from *Caenorhabditis elegans*) display sequence homology in all four BH domains, whereas the pro-apoptotic members (e.g., Bax, Bak and Bok) have homologous BH1–3 domains. Pro-apoptotic Bcl-2 family members, upon activation by apoptotic stimuli, are capable of forming heterodimers with anti-apoptotic members. The solution structure of Bcl-x_L reveals that the BH1–3 domains of Bcl-x_L form an elongated hydrophobic groove, which is the docking site for the BH3 domains of pro-apoptotic binding partners [11-12].

In addition to BH domains, many of these proteins contain a hydrophobic transmembrane domain (TMD) at their carboxy terminus. This C-terminal domain is supposedly responsible for membrane localization, for example, in the outer membrane of mitochondria, the nucleus or in the endoplasmic reticulum membranes [13–18], indicating that these proteins may exist as integral membrane proteins [19–21]. The pattern of membrane

Abbreviations: CF, 5(6)-carboxyfluorescein; DAG, diacylglycerol obtained from egg yolk phosphatidylcholine; DPH, 1,6-diphenyl-1,3,5-hexatriene; EYPC, egg yolk phosphatidylcholine; LUVs, large unilamellar vesicles; MLVs, multilamellar vesicles; *P*, polarization of the emitted fluorescence; TFE, 2,2,2-trifluoroethanol; THF, tetrahydrofuran

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localization differs among pro- and anti-apoptotic members. In this sense, Bcl-2 has been shown to reside on the cytoplasmic face of the mitochondrial outer membrane, whereas Bax or Bid is found mainly in the cytosol or as membrane peripherals in healthy living cells, and only interacts with the mitochondrial membrane when they are activated [22–25].

Some of the Bcl-2 family members have been cloned and expressed for studies at high resolution. However, many of these studies used truncated forms which do not include the hydrophobic C-terminal domain in order to obtain the high quantities of soluble protein required for the structural studies, because full proteins are of impaired solubility. Nevertheless, the structures of a few full-length proteins have been determined, and in all cases, the C-terminal segments were shown to fold back onto a groove on the structures, similarly to Bax [26].

Homeostasis is maintained by controlling the amount of active pro- and anti-apoptotic family members in different tissues. The ratio between pro- and anti-apoptotic proteins helps to determine, in part, the susceptibility of cells to a death signal and, when called for, programmed cell death. These apoptotic signals target various intracellular components, including the pro-apoptotic Bcl-2 members.

The hydrophobic C-terminal domain is, it is supposed, very important in targeting these proteins to differing cell membranes, as mentioned above. In this sense, a comparative study of different C-terminal domains of pro- and anti-apoptotic Bcl-2 proteins would be very interesting to help elucidate the role of each one. In the present study, we have used five synthetic peptides imitating the C-terminal domain of some of the Bcl-2 family proteins, namely Bcl-2-C, Bak-C, Bax-C and two mutants of this last protein (Fig. 1), and studied their interaction with different model membranes and their ability to disrupt membrane barrier properties using fluorescence spectroscopy. The results showed that the interaction of each peptide with the model membranes, as seen through the release of encapsulated carboxyfluorescein, differed, with Bcl-2-C and BaxS184K-C showing the weakest effects.

2. Materials and methods

2.1. Materials

The synthetic Bcl-2 C-terminal domain peptide (Bcl-2-C) encompassed residues 217–239 of Bcl-2 ($^{+}{}_{3}$HN-^{217}LTKLLSLALVGACITLGAYLGHK^{239}-COO^{-}), whereas the synthetic Bak C-terminal domain peptide (Bak-C) included residues 188–211 of Bak ($^{+}{}_{3}$HN-^{188}ILNVLVVLGVVLLGQFVVRRFFKS^{211}-COO^{-}). The three remaining synthetic peptides were related, because one, the Bax C-terminal domain peptide (Bax-C), encompassed residues 172–192 of Bax ($^{+}{}_{3}$HN-^{172}TVTIFVAGVLTASLTIWKKMG^{192}-COO^{-}) and the other two were mutants of the previous one, so that Ser184 was replaced by Lys184 (Bax S184K-C) and in the second Ser184 was deleted (BaxΔS184-C). The five synthetic peptides were purchased from Genemed (San Francisco, CA, USA)

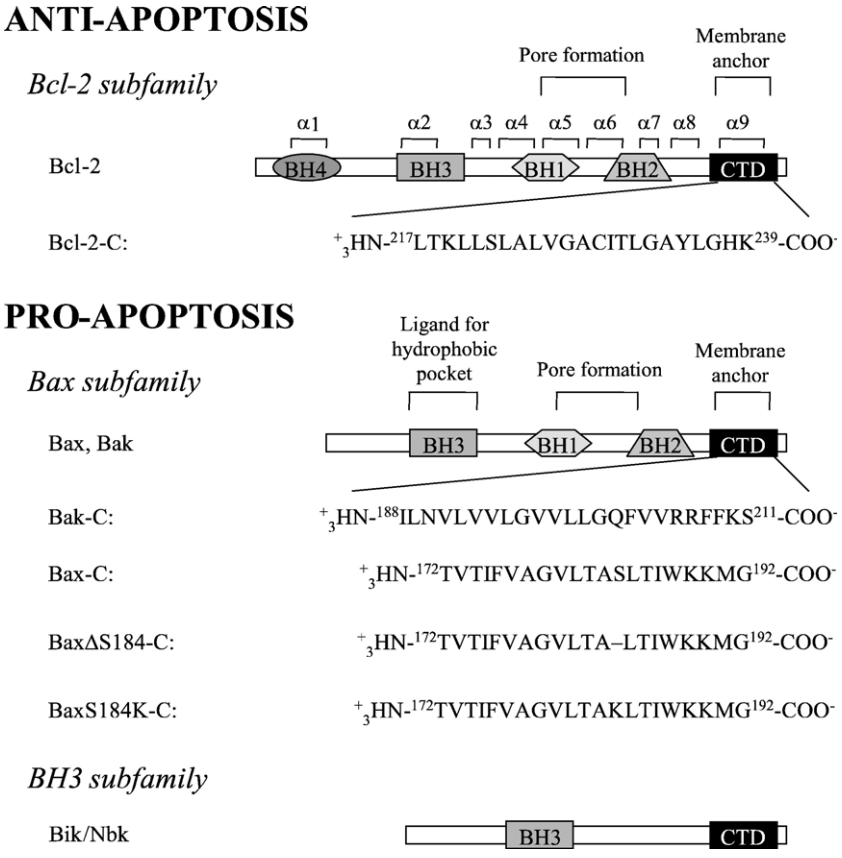

Fig. 1. Classification of the Bcl-2 family, showing the most important member of each subfamily. Bcl-2 homology domains (BH) 1 to 4, and hydrophobic C-terminal transmembrane domain (CTD) are drawn. The position of α-helices and the function of the most important regions are also shown. The sequences of the carboxy terminal peptides used in this study are also displayed.