Effects of microinjection of synthetic Bcl-2 domain peptides on apoptosis of renal tubular epithelial cells

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Pehorstfer, Elisabeth, Bernd Mayer, Stefan Boehm, Arno Lukas, Peter Hauser, Gert Mayer, and Rainer Oberbauer. Effects of microinjection of synthetic Bcl-2 domain peptides on apoptosis of renal tubular epithelial cells. Am J Physiol Renal Physiol 283: F190–F196, 2002.—Bcl-2 protein family members are among the key regulators of the apoptosis effector phase. Therefore, we investigated the ability of synthetic peptides derived from proteins of the Bcl-2 family, namely, the NH2-terminal region of Bcl-2 (Bcl2_syn), a central domain of Bax (Bax_syn), and a central domain of Bak (Bak_syn) to interfere with the apoptotic process in LLC-PK1 cells. Apoptosis was induced by tacrolimus or lipopolysaccharide treatment, and microinjection of Bax_syn into stimulated LLC-PK1 cells significantly reduced the percentage of apoptotic cells detected within 4 h after the treatment. Microinjection of Bax_syn or Bax_syn, in contrast, induced apoptosis in otherwise untreated LLC-PK1 cells during the same period of time. A random sequence control peptide (Control_syn), which served as a negative control, as well as FITC-labeled dextran, which was co-injected in all experiments for visualization, were ineffective in either preventing or inducing apoptosis. These results suggest that synthetic peptides mimicking the functional domains of proteins of the Bcl-2 family are capable of regulating apoptosis when microinjected into LLC-PK1 cells in vivo. Analogs to these regulatory peptides could therefore provide valuable lead compounds in the therapeutical context.

apoptosis regulation; B cell lymphoma/leukemia-2; Bax; Bak; synthetic peptides

Apoptosis is regulated by external stimuli but also within the cell itself. Bcl-2 was first discovered because of its involvement in B cell malignancies, whereby chromosomal translocations activate the gene in the majority of follicular non-Hodgkin’s B cell lymphomas (35). In 1988, Vaux et al. (36) were the first to report that Bcl-2 can prolong cell survival, and Hockenbery et al. (12) later demonstrated that this effect is mediated by its capacity to block apoptosis. As the Bcl-2 protein is expressed especially in long-lived cells and/or proliferating cell zones (13), it is not surprising that it is also found in human renal proximal tubule cells (29). Hence, Bcl-2 is an excellent candidate that may be involved in mechanisms that regulate apoptosis in kidney cells, such as LLC-PK1 (5). The Bcl-2 protein as well as other members of this family (such as Bag-1 and Bcl-xL) protects cells against apoptosis. In contrast, other Bcl-2-like proteins (such as Bak, Bad, and Bcl-xS) promote cell death. Thus, for any given apoptotic stimulus, the balance between death and survival appears to be determined by the ratio of apoptosis stimulating and suppressing Bcl-2 family members (30).

Cytoplasmic events, such as protease activation, rather than nuclear events, such as endonuclease activation, are assumed to play the initiating and primary role in mediating apoptotic cell death (24). Hence, a number of studies were carried out to elucidate the intracellular distribution of Bcl-2 homologues by antibody labeling and laser scanning microscopy techniques (17, 19, 22). The most prominent location of Bcl-2 proteins is the outer mitochondrial membrane, in which they show a patchy, nonuniform distribution. In addition, Bcl-2 is located at the endoplasmic reticulum but not in the plasma membrane, Golgi vesicles, or other organelles. A fraction of Bcl-2 is associated with nuclei as well (1), and the protein appears to span the inner and outer nuclear membrane, possibly forming a nuclear pore complex. The distribution of Bcl-2 over the nuclear membrane is not uniform, which suggests that it is targeted to specialized regions, although Bcl-2 lacks any of the common organelle-specific targeting sequences. Hence, Bcl-2 might specifically interact with certain protein complexes in intracellular membranes, such as the nuclear pore complex or the junctional complex in mitochondria. These complexes are both involved in protein transport across membranes (32).

Bcl-2 proteins display a complex domain structure, whereby the individual domains may subserve specific functions, such as homo- or heterodimerization, that...
are important in the apoptosis effector phase. This paper focuses on the distinct characteristics and functions of the three isolated domains Bcl2_syn, Bax_syn, and Bak_syn of the Bcl-2 family members Bcl-2, itself, Bax, and Bak, respectively. First, a structural and electrostatic analysis of the synthetically derived peptides in vitro is given, because both parameters are crucial to define specific molecular recognition sites. Thereafter, microinjection of these three peptides into renal proximal tubule epithelial cells was performed to investigate whether the designed peptides might regulate apoptosis in vivo.

EXPERIMENTAL PROCEDURES

Computational techniques. Secondary structure prediction of the peptide sequences Bcl2_syn, Bax_syn, and Bak_syn was performed by applying the neural network-based prediction routine PHDsec (33). The EMBL PredictProtein server was used to align the sequences of interest to homologous sequences with known secondary structures available in the SwissProt database.

Refinement of the secondary structure prediction as well as further analysis of the conformational space of the peptides was on the basis of a Monte Carlo simulated annealing (MCSA) algorithm using the program package MultiMize (Mayer B., personal communication). Peptide structure optimization was realized by using a combined optimization of potential energies (computed by means of the ECEPP-3 force field, see Ref. 28) and free energies of solvation (calculated by means of a continuum approximation applying the Woedtken-Eisenberg free solvation energy parameter set with the adjustment of Sharp; see Ref. 38) with an extended Metropolis criterion (see Ref. 25 for details of the method). In each MCSA step, one freely rotatable bond was altered in the interval (−180°, 180°), and the acceptance probability of each newly generated structure was evaluated within the extended Metropolis criterion considering both potential and solvation energies. A multiple exponential cooling scheme was applied in the temperature interval (1,000, 280 K), realized within $5 \times 10^5$ MCSA steps, with a sudden heating back to 1,000 K every $10^6$ steps.

The electrostatic potential of low-energy conformations was calculated on the basis of solving the Poisson-Boltzmann equation as provided within the program package MOLMOL (18).

Synthetic peptides. Bcl2_syn, Bax_syn, and Bak_syn were synthesized by Genemed Synthesis (South San Francisco, CA). Synthesis was checked by means of mass spectroscopy, and a purity of >95% was ensured by means of HPLC. Bcl2_syn is defined by the sequence NH$_2$-DNREIVMKYLYKLYKLSQRGYEW-COOH. This peptide resembles the BH4 domain of human Bcl-2 (9), in which 5 residues were shown to be critical for Bcl-2 activity, namely, Ile-14, Val-15, Tyr-18, Ile-19, and Leu-23, whereas the remaining 19 residues are not specifically required (20). A recent study analyzed the structure of this BH4 domain, and secondary structure prediction as well as circular dichroism spectroscopy suggested that this domain might be highly α-helical, in particular in the presence of hydrophobic (micellar) surfaces. Especially within the residues 11–26, the helical content is high. These data suggest that the BH4 domain of Bcl-2, and consecutively our Bcl2_syn, exhibits an autonomous structural entity within the protein itself (11). From the functional viewpoint, the BH4 domain was shown to be critical for the interaction with CED-4 (15) as well as for heterodimerization with Bax and consecutive inhibition of apoptosis (11).

Bax_syn is defined by the sequence NH$_2$-STKKLSE-CLKRIGDELSNMELQ-COOH as given by a stretch of human Bax (SwissProt Q07814). This peptide resembles the suicide domain within Bax and is essential for the apoptosis promoting effect of Bax (16). This domain spans as few as 23 amino acids (residues 55–77) within Bax. The construction of chimeric Bax/Bcl-2 proteins, in which this suicide domain of Bax is transferred into the corresponding amino acid region within Bcl-2, drastically alters the function of Bcl-2. The suicide domain of Bax converts the apoptosis inhibitor Bcl-2 into a promoter of apoptosis (16).

Bak_syn is defined by the sequence NH$_2$-GQYGRQALIAG-DDINR-COOH, as given within human Bak (SwissProt Q16611). A minimal region of Bak required to bind to Bcl-x was recently determined; in particular, a peptide composed of the amino acids 72–87 was determined (34). This sequence resembles Bak_syn. NMR studies indicate that this peptide binds in a hydrophobic cleft formed by the BH1, BH2, and BH3 regions of Bcl-x. The peptide is a random coil in solution but again forms an α-helix when complexed to Bcl-x. The NH$_2$-terminal part interacts in particular with Val-126, Glu-129, Leu-130, and Phe-146, and the COOH terminus complexes in particular to Phe-97, Arg-100, Tyr-101, and Phe-105 of Bcl-x. The hydrophobic side chains of the peptide (Val-74, Leu-78, Ile-81, and Ile-85) point into the hydrophobic cleft of Bcl-x and stabilize complex formation. In addition to these hydrophobic interactions, the charged side chains of the peptide, Arg-76, Aps-83, and Asp-84, are close to oppositely charged residues of Bcl-x (Glu-129, Arg-139, and Asp-100).

Control_syn is a random sequence peptide with no known function. The secondary structure of the peptide in aqueous solution is due to the short sequence, which is most likely a random coil. The sequence of the peptide is given as NH$_2$-ALILTLVS-COOH. Control_syn was solely used as a negative control in the subsequent microinjection experiments.

Cell cultures. LLC-PK1 are immortalized cells derived from pig renal proximal tubular epithelium (a kind gift from Drs. Walter Pfaller and Gerhard Gstraunthaler, University of Innsbruck). Nineteen 6-cm$^2$ dishes with grid patterns forming 4-mm$^2$ squares were used to seed the LLC-PK1 cells, which were cultured in 5 ml DMEM (GIBCO) containing 100 IU/ml penicillin-streptomycin (GIBCO), 4 mmol glutamine, and 5% FCS (GIBCO). Cells were incubated at 37°C in a humidified atmosphere and 5% CO$_2$ for 48 h. For all experiments, confluent cell monolayers were used. The phenotype of the cells, the phenotypical change during adaptation to osmostress, and the typical appearance of apoptotic cells were determined previously (10, 39). Viability of cells was determined by light microscopy with and without trypan blue staining.

Before microinjection, DMEM was removed from LLC-PK1 cells and replaced by a buffer containing (in mM) 140 NaCl, 6.0 KCl, 2.0 CaCl$_2$, 2.0 MgCl$_2$, 20 glucose, and 10 HEPES, adjusted to pH 7.4 with NaOH.

Apoptosis induction and microinjection. Injection pipettes were made shortly before the experiments with a Sutter P-97 programmable puller (Sutter Instruments). Microcapillaries (Type GB 100-TF8P, Science Products) were pulled to yield tip resistances of 50–80 MΩ. Pipettes were loaded by retrograde filling with 3 µl of a sterile filtered buffer [in (mM) 140 KCl, 1.59 CaCl$_2$, 10 EGTA, and 10 HEPES, adjusted to pH 7.3 with NaOH] containing 0.5% FITC-labeled dextran (70 kDa, 10 µM), to which were added the peptides (in mM) 0.8 cytochrome c, 2.5 Bcl2_syn, 5.6 Bax_syn, 5.6 Bak_syn, and 10 HEPES, adjusted to pH 7.4 with NaOH.
RESULTS

Structural properties of the isolated peptides. Figure 1 gives the \(\alpha\)-helix formation propensities (scaled in an interval \((0, 9)\)) of the three isolated peptides Bcl2_syn, Bax_syn, and Bak_syn applying PHDsec. Clearly evident is the high \(\alpha\)-helix propensity predicted for Bcl2_syn and Bax_syn along the core of the peptides, i.e., the sequence \(3\text{REIVMKYIH11}\) for Bcl2_syn and \(3\text{KLSECLRGIDEL16}\) for Bax_syn. Less well defined is the \(\alpha\)-helix in Bak_syn, showing overall decreased propensity and, in addition, an \(\alpha\)-helix break along amino acids 9–13 (IIGDD).

PHDsec predicted a low propensity for other secondary structures, such as extended (backbone dihedral angles around 180°) and loop (random backbone dihedrals) elements, within the three peptides. A high propensity for forming a loop was predicted only for both termini of Bcl2_syn and Bax_syn, respectively. This can be attributed to end group effects within the relatively short peptides. Comparable findings were obtained with Bak_syn, leaving only the stretch RQAAI with a reasonable \(\alpha\)-helix propensity.

MCSA calculations of the three sequences given above were performed within MultiMize, considering fully \(\alpha\)-helical peptides as start structures, and \(5 \times 10^5\) MCSA steps were performed with an exponential cooling scheme. This type of simulation reveals the stability of the \(\alpha\)-helical start structures. The largest energetic difference between an ideal \(\alpha\)-helix and the MCSA-computed low-energy structure was found for Bcl2_syn (energy difference = \(-17.07\) kcal/mol), followed by Bax_syn (energy difference = \(-8.59\) kcal/mol). Nearly no energetic difference is given for Bak_syn, with a total folding energy difference of \(-1.46\) kcal/mol.

The backbone models (ribbons) of the three peptides give a clear picture of the secondary structural elements realized in solution: Bcl2_syn as well as Bax_syn shows a high degree of \(\alpha\)-helicity. Furthermore, Bcl2_syn maintains an overall extended helix, whereas Bax_syn shows a significant overall bend. Bak_syn, on the contrary, realizes an \(\alpha\)-helix only for the NH\(_2\)-terminal part, whereas the second half of the peptide appears to fold into a random coil regime.

The electrostatic profiles plotted on the solvent-accessible surface areas show additional distinct differences among the three peptides. Bcl2_syn is split into a hydrophobic rim and, on average, a positively charged rim. This pattern could indicate a distinct intermolecular interaction profile to putative dimerization partners. Another distinct electrostatic profile was revealed for Bax_syn, which displayed an overall positive charge distribution at the NH\(_2\) terminus and, on average, a negatively charged COOH-terminal side of the peptide. Finally, Bak_syn did not show a clear pattern of local charge distributions.
After the structural analysis, Bcl2_syn and Bax_syn do exhibit a stable secondary structure and defined molecular recognition sites, in close resemblance to the structure in the respective full proteins Bcl-2 and Bax. These structural prerequisites should also enable the putative functionality of these autonomous domains as isolated peptide sequences.

In contrast, Bak_syn did not give a defined secondary structure or electrostatics profile, which indicates that this domain is structurally stable only in the context of the full protein. However, a recent experimental setting (34) did show that a Bak_syn-like sequence, although a random coil in solution, does adopt an α-helix when complexed to Bcl-x.

Microinjection of Bcl2_syn peptide into tacrolimus- or LPS-treated cells. Tacrolimus treatment induced a time-dependent increase in the number of apoptotic cells. The number of apoptotic cells was not different between tacrolimus-treated uninjected cells and tacrolimus-treated cells that were injected with Control_syn (not shown); the percentages of apoptotic cells were roughly 3% at 1 h, 6% at 2 h, 9% at 3 h, and 10% at 4 h after injection. Thus injection of the Control_syn peptide as well as microinjection of FITC-dextran alone was not effective in preventing tacrolimus-induced apoptosis. LPS stimulation led to a stronger increase in apoptotic cell death compared with tacrolimus induction (Fig. 2, A and B).

**Fig. 2.** Time course of apoptotic cell death. All microinjections were visualized by coinjection of FITC-labeled dextran. Apoptosis induction was performed by tacrolimus (A) or lipopolysaccharide (LPS) treatment (B). No Tacrolimus/LPS + Control_syn, untreated cells were injected with a synthetically derived nonsense peptide that served as negative control (1 fl of 5.6 mM); Tacrolimus/LPS + Bcl2_syn, apoptosis was prevented by microinjection of the synthetically derived nonsense control peptide Control_syn (1 fl of 5.6 mM); Tacrolimus/LPS + Bcl2_syn, apoptosis was prevented by microinjection of the synthetically derived peptide Bcl2_syn (1 fl of 2.5 mM). n = 5 in each experiment; *P < 0.05 ± SE, Bcl-2_syn vs. Control_syn. C: synthetically derived nonsense peptide, Control_syn, which served as negative control, was ineffective in apoptosis induction (1 fl of 5.6 mM). Bax_syn and Bak_syn, apoptosis induction by microinjection of 1 fl of 5.6 mM synthetical peptide; Cytochr. C, apoptosis induction by microinjection of 1 fl of 0.8 mM cytochrome c. Values are means ± SE; n = 5 in each experiment; *P < 0.05, Bax_syn and Bak_syn vs. Control_syn. D: percentage of apoptotic cells after LPS stimulation determined by morphological criteria and annexin V staining. Phosphatidylexternalization (annexin positivity) is an earlier event than cytoplasmic blebbing and budding, which explains the higher percentage of apoptotic cells determined by annexin V staining compared with morphology. Values are means ± SE; n = 3 in each experiment. *P < 0.05, morphology vs. annexin V.
Microinjection of Bcl2_syn peptide, however, significantly reduced the number of cells undergoing apoptosis 2–4 h after tacrolimus as well as after LPS treatment (Fig. 2, A and B). Cells injected with Control_syn but not treated with tacrolimus or LPS also showed a time-dependent increase in the rate of apoptosis, with 4% of the cells being apoptotic 4 h after injection. However, the number of spontaneous apoptosis in cultured LLC-PK1 cells over time was not different from that observed in cells injected with Control_syn or FITC-dextran (data not shown).

Microinjection of Bax_syn, Bak_syn peptide, and cytochrome c. The time course of the induction of apoptosis after microinjection of various peptides is depicted in Fig. 2C. A view of Bax_syn-injected apoptotic LLC-PK1 cells with FITC and annexin V staining, as well as of nonapoptotic LLC-PK1 cells injected with Control_syn cell, is given in Fig. 3. For comparison, regularly cultured nonmicroinjected native LLC-PK1 cells are also shown. Cytochrome c led to a dramatic increase in the number of apoptotic cells as early as 2 h after microinjection. The percentages of apoptotic cells 2, 3, and 4 h after microinjection of cytochrome c were 8, 16, and 25% compared with 1, 2, and 4% for cells injected with Control_syn. As mentioned above, the time course of spontaneous apoptosis in untreated, uninjected cells was not statistically different from that in cells injected with Control_syn and/or FITC-dextran (data not shown).

The synthetically derived peptide Bax_syn was effective in triggering apoptosis after microinjection. After 2, 3, and 4 h, ~7, 7.5, and 10%, respectively, of injected cells showed typical features of apoptosis. Microinjection of the other synthetically derived proapoptotic peptide Bak_syn revealed similar effects on apoptosis induction. The corresponding numbers of apoptotic cells after 2, 3, and 4 h were 6.5, 7, and 8.5%, respectively. These values were significantly different from those of Control_syn-injected cells ($P < 0.05$).

**DISCUSSION**

We used synthetic peptides corresponding to the BH4 domain of Bcl-2 (Bcl2_syn) as well as peptides corresponding to functional segments of Bax and Bak (Bax_syn and Bak_syn, respectively) to interfere with the apoptosis effector phase. First, the secondary structures of these peptides were resolved to define sites potentially capable of molecular recognition, and then the peptides were microinjected into LLC-PK1 cells to investigate their functions in the regulation of apoptosis. The combination of these techniques allowed us to define basic requirements for the design of peptide mimetics as key regulatory elements of apoptosis.

A central issue of this study was the question of whether the peptides may adopt stable secondary structures in solution to provide functional molecular recognition sites. Our computational analysis demonstrated the formation of extended $\alpha$-helices in Bcl2_syn. Previously, an X-ray analysis of Bcl-xL (27), a protein highly homologous to Bcl-2, suggested a high degree of helicality for the BH4 region, which corresponds to the sequence of Bcl2_syn. This was corroborated by circular dichroism spectroscopy, which indicated a pronounced helicity for an isolated peptide corresponding to the BH4 region of Bcl-2 (20). Another important structural aspect of Bcl2_syn is the presence of two opposing electrostatic elements. A similar charge distribution has been reported for the BH4 domain of Bcl-2 (20). Maintenance of the helical character as well as of this characteristic charge must be assumed to be crucial for the functions of the BH4 domain of Bcl-2 and, of course, for the isolated peptide Bcl2_syn. The results obtained in our microinjection experiments support this hypothesis by showing that
Bcl2_syn is capable of reducing the rate of apoptosis in tacrolimus-treated LLC-PK1 cells.

Our computations also revealed a strong tendency to form \( \alpha \)-helices in solution for Bax_syn (Figs. 1 and 2B). The sequence of this peptide shows close homology to corresponding regions of Bak and Bik (7) and is supposedly participating in Bcl-2 dimerization, which may promote apoptosis. Bax_syn is also homologous to the BH3 region of Bcl-2, and the BH3 domain also displays an \( \alpha \)-helix in Bcl-xL. Still, the BH3 region exhibits a function opposite to that of Bax_syn, which induced apoptosis in LLC-PK1 cells. Charged amino acids within Bax_syn are distributed in a way that the peptide exhibits an overall positively charged NH\(_2\) terminus and a negatively charged COOH terminus. This appears to represent a functional structural unit that is sufficient to trigger apoptosis in LLC-PK1 cells.

The secondary structure of Bak_syn does not give a clear picture. Our computational results indicate the formation of a random coil structure in solution (Figs. 1 and 2C), and the electrostatics profile also fails to provide any insight into predefined molecular recognition sites. Nevertheless, an NMR study by Sattler et al. (34) showed that this peptide is able to bind to the hydrophobic cleft formed by the BH1, BH2, and BH3 regions of Bcl-xL, and, when complexed to Bcl-xL, this peptide may also form an \( \alpha \)-helix. Our functional data showing the promotion of apoptosis by Bak_syn support the idea that this peptide may change from random coil to \( \alpha \)-helix when recognizing and binding to a respective complexation site on an apoptosis inhibitor (such as Bcl-xL) of the Bcl-2 family.

During apoptosis, cytochrome c is released from mitochondria, and this release is supposed to be a critical trigger event for the activation of proapoptotic caspases. Accordingly, the microinjection of cytochrome c into the cytosol of human embryonic kidney 293 cells resulted in a dose-dependent induction of apoptosis (21). Similarly, microinjection of cytochrome c into normal kidney cells (NRK-52E) produced rapid apoptosis, which usually began within 30 min and reached a maximum of 60–70% after 3 h. Cells first showed shrinkage, then displayed multiple pseudopods, which rapidly extended and retracted, giving the cells a bosselated appearance (6). The same morphological features were detected in our studies on LLC-PK1 cells when injected with cytochrome c. In NRK-52E cells, apoptosis induced by cytochrome c was prevented by a caspase 3 inhibitor but not by the overexpression of Bcl-2, which indicates that cytochrome c acts further downstream within the apoptosis cascade than Bcl-2. Therefore, we used tacrolimus to provide an external trigger to stimulated apoptosis when investigating potentially antiapoptotic effects of Bcl2_syn.

Tacrolimus as well as LPS treatment and injection of Bax_syn and Bak_syn led to a significant increase in the number of apoptotic cells for as long as 4 h after injection. During this period of time, Bcl2_syn prevented apoptosis. Because the different peptides affected apoptosis in opposite directions at almost equimolar concentrations and the negative control peptide was ineffective in either direction, it is unlikely that intracellular osmotic or volume changes were responsible for the effects observed in Bax_syn- or Bak_syn-injected cells. The percentage of apoptotic cells after tacrolimus or Bax_syn and Bak_syn injection appear low on the first view. However, assuming a process time of 60–90 min from apoptosis induction to complete clearance and disappearance, a considerable number of cells would have undergone apoptosis after 4 h in culture (4, 8). This is especially important in the clinical setting of renal tubular cell injury, whereby a considerable number of cells are cleared by apoptosis.

Many studies have indicated the central role of members of the Bcl-2 family in the regulation of apoptosis in renal tubule cells in vivo (29). Bcl-2 is expressed in the normal and diseased kidney, particularly in distal and proximal tubule epithelial cells (5, 23, 28). Bax is expressed only faintly in healthy human renal tubule but is immediately upregulated after ischemic injury (data not shown). Bak, on the other hand, is expressed only in the distal convoluted tubule (19). Our present results indicate that synthetic peptides derived from members of the Bcl-2 family may be used to control the apoptotic process in renal tubule cells.

In conclusion, the artificial Bcl-2 peptide Bcl2_syn adopts a functional conformation in vitro and inhibits apoptosis in renal proximal tubular epithelial cells under quasi in vivo conditions. Bax_syn and Bak_syn are also functional peptides and induce apoptosis in these cells. Thus analogs to these regulatory peptides may provide valuable lead compounds in the therapeutic context.

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REFERENCES


