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Neuronal and muscular alterations caused by two wheat endosperm proteins, puroindoline-a and alpha1-purothionin, are due to ion pore formation

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Abstract Using the patch-clamp technique it was found that the toxicity of the two wheat endosperm proteins puroindoline-a and alpha1-purothionin probably results from the dissipation of ion concentration gradients essential for the maintenance of cellular homeostasis.

Keywords Giant liposomes · Mouse neuromuscular junction · Neuroblastoma cells · Pore formation · Wheat endosperm proteins

Abbreviations *PIN-a* puroindoline-a · *PTH* alpha1-purothionin

Introduction

Puroindoline-a (PIN-a) and alpha1-purothionin (PTH), two basic cysteine-rich proteins isolated from the wheat endosperm of *Triticum aestivum* sp., have been suggested to play a role in plant defence mechanisms against phytopathogenic organisms (Blochet et al. 1993; Bohlmann 1994; Dubreil et al. 1998). The two proteins were shown to have a marked toxicity to vertebrate cells, as revealed by studying their effects on the morphological and functional properties of neuroblastoma NG108-15 cells, frog myelinated axons, C2 myotubes and mammalian neuromuscular junctions, using confocal laser scanning microscopy and conventional electrophysiology (Mattei et al. 1998; Benoit et al. 2001). In order to obtain insight into the mechanisms involved in the cellular toxicity of PIN-a and PTH, the proteins were incorporated into asolectin giant liposomes (Riquelme et al. 1990) and their pore-forming ability was investigated using the patch-clamp technique in excised patch configuration.

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Results

Current recordings from liposomes containing PIN-a revealed that the protein forms single channels (Fig. 1A) with a unitary conductance of about 15 pS between −80 and 80 mV. The reversal potential of the current recorded in response to potential-ramps was shifted from 0 to −24 mV when the bath concentration of NaCl was increased from 140 to 440 mM, and from 0 to −9 mV when external NaCl was replaced by KCl. This indicates that PIN-a forms cationic channels whose selectivity is $K^+ > Na^+ > > Cl^-$. Current recordings from liposomes containing PTH revealed that this protein also forms single channels (Fig. 1B, C) with a unitary conductance at −100 mV of about 35 pS in 2/3 of the recordings and about 100 pS in 1/3 of the recordings. The ionic selectivity was $Na^+ \approx K^+ > > Cl^-$ for the low conductance channels and $Cl^- > > Na^+$ for the large conductance channels, indicating that these channels correspond to cationic and anionic channels, respectively.

Conclusion

It is concluded that the toxicity of the two wheat endosperm proteins, PIN-a and PTH, to fungi, yeast, bacteria and vertebrate cells (Blochet et al. 1993; Bohlmann 1994; Dubreil et al. 1998; Mattei et al. 1998;

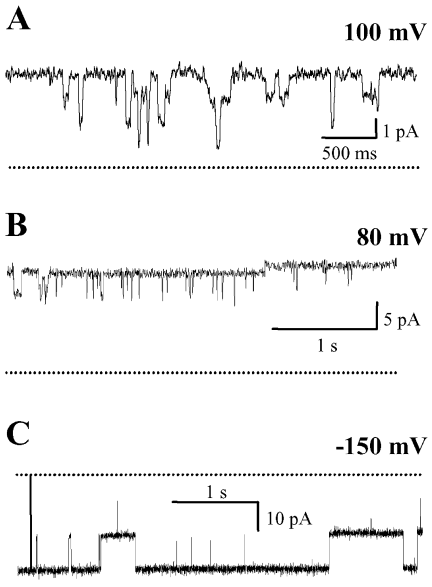

Fig. 1 Ion-channel activities exhibited by giant liposomes containing either PIN-a (**A**) or PTH (**B** and **C**). The single-current traces were recorded at the indicated holding potential. The zero current level is indicated by the *dotted lines*

Benoit et al. 2001) is likely to result from the dissipation of ion concentration gradients essential for the maintenance of cellular homeostasis.

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