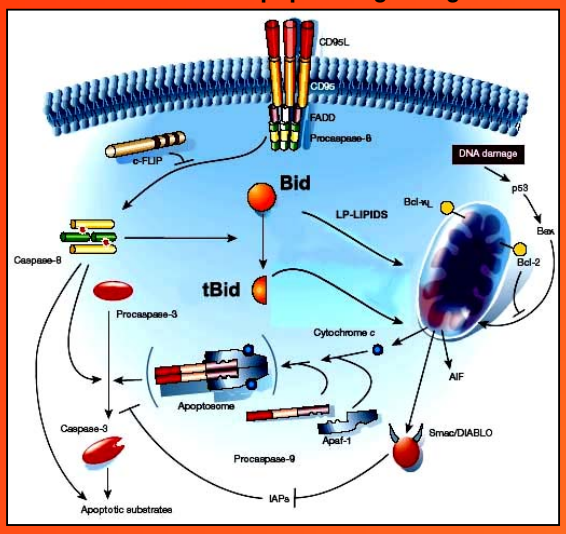
Lipid exchange in mitochondrial cytocrome c release: apoptotic effect of maize lipid transfer protein

MASSIMO CRIMI[1], GIANNI ZOCCATELLI[1], ALESSANDRA ASTEGNO[1], MAURO DEGLI ESPOSTI[2]

[1] Dept.Scientific & Technologic, University of Verona (Verona)
[2] Faculty of Life Sciences, University of Manchester (Manchester, UK)

Membrane lipids and protein-lipid interactions are attracting increasing interest in the field of cell death and apoptosis.

Some mammalian pro-apoptotic proteins of the Bcl-2 family, like **Bid**, appear to have an intrinsic capacity of binding and exchange lipids [1] but it is still unclear whether this function could be relevant for apoptotic signalling cascade.

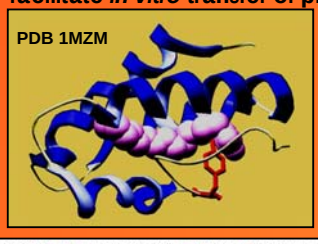


Modified from M.O. Hengartner, (2000) Nature 407, 770-776.

We have studied the ability of a ***plant lipid transfer protein [2]*** , not related to animal apoptotic cascade, to induce cytochrome c release from mammalian mitochondria.

Non-specific lipid transfer proteins (**nsLTPs**) are ubiquitous plant proteins that have been shown to bind, *in vitro*, various amphiphilic molecules including lysolipids and glycolipids and to facilitate *in vitro* transfer of phospholipids between membranes [3].

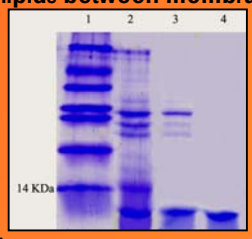
A



B

```
LIP  39  RTTADRRAACNCLKNAAAGVSGLNAGNAASIPSKCGVSIPYTI-STSTDC
BH3  71  RIEADSESQEDIIRNIARHLAQVGDSMDRSIPPGL-VN-GLALQLRNTSR
```

A) Maize nsLTP structure complexed with palmitic acid
B) Alignment of Bid to maize LTP lipid binding region

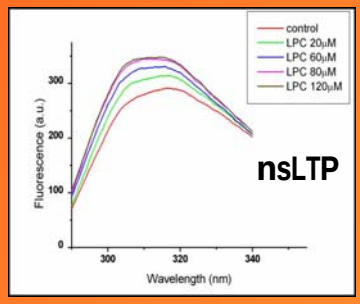


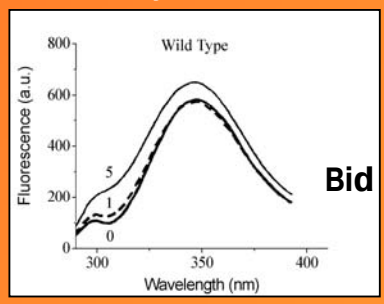
Purification of nsLTP from maize flour 1. *MWS*; 2. total extract; 3. MonoS column; 4. Superose 12 HR.

Results

- A sequence similarity between Bid, and plant LTPs has been observed in the region involved in lipid binding [4]. Both Bid and nsLTP, in fact, have the ability to bind and exchange lysolipids as demonstrated by LPC-induced changes in the fluorescence emission of the aromatic residues.
- We found that maize nsLTP induces the release of cytochrome *c*; this activity is strongly dependent on the presence of (lyso)lipids.
- The effect of LTP is stronger than that of full length Bid either in the absence or in the presence of exogenous lysolipids.
- The length of the aliphatic chain of LPC does not modify the cytochrome c release induced by nsLTP.
- The removal of endogenous free lipids with BSA-washing of mitochondria completely suppresses the LTP-induced release of cytochrome *c*

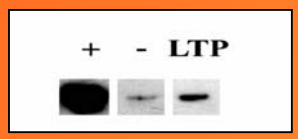
Fluorescence emission analysis

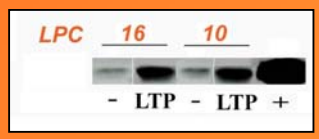




Maize nsLTP and Bid lysophospholipids (LPC-C16) binding analysis monitored through tyrosine and thryptophan fluorescence changes.

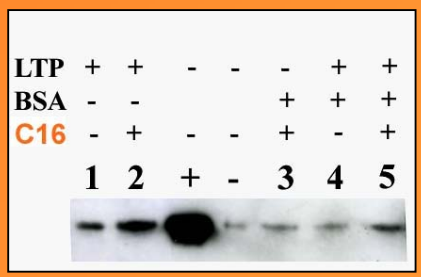
Western blot analysis of cytochrome *c* release from mice mitochondria





Effect of LTP in the absence or in the presence of exogenous lysolipids (*LPC C16 or C10*).

Comparison between the effect of LTP and Bid with or without LPC-C16.



Analysis of nsLTP induced cytochrome *c* release from control (1 and 2) and BSA washed (3, 4 and 5) mitochondria with or without exogenous added LPC-C16 (C16).

Conclusions

We found that the lipid transfer activity of maize nsLTP is able to induce cytochrome c release from mammalian mitochondria. The presence of (lyso)lipids (i.e. lysophosphatydilcholine) is required to observe this effect.

The present results highlight the concept that the dynamic capacity of exchanging lipids, which is intrinsic to diverse lipid transfer proteins, could alter the surface composition of the mitochondrial lipid membrane. This may represent a common biochemical mechanism of promoting pro-apoptotic reactions relevant to diverse forms of mitochondria-dependent death.

References:

1. *M. Degli Esposti, J.T. Erler, J.A. Hickman and C. Dive (2001) Mol. Cell Biol. 21, 7268-7276.*
2. *G.W. Han, J.Y. Lee, H.K. Song, C. Chang, K. Min, J. Moon, D.H. Shin, M.L. Kopka, M.R. Sawaya, H.S. Yuan, T.D. Kim, J. Choe, D. Lim, H.J. Moon and S.W. Suh (2001) J. Mol. Biol. 308, 263-278.*
3. *F. Guerbette, M. Grosbois, A. Jolliot-Croquin, J.C. Kader and A. Zachowski (1999) Biochemistry 38, 14131-14137.*
4. *M. Degli Esposti (2002) Biochim. Biophys. Acta 1553, 331-340.*