Research line No. 5
Structure, function and manipulation of peptides and proteins

Programs

5.1 Chemical and kinetic characterization of proteases of high specificity from the venom of mexican reptiles.

5.2 Purification and chemical characterization of toxins from scorpion venom.

5.3 Isolation and characterization of specific receptors through the utilization of peptidic toxins.

5.4 Functional characterization of toxins peptides.

5.5 Purification and characterization of the plasminogen activator from the saliva of hematophage bats.

5.6 Development and optimization of proteins and peptides purification methods.

5.7 Production of monoclonal antibodies against peptides and proteins.

5.8 Protein engineering.

Program 5.1 Chemical and kinetic characterization of proteases of high specificity from the venom of mexican reptiles.

The venoms from poisonous saurian and ophidian of México are highly rich in proteolytic enzymes, although they are poorly studied, so far.

Through the use of affinity chromatography and conventional purification methods, a kallikrein and two plasminogen activators from saurian Heloderma horridum venom have
been obtained in homogenous form. Their characterization helps us explain at a molecular level the phylogenetic relations between Heloderma and other organisms, and its participation in the intoxication physiopathology of this animal's bite.

We are performing a screening study to detect these and other proteolytic activities in the venom of about twenty vipers, endemic in México. We explore their potential in basic research and the application of these highly selective tools. We have discovered a new type of toxin in the Heloderma horridum venom.

Specific projects

Sequencing of helodermatine, a new kallikrein present in Heloderma horridum's venom.
A. Alagón, L.D. Possani and W.D. Schleuning
1985/P/S/DBP

Helodermatine's molecular action on natural substrate.
B. Sosa, A. Alagón and W.D. Schleuning
1985/P/S/DBP

Purification and characterization of a toxin responsible for hypothermia from the venom of Heloderma horridum horridum.
J.M. Mochca, B.M. Martín and L.D. Possani
1981/P/S/DBP/UB

Program 5.2 Purification and chemical characterization of toxins from scorpion venom.

The venoms from many scorpion species contain polypeptides and proteins highly toxic to humans. The isolation and chemical characterization of these toxic components have permitted the discovery of their molecular mechanisms of action. Among the animals whose venoms have been studied extensively, are the snakes and scorpions. The use of chromatographic and electrophoretic techniques has made possible the separation of a large number of polypeptides and neurotoxic proteins. Many of them effect the acetylcholine receptor, ionic channels (Na+, K+, Ca2+) or participate in a series of important physiological functions like pancreatic secretion, hypothermia, and liberation of neurotransmitters.
The toxins have been purified to homogeneity; their amino-acids composition and primary sequences have been or are on the way of being determined.

### Specific projects

**Isolation and chemical characterization of toxins from *Centruroides noxius* Hoffmann scorpion venom.**
A. N. Ramírez, B. M. Martín, G. B. Gurrola and L. D. Possani
1984/P/S/DBP

**Isolation and characterization of two toxins from the mexican scorpion *Centruroides limpidus* Karsch.**
A. Alagón, H. S. Guzmán, B. M. Martín, A. N. Ramírez, E. Carbone and L. D. Possani
1983/T/S/DBP
Total amino acid sequence of amino acids from a toxin isolated from Centruroides limpidus tecomanus Hoffmann scorpion venom.

Isolation and characterization of toxins from Centruroides infamatus infamatus scorpion venom.
M.D. Dehesa, B.M. Brian and L.D. Possani 1985/P/S/DEBP/UB

Primary structure of toxins from the scorpion Tityus serrulatus Lutz and Mello.

Purification and characterization of taicatoxin; a new and selective blocking peptide for the calcium channel.

Program 5.3 Isolation and characterization of specific receptors through the use of peptidic toxins.

The peptidic toxins purified to homogeneity, up to date, are components that recognize in a specific way some membrane receptors. For this reason, they have been transformed into useful tools for the isolation and functional characterization of receptor molecules. The alpha-toxin of elapides (Naja naja siamesis) is among the isolated and characterized toxins. It has been utilized in the isolation of the acetylcholine receptor. The gamma toxin from Tityus serrulatus has been used in the isolation of the sodium channel. The specific Noxustoxin for the potassium channel and, more recently, the blocking Taica toxin for the calcium channel, are new tools being used for the isolation of channel proteins.

All these natural peptides have been marked with radioactive isotopes or fluorescent chromophores for their use as biological tracers, or they have been utilized for the synthesis of affinity chromatography supports.
Specific projects

Isolation and characterization of potassium channel from mice brain.
H.H.F. Valdivia, A. Zentella, G., Szabo and L.D. Possani
1984/P/S/DBP/URIA/UB

The utilization of noxiustoxin and of taicatoxin for the study on the distribution of potassium and calcium channels in excitable membranes.
1986/I/S/DBP

Program 5.4 Functional characterization of toxin peptides.

Natural and synthetic peptides have been utilized as tools in the characterization of biological functions, from an electrophysiological, neurochemical, and morphological point of view.

The study of the opening and closing mechanism of ionic channels from excitable membranes has benefited from the discovery of peptidic toxins. Likewise, the studies on the liberation of neurotransmitters and experimental pancreatitis have been implemented thanks to the use of natural and synthetic peptides. Finally, morphologic alterations and immunohistochemical localizations have been visualized or understood thanks to the use of the above mentioned peptides.

Specific projects

Blockade of potassium channel of the squid’s axon by Noxiustoxin; a toxin from Centruroides noxius scorpion venom.
E. Carbone, G. Prestipino, L. Spadavecchia, F. Franciolini and L. D. Possani
1982/T/S/DBP
The effect of two toxins from the new world scorpions in sodium channels from the heart.
A. Yatani, L.D. Possani, G. Kirsch and A.M. Brown
1985/P/S/DBP

The effect of toxin II-10 and II-9.2.2 from C. noxius scorpion venom in the GABA liberation of synaptosomes from rat brain.
M. Sitges, L.D. Possani and A. Bayón
1984/P/S/DBP

Neurotoxins that act selectively on the voltage-dependent calcium channel of the heart.
A. Brown, A. Yatani, A. Lacerda, G.B. Gurrola and L.D. Possani
1985/P/S/DBP

Localization of scorpion toxin binding sites in the central nervous system of rats, by labelling anti-toxin monoclonal antibodies.
1985/P/S/DBP

The effect of Tityus serrulatus toxins on pancreatic secretion.
P.L. Fletcher, M. Fletcher and L.D. Possani
1984/P/S/DBP

Program 5.5 Purification and characterization of the plasminogen activator from the saliva of hematophage bats.

The Desmodus rotundus activator (desmokinase) degrades with great efficiency sanguineus clots from mammals. Our plan is to study this enzyme’s molecular biochemistry and explore its possible use as a thrombolytic agent. The great dependence of desmokinases on fibrin specificity and low immunogenicity allows its routine use in patients with profound thrombosis.
The effect of two toxins from the new world scorpions in sodium channels from the heart.
A. Yatani, L.D. Possani, G. Kirsch and A.M. Brown
1985/P/S/DBP

The effect of toxin II-10 and II-9.2.2 from C. noxius scorpion venom in the GABA liberation of synaptosomes from rat brain.
M. Sitges, L.D. Possani and A. Bayón
1984/P/S/DBP

Neurotoxins that act selectively on the voltage-dependent calcium channel of the heart.
A. Brown, A. Yatani, A. Lacerda, G.B. Gurrola and L.D. Possani
1985/P/S/DBP

Localization of scorpion toxin binding sites in the central nervous system of rats, by labelling anti-toxin monoclonal antibodies.
1985/P/S/DBP

The effect of Tityus serrulatus toxins on pancreatic secretion.
P.L. Fletcher, M. Fletcher and L.D. Possani
1984/P/S/DBP

Program 5.5 Purification and characterization of the plasminogen activator from the saliva of hematophage bats.

The Desmodus rotundus activator (desmokinase) degrades with great efficiency sanguineous clots from mammals. Our plan is to study this enzyme's molecular biochemistry and explore its possible use as a thrombolytic agent. The great dependence of desmokinases on fibrin specificity and low immunogenicity allows its routine use in patients with profound thrombosis.
Specific projects

The purification and chemical characterization of desmokinsa, the plasminogen activator from the saliva of Desmodus rotundus vampire.
B. Sosa, R. Medellin and A. Alagón
1985/P/S/DBP

The dependence on the requirements of fibrin for the enzymatic action of desmokinsa.
B. Sosa, A. Alagón and W.D. Schleuning
1985/P/S/DBP

Program 5.6 The development and optimization of methods and purification systems for proteins and peptides.

We intent to develop both general and specific methodologies for the purification of polypeptides utilizing mainly techniques such as: affinity chromatography, ionic interchange chromatography, gel permeation chromatography, high resolution chromatography, electrophoresis and diffusion through membranes. Likewise we work in the scaling up of purification methods for specific peptides.

Specific projects

Purification by ion exchange chromatography of human insulin chains produced in bacteria.
L. Güereca, X. Alvarado, G. Estrada, N. Cruz and F. Bolívar
1984/T/S/DBP/UPP

Analytical and semipreparative purification by HPLC of the human A and B insulin peptides produced in bacteria and the products of chemical association.
S. Antonio, N. Cruz and L. Güereca
1985/T/S/DBP/UPP

Separation of TRH and its metabolites by ion-paring reversed-phase HPLC.
S. Antonio, L. Güereca, M. Cisneros and J. L. Charli
1985/T/S/DBP/UPP

Development of immunoaffinity columns for TRH.
P. Joseph
1984/P/DBP/URIA

Design, synthesis and evaluation of supports for pseudo-
affinity chromatography.
N. Cruz and L. Güereca
1985/P/S/DBP/UPP

Optimization of enzyme purification methods in nucleic acids
research.
I. Vichido and N. Cruz
1986/I/DGBM/DBP/UPP/UCCRB

Program 5.7 Production of monoclonal antibodies against
peptides and proteins.

We work on developing methodologies for the production of
monoclonal antibodies directed against specific polypeptides.
They will be used for quantification, characterization, and
purification of such polypeptides.
Specific projects

Production of monoclonal antibodies against LHRH and its use in the hormone purification by affinity chromatography. P. Héron, R. Saavedra and P. Joseph 1983/P/S/DBP/UB/URIA

Program 5.8 Protein engineering.

This field has great implications in the molecular interpretation of physiological phenomena and in the biotechnological application of specific proteins. Our aim is to explore in depth the structural-functional relation of specific proteins. We intent to apply this knowledge in the design of improved proteins for diverse purposes. Genetic engineering and classic genetic methods will be applied during the initial period and graphic and dynamic molecular methods will be implemented in subsequent stages.

Specific project

Saturation mutagenesis for the selection of specificity mutants of EcoRI endonuclease.
M. Alonso and X. Soberón 1985/P/DGBM/USQM

Isolation of the DNA which codes for non toxic immunogenic fragments from tetanus toxin.
J. Osuna and X. Soberón 1985/P/S/DGBM