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Five hundred mg of B.pirajai venom were gel filtered and
ven fractions, SI to SVII, were obtained. The fractions were
ayed for PLA_2, esterasic and coagulant activities. Fraction
, which showed low PLA_2 and neither esterasic nor coagulant
tivities, revealed a molecular weight around 12,000, which
rresponds to the molecular weight of myotoxins of snake venoms
m the same genus. It was submitted to an ion-exchange
romatography. Five sub-fractions were obtained, SIV-1 to SIV-5.
sed on electrophoretic migration similarities of SIV-3 and SIV-5
th BthTx-II and BthTx-I, respectively, both myotoxins isolated
m B.jararacussu snake venom, the initial N-terminal sequencing
SIV-3 and SIV-5 was carried out. SIV-3 showed the first
fference with the BthTx-II sequence in the fourth amino acid
sidue, whereas SIV-5 was identical to BthTx-I at least until the
th amino acid residue. The structural and functional characteriza
n of these myotoxins is now in progress.

ancial support: CNPq, CAPES, FAPESP

§ Depto. Ciências Fisiológicas - UFU - Uberlândia,MG

Bothropstoxin I (BthTx-I) is a myotoxin isolated from the venom of the snake Bothrops jararacussu (Toxicon 26: 615,1988). BthTx-I is a Lys - 49 phospholipase A_2 (PLA_2) - like protein composed of a single polypeptide chain of 121 amino acids residues (M.W. 13,720 and pI 8.2) containing 14 half - cystines and one methionine residue. Its amino acid sequence was determined recently (J.Protein Chem.12:57,1993). Despite its PLA_2- like structure ,BthTx-I is deprived of any PLA_2 activity, and in addition to its myotoxic effects , this toxin also inhibits coagulation in citrated plasma. Concentrations over 50 ug/ml of BthTx-I significantly altered the recalcification time of citrated rat plasma. The mean clotting time increased from 75 sec (control) to more than 2 hours. In order to determine which region of the molecule is responsible for this activity, we tried to get some peptides from BthTx-I by limited proteolysis. Reduced and carboxymethylated BthTx-I was partially digested for 4 hours at 37º with Staphylococcus aureus V8 protease which acts mainly upon Glu-CONH peptide bonds. After the digestion, the mixture was lyophilized and fractionated on a Sephadex G-25 gel filtration column by using a 50 mM pH 7.8 ammonium bicarbonate buffer. Only one (SIII) out of eight fractions obtained showed anticoagulant activity in citrated rat plasma similar to the intact toxin. These results indicate that such a cleavage in the original molecule can generate some active peptides. SIII was fractionated into six major peaks by using a reverse phase C18 analytical column in a HPLC system. Due to the small amount of the material further assays are in progress in order to test the activity of these fractions and to get the amino acid sequence of the isolated peptides.

Financial support : FAPESP, CAPES

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YSTAL STRUCTURE DETERMINATION OF Lys49 PHOSPHOLIPASES FROM Bothrops asper and Bothrops godmani.

R.K. Arni[M] and J.M. Gutierrez[O]

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Instituto Clodomiro Picado, San Jose, Costa Rica

hospholipases A2 (PLA) are small, stable, calcium dependent lytic
nzymes that specifically hydrolyze the *sn-2* ester of
nospholipids. These molecules are widely distributed and play an
portant role in a number of physiological cellular processes
ch as inflammation, blood platlet aggregation and acute
ypersensitivity (Hirata & Axelrod, 1980).
ecent sequence analysis have indicated the presence of a variant
LA whose characteristic feature is the complete absence of
talytic activity due to substitution of key amino acids in the
lcium binding loop (Maraganore et al., 1987; Francis et al.,
991).
e have purified, crystallized and solved the three-dimensional
tructures by X-ray crystallographic methods of two Lys49 PLA
riants from *Bothrops asper* and *Bothrops godmani* venoms (Arni &
utierrez, 1993). These represent the first crystal structure
termination of proteins in South America.
tructural implications of the substitution of key amino acids in
he calcium binding loop will be presented.

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M - 28

EFFECTS OF IONIZING RADIATION ON THE BIOLOGICAL AND BIOCHEMICAL PROPERTIES OF CROTOXIN

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Ionizing radiation has been shown to inactivate various protein toxins. We have examined several biological and biochemical properties of purified crotoxin, the principal toxin from the venom of *Crotalus durissus terrificus*, following irradiation of a 2 mg/ml solution in 0.15 M Na using a dose of 2000 Gy of ^{60}Co. Electrophoretic analysis of irradiated crotoxin showed the presence of high molecular weight bands that were absent in native protein. Further, the irradia crotoxin exhibited about one-half the phospholipase activity and toxicity as native material. Wh irradiated crotoxin was subjected to molecular exclusion chromatography on either Sephadex G or G100, a broad early eluting peak indicated the presence of aggregated material. Electrophore analysis of material in this early eluting peak confirmed the presence of higher molecular weigh aggregates not dissociated in the presence of sodium dodecyl sulfate. This same aggregated material was not toxic when injected into mice i.v. at concentrations up to 2.5μg/g and had grea diminished phospholipase activity. Non-aggregated, irradiated material recovered from Sephad chromatography had normal i.v. LD_{50}-values in mice of *ca.* 0.06μg/g and phospholipase activities. Thus, active and inactive forms of crotoxin could be recovered from irradiated croto by Sephadex chromatograpy. The antigenicity and other biological properties of these different forms of crotoxin are currently under investigation.

Supported in part by the International Atomic Energy Agency.

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URIFICATION AND AMINO ACID SEQUENCES OF SIX TX3 TYPE EUROTOXINS FROM THE VENOM OF THE BRAZILIAN "ARMED" SPIDER *PHONEUTRIA NIGRIVENTER* (KEYS)

.G.Figueiredo[1], M.N.Cordeiro[1], A.C.Valentin[1], C.R.Diniz[1], .R.C.Von Eickstedt[2], J.Gilroy[3] and M.Richardson[3]
Fund. Ezequiel Dias, Belo Horizonte, M.G.; [2]Inst. Butantan, São aulo, S.P. and [3]University of Duhran, Durhan, U.K.

ix neurotoxic peptides (Tx3-1 to Tx3-6) were purified from the enom of the spider *P. nigriventer* by a combination of gel iltration, reverse phase FPLC on PEP-RPC and PRO-RPC columns, everse phase HPLC on Vydac C 18. and ion exchange HPLC on ationic and anionic columns. These toxins caused different eurological symptoms in mice after intracerebroventricular njection. At dose levels of 5 ug/mouse, Tx3-3 and Tx3-4 caused apid general flaccid paralysis followed by death in 10-30 min; Tx3-2 induced immediate clockwise gyration and flaccid paralysis fter 6 hr; Tx3-1, Tx3-5 and Tx3-6 produced paralysis only in the osterior limbs and gradual decreases in moviment and agression uring 24 hr. The mol. wt of these cystine-rich peptides were found to be in the range of 3500-8500 by mass spectroscopy and DS-PAGE. The complete amino acid sequences of the neurotoxins Tx3-1 (40 residues), Tx3-2 (34 res.) and Tx3-6 (55 res.), and the -terminal sequences of Tx3-3 (34 res.) Tx3-4 (40 res.) and Tx3-5 (36 res.) were established by direct automated Edman egradation, and manual DABIT/PITC microsequence analyses of eptides obtained from digests with various proteases. The tructures of these Tx3 neurotoxins from *P. nigriventer* exhibited equence similarities to one another and to the neurotoxins from the venoms spiders *Hololena curta* and *Agelenopsis aperta*, which ere most evident in the location of the Cys residues.

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M - 30

CHARACTERIZATION OF *Bothrops erythromelas* VENOM

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[2]Departamento de Zoologia, UFBA, Salvador-BA

The *Bothrops erythromelas* (B.ery) venom known as "jararaca seca" is found throughout the northeast region of the contry, be responsible for the majority of the registered ophidics accident the state of Pernambuco. The objective of this work was determin and comparing the enzymatic and biological activities of the refer venom with the venom of *B. jararaca* (B.jar). With this purpose the follo activities were analized: coagulant on human plasma; proteolytic casein; toxic by determination of LD_{50} (i.p.) and edema-forming intraplantar injection of the venom and determination of the weight increase. Among the tested activities, the coagulant one sho to be more significant, being 2.8 times bigger than one obtained the *B. jararaca* venom (B.ery= 57.5±1,9s[1]; B.jar= 19.9±1.5s[1]). While the proteolytic activity it was not observed significant differ (B.ery= 46.5±4.9U/mg; B.jar= 43.2±3U/mg). By the other hand, toxic and edema-forming activities of the *B. erythromelas* venom w aproximately 5.8 and 2.3 times smaller than the one observed in *B. jararaca* venom (LD_{50}: B.ery= 11.22±3.04mg/Kg, B.jar= 1.94±0.42mg/ edema: B.ery= 10±2.9U/mg, B.jar= 23.2±3U/mg). These results ag with description of the ophidics accident caused by *B. erythrome* that described only not serious poisoning cases.

Supported by: CNPq

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EFFECTS OF IONIZING RADIATION ON THE BIOLOGICAL AND BIOCHEMICAL PROPERTIES OF CROTOXIN

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Ionizing radiation has been shown to inactivate various protein toxins. We have examined several biological and biochemical properties of purified crotoxin, the principal toxin from the venom of *Crotalus durissus terrificus*, following irradiation of a 2 mg/ml solution in 0.15 M NaCl using a dose of 2000 Gy of ^{60}Co. Electrophoretic analysis of irradiated crotoxin showed the presence of high molecular weight bands that were absent in native protein. Further, the irradiated crotoxin exhibited about one-half the phospholipase activity and toxicity as native material. When irradiated crotoxin was subjected to molecular exclusion chromatography on either Sephadex G75 or G100, a broad early eluting peak indicated the presence of aggregated material. Electrophoretic analysis of material in this early eluting peak confirmed the presence of higher molecular weight aggregates not dissociated in the presence of sodium dodecyl sulfate. This same aggregated material was not toxic when injected into mice i.v. at concentrations up to 2.5μg/g and had greatly diminished phospholipase activity. Non-aggregated, irradiated material recovered from Sephadex chromatography had normal i.v. LD_{50}-values in mice of *ca.* 0.06μg/g and phospholipase activities. Thus, active and inactive forms of crotoxin could be recovered from irradiated crotoxin by Sephadex chromatograpy. The antigenicity and other biological properties of these different forms of crotoxin are currently under investigation.

Supported in-part by the International Atomic Energy Agency.