Annexin III is the only member of the annexin family for which an enzymatic activity has been proposed: it was identified as an inositol 1,2-cyclic phosphate 2-phosphohydrolase (1).

Annexins are a family of calcium and phospholipid binding proteins whose biological function is not well defined yet. They consist of a highly conserved C-terminal core of four (exceptionally eight) repeats of about 70 amino acids, and a more variable N-terminus (2). Unlike most members of the family, annexin III is expressed preferentially in neutrophils, in their final stage of differentiation (3). The 3D structure of the conserved core of annexins V and I shows a protein formed of 4 structural domains, with a hydrophilic channel between modules formed of domains 1+4 and 2+3. The calcium binding sites are all located on one side of the molecule that has thus been defined as the membrane-binding surface.

The overall structure of annexin III is similar to that of annexin V, but with a well defined catalytic site in domain 3, which is observed only under high calcium conditions for annexin V. The N-terminus of annexin III carries a Trp which lies with its side-chain inserted into the hydrophilic surface between the modules described above, which may affect any potential ion channel activity. The relative position of domain 3 with respect to domain 2 is different from annexins I and V, suggesting a molecular flexibility that may be important for the attachment of annexins on membrane surfaces (4).

The crystals grown in the presence of inositol-1,2-phosphate (InP) are not isomorphous to native ones, yet we find only one molecule of InP in the structure. It lies on the calcium-binding surface of the protein, interacting with a secondary calcium site on this surface. Its location does not suggest an enzyme active site, but on the basis of its presence on the membrane-binding surface of annexin we can model the interaction of annexins with phospholipids embedded in a membrane.

BmK M8 was purified from Chinese scorpion Buthus martensii Karsch. The crystals of BmK M8 belong to space group P21 and contains one molecule per asymmetric unit. The structure model of BmK M8 has been refined to a R factor of 18.1% with data from 8A to 1.85A.

BmK (Rana catesbeiana) egg lectin (RCEL) is a member of the pancreatic ribonuclease superfamily. RCEL exhibits RNA-degrading activity as well as multivalent carbohydrate-binding activity (1). Since RCEL preferentially inhibits protein synthesis in tumor cells, it is suggested that RCEL binds cell surface receptors, enters cell cytosol through receptor-mediated endocytosis, then degrades RNA, and finally kills the cell. RCEL shows 28% and 53% sequence identities respectively to pancreatic ribonuclease A (RNase A) and a cytotoxic ribonuclease (onconase) from frog (Rana pipiens) eggs (2). The amino acid residues, His12, Lys41 and His119 (RNase A numbering), essential for RNA-degrading activity are structurally conserved among RNase A's and onconase. The disposition of the receptor binding sites of RCEL, however, has remained uncertain because the structures of the homologous proteins, human angiogenin and onconase, were revealed as non-liganded forms. Using a hanging-drop vapor diffusion method, crystals of RCEL grew in a few weeks at 20 °C under the condition [0.1 M R-AXIS IIc area detector. The crystal structure of RCEL was solved by molecular replacement techniques using the known structure of onconase as a search model. The current refined model of RCEL revealed, as expected, the catalytic active site similar to those found in the other members of the superfamily and the binding sites for two sialic acid molecules suggesting putative receptor binding sites.

The crystal structure of an acidic scorpion neurotoxin BmK M8 has been determined by using molecular replacement method. It is the first acidic α-neurotoxin neurotoxin reported so far. Compared with the basic and most potent α-toxin, AaH II, the BmK M8 is a relatively inactive toxin (1100 times less active than AaH II) with an unusually low isoelectric point (5.3). The much drop in toxin lethality is accompanying the acidic property of the molecule. The refined structure showed new functionally important area and some features of the toxin-receptor binding. In association with the information from antibody mapping for localization of binding site on sodium channel, a multiposition mode for the toxin-receptor interaction and three "toxic regions" related to the binding process, including Face A, Face B and Site C, are proposed. The Face A, mainly consisted of Tyr5, 35, 47, α-amino group, Arg2 and Asp3, may be more essential for the binding; the Face B, mainly comprised conserved residues Tyr14, 21, Lys28 and Val55, may contribute to the high efficacy of the binding process and substitutions of acidic residues in this area will strongly weaken the toxic activity; and the Site C formed by Lys58 and Arg62 from C-terminal and Arg41 and Tyr62 from loop 38-44 may be involved in the binding sitespecificity. On basis of these, a toxin-sodium channel model will be proposed.

**References**


**PS04.18.17** IS ANNEXIN III AN ENZYME? Anita Lewiti-Bentley*, Béatrice Perron§ & Françoise Russo-Marié§* LURE, Univ. Paris-Sud, 91405 Orsay, §§ICGM, 22 rue Méchain, 75014 Paris, France

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