11. Kesavan, P. C., Sharma, G. J. and Afzal, S. M. J., *Radiat. Res*, 1978, 75, 18–30
12. Conger, B V., Nilan, R. A. and Konzak, C. F., *Radiat. Bot.*, 1968, 8, 31–36
13. Bradford, M. M, *Anal. Biochem.*, 1976, 72, 248–254.
14. Scandalios, J. G., *Biochem. Genet*, 1969, 3, 37–79.
15. Hochanadel, C. J, *J. Phys. Chem.*, 1952, 56, 587–594.
16. Laemmli, U. K., *Nature*, 1970, 227, 680–685.
17. Powers, E. L. and Cross, M., *Int J. Radiat. Biol.*, 1970, 17, 501–514.
18 Ewing, D., Fielden, E. M and Roberts, P. B., *Radiat. Res.*, 1974, 58, 481–488.
19. Kesavan, P. C. and Powers, E. L., in Proceedings of the 8th International Congress of Radiation Research, Edinburgh (eds Fielden, E. M., Fowler, J. F., Hendry, J. H. and Scott, D) *Radiat Res.* 1, 1987, C30-4P.
20 Brustad, T. and Wold, E., *Radiat. Res.*, 1976, 66, 215–230.
21. Samuni, A. and Czapski, G., *Radiat. Res*, 1978, 76, 624–632.
22. Watanabe, H., Iizuka, H. and Takehisa, M., *Radiat. Res.*, 1982, 89, 325–333.
23. Roots, A., Chatterjee, A., Blakely, E., Change, P., Smith, K. and Tobias, C., *Radiat. Res.*, 1982, 71, 678–682.
24. Keene, J. P., *Radiat. Res*, 1964, 22, 1–13.
25. Antoku, S., *Radiat. Res.*, 1977, 71, 678–682.
26. Ewing, D., Koval T. M. and Walton, H L., *Radiat. Res.*, 1986, 106, 356–365.
27. Simone, G. and Quintiliani, M., *Int. J Radiat. Biol.*, 1977, 31, 1–10.
28. Tibly, M. J., Loverock, P. S. and Fielden, E. M, *Radiat. Res.*, 1982, 89, 488–502.
29. Singh, S. P. and Kesavan, P. C., *Curr. Sci.*, 1992, 62, 486–488
30. Ogawa, M. and Uritani, J., *Radiat. Res.*, 1970, 41, 342–351.
31. Ropp, M., Courgeon, A. M, Clavayrae, R. and Best Belpomme, M., *Can. J. Biochem. Cell. Biol.*, 1983, 61, 456–461.

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Tertiary structural categories of leiurotoxin and some other scorpion venom toxins

P. Narayanan
Department of Life Sciences, University of Bombay, Bombay 400 098, India

Similarly-placed cysteine residues (and in-register disulphide bridges) in CnII-11 (C. noxius), noxiustoxin, charybdotoxin and leiurotoxin I, and amino acid sequence homology among these scorpion venom toxins enable classification of these molecules under CnII-11 type tertiary structural category under scorpion-toxin type proteins. Similar structural motif is also found in bee venom toxin apamin and apamin-type peptides. Neurotoxin P2 (Amm P2) should be classified under the I5A type tertiary structure.

THE unique folding of macromolecules is due to the cooperative process of packing interactions of side chains, motifs, modules and domains. Therefore, prediction of tertiary structures of proteins from their amino acid sequence data is an extremely complex and challenging task. Prediction of secondary structural elements (helix, sheet and turns) of proteins from their primary structural data is one of the strategies towards the goal of predicting their tertiary structures (vide literature). But these procedures have inherent limitations because structural topologies (motifs, modules, domains, etc.), and not amino acid sequence homologies, are better conserved in folding (evolution). Even in cases where no obvious amino acid sequence homologies may be found, the unknown structures can be modelled based on structural motifs and topologies. To emphasize, the essence of structure prediction is one of pattern matchings and, therefore, the logical way of addressing this complex problem is to identify proteins by motifs, modules and shapes and align the amino acid sequences to fit the topologies. Prediction of tertiary structure (protein folding) from the primary structure data can be attempted on certain classes of proteins with better success. One of the examples is immunoglobulins. On the basis of comparative studies of known antibody structures and application of energy constraints and distance-geometry methods, the repertoire of conformations of the antibody-combining site for a given amino acid sequence of the hypervariable loop in immunoglobulins could be modelled[1,2].

Another class of proteins/peptides where the natural constraints, imposed by disulphide bridges, would simplify the protein folding problem is that of disulphide-containing proteins/peptides. In addition to empirical rules governing the packing interactions that occur between and among secondary structural elements to form motifs and modules, the incorporation of the structural role and hierarchies of disulphide bonds, where S-S bonds have predominant influence on the folding processes (to which belong the bee, scorpion, several of snake and sea snail venom toxins, hormones, growth factors, insect defensins, etc.), would improve the rate of success of structure prediction methods in the disulphide-containing proteins/peptides. The empirical 'knowledge' one could discern in the case of disulphide-containing molecules is: (i) S-S bridges are found as integral parts of structural motifs, creating hydrophobic moieties (hydrophobic effect) and (ii) there exists structural hierarchy of S-S bridges in stabilizing the structural moieties and tertiary folding. Emphasizing this empirical 'knowledge' and based on the structural motif that exists in the scorpion venom toxin, CsEV3[3], all the 'scorpion-toxin' type proteins can be classified under five tertiary structural categories from their amino acid sequence data[4]. Energy constraints, minimum accessibility of hydrophobic moieties to solvent and other procedures can be employed to model individual tertiary structures from these 'canonical' tertiary structural categories.

Table 1. Amino acid sequence comparison. CnII-11 type: (S-S bonds: C25-C46; C29-C48 & C16-C41. Numbering according to CsEV3 structure)

Toxin	Amino acid sequence	Refs
	12 16 25 29 41 4648 65	
CsEV3	KEGYLVKKSDGCKYGCLKLGENEGCDTECKAKNQGGSYGYCYAFACWCEGLPESTPTYPLPNKSC	7
CnII-11	TIINVKC---TSPKQCSKPCKELYGSSAGAKCMNGKCKCBN	8
Noxiustoxin	TIINVKC---TSPKQCSKPCKELYGSSAGAKCMNGKCKCYNN	9
Charybdotoxin	EFTNVSC---TTSKECWSVCQRLHNTSRG-KCMNKKCRCYS	10
Leiurotoxin I	AFC----NLRMCQLSCRSLGLLG---KCIGDKCECVKH	4
	5 11 15 1 3	
Apamin*	APETALCARRCQQH -CNCK	11
MCD Peptide(401)*	RHVIKPHICRKICGKN KCNCK	12

Single alphabet nomenclature of amino acids is followed.
*Structural motif is similar to the scorpion toxins, but sense of backbone direction is different (Numbering of apamin is also indicated).

An inhibitor of apamin binding from the scorpion venom, *Leiurus quinquestriatus hebraeus* (leiurotoxin I) has been compared for sequence homology with other scorpion venom toxins, namely, noxiustoxin, charybdotoxin and neurotoxin P2[5]. Noxiustoxin has a high sequence homology to scorpion venom toxin CnII-11 (C. noxius), but charybdotoxin and leiurotoxin I have only partial sequence homology to CnII-11 (Table 1). All the same, considering the similarly-placed arrangement of cysteine residues and partial amino acid sequence homology among these venom toxins to CnII-11 peptide and the existence of the structural motif that has been found in the scorpion venom toxin, CsEV3[3] in these peptides also, these peptides can be classified under the 'CnII-11' type tertiary structural category according to the hypothesis (Figure 1 *a*)[4].

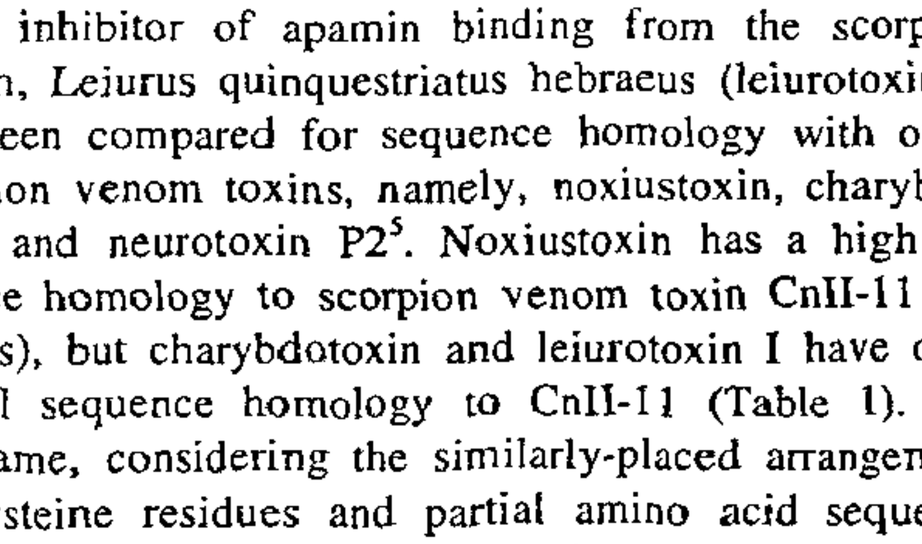

Figure 1 *a*. CnII-11 type structure. Numbering is according to CsEV3 structure

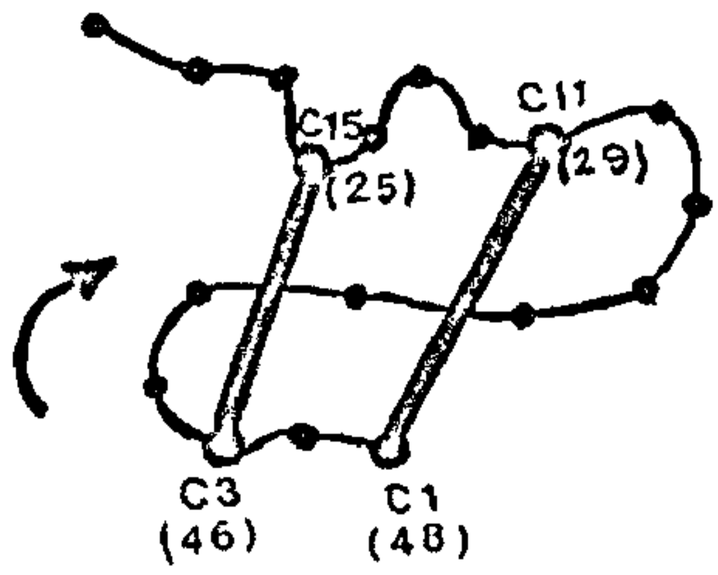

Figure 1 *b*. Apamin fold structure. Numbering corresponding to apamin structure is indicated. The corresponding numbers in CsEV3 structure are given in parentheses.

Chicchi *et al.*[5] have referred to the lack of structural homology between the bee venom toxin apamin and inhibitor of apamin binding, leiurotoxin I. This is questionable as the structural evidence available does not agree with their inference. Structural motif (apamin-fold), similar to the one found in CnII-11 structure is also found in apamin[3] and mast cell degranulating peptide (MCD–401)[4] (Figure 1 *b*). That there is a lack of amino acid sequence homology between apamin and inhibitor of apamin binding, leiurotoxin I, is true, but this should not necessarily imply lack of structural homology. Even in cases where no obvious amino acid sequence homologies may be found, the structures can have similar structural motifs and moieties (vide literature).

Also, inclusion of the neurotoxin P2 (Amm P2)[6] among leiurotoxin I, charybdotoxin and noxiustoxin[5] is not correct. Alignment of the relevant cysteine residues in register keeping in view the structural motif that exists in CsEV3 structure and also in the neurotoxin P2 structure, the structure should actually be classified under 'I5A' type tertiary structural category[4]. Classification of these disulphide-containing proteins/peptides under correct structural categories is relevant and important, because the predicted structures can be used as initial models to interpret the three-dimensional structure data by X-ray diffraction and NMR techniques.

1. Martin, A. C. R., Cheetham, J. C. and Rees, A. R., *Proc. Natl. Acad. Sci. USA*, 1989, 86, 9268–9272.
2. Chothia, C., Lesk, A. M., Tramontano, A., Levitt, M., Smith-Gill, S. J., Air, G., Shenff, S., Padlan, E. A., Davis, D., Tulip, W. R., Colman, P. M., Spinelli, S., Alzari, P. M. and Poljak, R. J., *Nature*, 1989, 342, 877–883.
3. Zell, A., Ealick, S. E. and Bugg, C. E., in *Molecular Architecture of Proteins and Enzymes* (eds Bradshaw, R. A. and Tang, J.), Academic Press, New York, 1985, pp. 65–97.
4. Narayanan, P. and Lala, K., *Life Sci.*, 1992, 50, 683–693.
5. Chicchi, G. G., Gimenez-Gallego, G., Ber, E., Garcia, M. L., Winquist, R. and Cascieri, M. A., *J. Biol. Chem.*, 1988, 263, 10192–10197.
6. Rochat, H., Bernard, P. and Couraud, F., in *Advances in Cytopharmacology* (eds Ceccarelli and Clement, F.), Raven Press, New York, 1979, pp. 325–334.
7. Fontecilla-Camps, J. C., Almassy, R. J., Suddath, F. L., Watt, D. D. and Bugg, C. L., *Proc. Natl. Acad. Sci. USA*, 1980, 77, 6496–6500.
8. Possani, L. D., Martin, B. M. and Svedsen, I., *Carlsberg Res. Commun.*, 1982, 47, 285–289.
9. Carbone, E., Prestipino, G., Spadavecchia, L., Franciolini, F. and Possani, L. D., *Pfluegers Arch. Eur. J. Physiol.*, 1987, 408, 423–431.
10. Gimenez-Gallego, G., Navia, M. A., Reuben, J. P., Katz, G. M., Kaczorowski, G. J. and Garcia, M. L., *Proc. Natl. Acad. Sci. USA*, 1988, 85, 3329–3333.
11. Callewaert, G. L., Shipolini, R. and Fernon, C. A., *FEBS Lett.*, 1968, 1, 111.
12. Argiola, A., Herring, P. and Pisano, J. J., *Peptides*, Suppl. 3, 1985, 6, 431–436.

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pH in the digestive system of some gastropod molluscs

Kumud Misra and Minakshi Shrivastava

Department of Zoology, University of Lucknow, Lucknow 226 007, India

Here we show that the gut and digestive gland of *Pila globosa*, *Bellamya bengalensis* and *Achatina fulica* is slightly acidic.

ENORMOUS work has been done on the digestive physiology of gastropod molluscs. Optimum conditions for the activity of a number of digestive enzymes have been determined[1,2] but there is no information regarding pH milleu in the digestive tract itself. Since efficiency of digestion depends upon pH, efforts were made to measure the pH of different parts of the gut of some gastropods *Pila globosa*, *Bellamya bengalensis* (Prosobranchia) and *Achatina fulica* (Pulmonata).

Freshly collected animals were refrigerated for 30 min at 5°C for immobilization. The entire alimentary canal along with the digestive glands was dissected out and kept on a clean slide. Digestive glands were separated and teased. Different parts of the gut were cut and slit open and the pH was determined using pH paper[3]. One snail of each species was dissected for one experiment and each experiment repeated six times.

Results are summarized in Table 1. Careful analysis of the data indicates that buccal mass is very slightly acidic (pH ranges 6.0 to 6.5) in *Pila* but almost neutral (pH 6.5 to 7.0) in *Bellamya* and *Achatina*. Oesophagus is slightly alkaline (pH 7.0 to 7.5) in *Bellamya* but acidic (pH 6.0 to 6.5) in *Pila* and *Achatina*. Stomach is the most acidic part in all the three species. In *Pila* and *Achatina*, pH is almost similar to digestive gland (5.5 to 6.0). In *Bellamya*, pH of stomach is higher (6.0 to 6.5) compared to digestive glands (5.5 to 6.0). Intestine is slightly acidic in *Pila* (pH 6.0 to 6.5) but almost neutral in *Bellamya* and *Achatina* (pH 6.5 to 7.0). The pH of hind gut is identical (6.0 to 6.5) and of digestive

Table 1. pH in the digestive system of some gastropod molluscs

Gastropod	pH range					
	Buccalmass	Oesophagus	Stomach	Intestine	Hind gut	Digestive gland
Pila globosa	6.0–6.5	6.0–6.5	5.5–5.8	6.0–6.5	6.0–6.5	5.8–6.0
Bellamya bengalensis	6.7–7.0	7.0–7.5	6.0–6.5	6.5–7.0	6.0–6.5	5.5–6.0
Achatina fulica	6.5–7.0	6.0–6.5	5.5–6.0	6.5–7.0	6.0–6.5	5.5–6.0