The conformation of apamin

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Energy minimisation techniques are used as a tool to distinguish between different proposed models for the structure of the bee venom polypeptide apamin. The influence of electrostatic interactions on the resultant energies is noted. The model of Hider and Ragnarsson [(1980) FEBS Lett. 111, 189–193] is found to be of consistently low energy.

Apamin *Secondary structure* *Energy minimization*

1. INTRODUCTION

Apamin is an 18 residue polypeptide component of bee venom [1]. It possesses powerful neurotoxic properties derived from its ability to block calcium-dependent potassium fluxes [2]. There is, therefore, considerable interest in the three dimensional structure of this polypeptide as a potential model for the study of potassium channels. Attempts to crystallise apamin have so far proved unsuccessful and there is consequently no X-ray determination of its structure. However, a number of studies of the structure of apamin have been attempted using spectroscopic techniques in particular NMR and CD studies [3–6]. Here we report the application of energy minimisation methods to this problem. Our results indicate the considerable potential of this technique in the study of conformational properties of polypeptides when it is used in conjunction with experimental techniques.

Apamin is remarkably unaffected by its environment, being resistant to extremes of pH and dielectric constant [6]. Also synthetic apamin folds to exhibit native activity and CD spectra [7], implying that the information for the tertiary structure is contained in the primary sequence. Moreover, the insensitivity to environment suggests that the effects of solvation on the conformation of the molecule may be relatively small. The combination of these facts has encouraged predictions of the structure of apamin based on its primary sequence. Previous attempts at such predictions have been based on the observed statistical preferences of amino acids [5] and 'ab initio' energy minimisation calculations [8,9]. A summary of proposed apamin models and their methods of derivation is given in table 1.

2. METHOD

The energy minimisation study reported here differs from that of many previous studies using the technique. Rather than search for the global minimum of a given energy function, a very lengthy task even for a molecule the size of apamin, the different available models of apamin have been subjected to energy minimisation. The resultant minimum energy structures can then be compared. We consider that this approach is appropriate since, although energy minimisation studies have proved extremely successful in fields as widely different as the study of small organic molecules [13] and extended ionic lattices [14], the application of the method to polypeptides and proteins is hindered by the inherent flexibility of the polypeptide backbone. This flexibility produces many

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Table 1

Summary of proposed apamin structures

Model	Method	Summary	Refs
1	CD and secondary structure prediction	reverse turns 1–4, 5–8; α-helix 9–17	5
2	Ab initio energy minimisation	planar structure	9
3	Conventional NMR	reverse turn 2–5; α-helix 6–16	3,4
4	2D NMR	reverse turns 3–5, 6; α-helix 9–18	10
5	Sequence homology and energy minimisation		11
6	Model building	reverse turns 1–4, 5–7; α-helix 9–17	12

possible conformations and makes the object of finding the global energy minimum extremely difficult to achieve. By concentrating on the structures proposed by both spectroscopic and theoretical considerations energy minimisation can be used as a comparative rather than an ab initio predictive tool.

Starting structures for energy minimisation were obtained from the published backbone dihedral angles for models 2 and 3. The backbone dihedral angles of the Zell et al./Kallenbach and Wemmer model (model 4,5) were kindly supplied by C.E. Bugg. Dihedral angles for the Hider and Ragnarsson (model 1) and the later Hider proposal (model 6) were taken from Nicholson models. For all the models, except that of Popov and Melnikov (model 2), side chain conformations were chosen to match those commonly found in proteins [15]. The dihedral angles of the proposed apamin models, presented in table 2, were used to prepare cartesian coordinates for each model using the standard residue geometries of the ECEPP Momany et al. program [16].

To obtain starting structures for the side chains, we used the observed conformation preferences of amino acids in proteins rather than attempting to search all possible side chain conformations. In doing this we are taking cognisance of the fact that apamin is a small peptide and is unlikely to have buried side chains. This means that the side chains will be largely exposed to the solvent environment and will therefore have access to a variety of possible conformations. The implication of this is not that different side chain conformations will not affect the energy of proposed model structures, but that this effect will be small in comparison with the overriding conformational constraint of the two disulphide bridges. As already noted the conformation of apamin is largely unaffected by the type of solvent implying that the conformation of the molecule is dominated by intramolecular rather than intermolecular interactions.

The calculations on apamin employed a standard cartesian coordinate energy code based on non-bonded, bond angle, bond length and torsional angle contributions to a potential energy function. The potential energy parameters used were those of Weiner et al. [17]. The non-bonded energy is calculated as the sum of electrostatic and short range contributions. For the calculation of electrostatic interactions we used the common procedure of taking the dielectric constant, D, as a linear function of interatomic separation, r, in an attempt to account for the effect of solvent and atomic polarisability (see [17]). As discussed below, two sets of calculations were performed with the constant of proportionality set to 1.0 and 2.0. Energy minimisation was achieved using the conjugate gradient method of Powell [18].

3. RESULTS AND DISCUSSION

The energies obtained for the different models are presented in table 3 for the dielectric constants $1.0r$ and $2.0r$. Dihedral angles for the different models calculated with $D = 1.0r$ are presented in table 4. It can be seen from table 4 that large changes in specific dihedral angles have occurred on energy minimisation. These changes are, however, quite local and the overall conformation of each model is maintained. The results in table 3 suggest that the structure proposed by Hider and Ragnarsson (model 1) has the greatest stability (for $D = 1.0r$); a schematic representation of the structure after energy minimisation is presented in fig.1. One of the most interesting features of the calculation is the effect of the dielectric constant on the

Table 2
Dihedral angles of proposed apamin models in degrees, before energy minimisation

	ϕ	ψ	ω	χ_1	χ_2	χ_3	χ_4	χ_5
Model 1								
Cys	60.0	65.0	180.0	90.0				
Asn	15.0	−120.0	180.0	−180.0	60.0			
Cys	−100.1	80.0	−180.0	0.0				
Lys	130.0	80.0	180.0	−180.0	180.0	180.0	180.0	180.0
Ala	−145.0	−130.0	−180.0					
Pro	−75.0	−40.0	180.0					
Glu	−70.0	−20.0	180.0	−60.0	60.0	60.0		
Thr	65.0	−90.0	−180.0	−60.0	0.0			
Ala	−60.0	−60.0	−180.0					
Leu	−60.0	−60.0	180.0	−60.0	180.0			
Cys	−60.0	−60.0	180.0	−90.0				
Ala	−60.0	−60.0	180.0					
Arg	−60.0	−60.0	180.0	180.0	180.0	180.0	60.0	
Arg	−60.1	−60.0	−180.0	−60.0	180.0	180.0	180.0	
Cys	−60.0	−59.9	−180.0	−90.0				
Gln	−60.1	−60.0	−180.0	180.0	60.0	−60.0	0.0	
Gln	−60.0	−60.0	180.0	−60.0	60.0	60.0	0.0	
His	−170.0	80.0	−180.0	−60.0	−90.0			
Model 2								
Cys	60.0	−46.9	−180.0	174.4				
Asn	−100.0	140.0	−180.0	−170.0	−42.0			
Cys	−57.0	−45.0	180.0	−74.1				
Lys	−66.0	−36.0	−180.0	−179.0	178.0	180.0	180.0	180.0
Ala	−138.1	91.0	−180.0					
Pro	−75.0	−36.0	180.0					
Glu	−81.0	62.0	180.0	−67.0	66.0	44.0		
Thr	−128.9	100.0	−180.0	−61.0	63.0			
Ala	−66.0	−59.5	180.0					
Leu	−81.7	119.1	180.0	−60.0	146.0			
Cys	−155.5	152.7	180.0	40.6				
Ala	−65.1	−44.1	−180.0					
Arg	−159.9	143.3	180.0	165.1	179.9	160.0	−77.1	
Arg	−68.8	146.3	180.0	−61.1	176.0	177.3	172.8	
Cys	−58.3	−50.0	180.0	−145.3				
Gln	−116.6	141.2	−180.0	178.5	56.5	−99.1	0.0	
Gln	−93.2	−32.3	180.0	−72.3	62.0	79.0	0.1	
His	66.5	58.5	−180.0	−46.7	−87.3			
Model 3								
Cys	60.0	−135.0	−180.0	180.0				
Asn	−108.00	99.0	180.0	−180.0	−60.0			
Cys	−67.5	−27.0	180.0	−75.0				
Lys	−81.0	−9.0	−180.0	−180.0	180.0	180.0	180.0	180.0
Ala	−76.5	144.0	180.0					
Pro	−75.0	−54.0	180.0					
Glu	−76.5	−54.0	180.0	−60.0	60.0	60.0		
Thr	−90.0	−36.0	−180.0	−60.0	60.0			
Ala	−54.0	−54.0	−180.0					

(continued on p. 292)

Table 2 (continued)

	ϕ	ψ	ω	χ_1	χ_2	χ_3	χ_4	χ_5
Leu	−66.9	−40.5	−180.0	−60.0	180.0			
Cys	−72.0	−54.0	−180.0	60.0				
Ala	−54.1	−44.9	180.0					
Arg	−63.0	−27.0	−180.0	180.0	180.0	180.0	−60.0	
Arg	−58.5	−27.0	−180.0	−60.0	180.0	180.0	180.0	
Cys	−67.5	−27.0	180.0	−120.0				
Gln	−76.5	−9.0	−180.0	180.0	60.0	−60.0	0.0	
Gln	−76.5	−54.0	−180.0	−60.0	60.0	60.0	0.0	
His	66.4	58.5	180.0	60.0	−60.0			
Model 4,5								
Cys	60.0	151.1	−180.0	180.0				
Asn	−144.6	169.6	180.0	−180.0	60.0			
Cys	−52.3	−40.0	180.0	−60.0				
Lys	−65.6	−5.0	180.0	−180.0	−180.0	180.0	−180.0	−180.0
Ala	−32.3	101.2	180.0					
Pro	−75.0	−124.9	−180.0					
Glu	−13.9	−68.0	−180.0	−60.0	60.0	60.0		
Thr	−73.6	83.6	180.0	−60.0	0.0			
Ala	−56.9	−23.8	−180.0					
Leu	−36.5	−55.0	−180.0	−60.0	180.0			
Cys	−83.5	−2.7	−180.0	−180.0				
Ala	−81.4	−46.2	−180.0					
Arg	−59.1	−43.9	180.0	−180.0	180.0	180.0	−60.0	
Arg	−55.9	−50.1	−180.0	−60.0	180.0	−180.0	180.0	
Cys	−66.6	−35.8	−180.0	180.0				
Gln	−68.2	−23.9	180.0	−180.0	60.0	120.0	0.0	
Gln	−79.9	164.9	−180.0	−60.0	60.0	60.0	0.0	
His	−104.8	134.5	180.0	−60.0	−60.0			
Model 6								
Cys	60.0	85.0	180.0	120.0				
Asn	−65.0	−50.0	180.0	−180.0	−60.0			
Cys	−60.0	−25.0	180.0	10.0				
Lys	−170.0	−60.0	180.0	−180.0	180.0	180.0	180.0	−180.0
Ala	−170.0	−69.9	180.0					
Pro	−75.0	80.0	180.0					
Glu	−70.0	59.9	−180.0	−60.0	60.0	60.0		
Thr	−130.0	−170.0	180.0	−60.0	0.0			
Ala	−59.9	−60.0	−180.0					
Leu	−60.0	−60.0	180.0	−60.0	180.0			
Cys	−60.0	−60.0	−180.0	120.0				
Ala	−60.1	−60.0	−180.0					
Arg	−60.0	−60.0	−180.0	180.0	180.0	180.0	−60.0	
Arg	−60.0	−60.0	−180.0	−60.0	180.0	180.0	180.0	
Cys	−60.0	−60.0	180.0	10.0				
Gln	−60.0	−60.0	−180.0	180.0	60.0	−60.0	0.0	
Gln	−60.0	−59.9	180.0	−60.0	60.0	60.0	0.0	
His	−170.0	80.0	−180.0	−60.0	−60.0			

The listed dihedral angles conform to the IUPAC IUB convention (1970)

Table 4
Dihedral angles of proposed apamin models in degrees, after energy minimisation

	ϕ	ψ	ω	χ_1	χ_2	χ_3	χ_4	χ_5
Model 1								
Cys	133.5	−177.6	168.9	28.0				
Asn	−116.8	−61.2	178.6	−174.0	76.1			
Cys	−88.5	−36.9	178.7	−59.3				
Lys	172.5	144.8	179.1	−163.5	176.1	−161.6	167.8	−173.9
Ala	161.5	−105.3	−172.8					
Pro	−70.6	−75.6	−168.6					
Glu	−42.9	−57.9	−179.1	−65.9	81.4	102.4		
Thr	73.2	−57.0	−179.1	−57.2	64.5			
Ala	−62.9	−18.5	175.4					
Leu	−98.8	−49.1	−169.1	−59.8	177.8			
Cys	−67.6	−29.9	173.3	−47.5				
Ala	−62.0	−13.3	177.4					
Arg	−125.9	26.2	168.6	−179.1	178.2	55.9	−87.2	
Arg	−131.1	−90.0	−177.6	−45.6	−166.4	−142.6	108.7	
Cys	−61.7	−30.9	−179.8	77.6				
Gln	−72.7	−0.5	163.9	−55.9	−54.9	−85.0	4.6	
Gln	−111.3	−51.2	−163.8	−79.8	74.5	92.6	6.2	
His	58.7	41.8	179.9	−86.9	96.1			
Model 2								
Cys	36.5	38.4	160.2	−69.6				
Asn	−79.8	158.2	−178.7	166.8	−110.7			
Cys	−54.0	−34.8	179.3	−63.7				
Lys	−116.9	24.9	175.2	−170.8	−174.8	177.6	−178.2	179.9
Ala	−165.0	70.4	−174.2					
Pro	−86.1	22.2	−175.4					
Glu	−81.8	66.5	172.1	−59.9	67.3	27.2		
Thr	−144.6	168.8	−173.0	−43.6	−24.7			
Ala	−147.0	28.4	171.2					
Leu	−121.5	22.0	−156.1	−61.7	174.2			
Cys	−93.5	71.1	173.2	−45.3				
Ala	−53.2	−32.3	160.5					
Arg	−134.5	160.7	174.2	−176.2	−175.2	175.9	−90.8	
Arg	−124.4	167.5	−178.1	−63.9	174.5	175.2	−116.9	
Cys	−24.1	−66.6	−177.0	165.4				
Gln	−152.9	145.9	178.9	179.8	50.2	−104.1	1.5	
Gln	−90.3	−30.2	175.2	−68.8	58.3	67.8	1.7	
His	70.6	37.1	−179.9	−47.0	−91.2			
Model 3								
Cys	112.4	−161.1	175.7	−160.8				
Asn	−64.9	111.1	−170.5	173.6	−88.2			
Cys	−81.5	42.1	178.4	−145.6				
Lys	−138.2	−61.2	−167.2	179.1	−176.1	178.5	−178.7	−180.0
Ala	−74.0	140.6	175.5					
Pro	−58.7	−32.1	179.9					
Glu	−62.1	−46.6	−179.6	−59.3	68.6	21.6		
Thr	−78.2	−21.0	168.6	28.0	−82.0			
Ala	−62.2	−39.7	173.6					

(continued on p. 294)

Table 4 (continued)

	ϕ	ψ	ω	χ_1	χ_2	χ_3	χ_4	χ_5
Leu	−62.4	−45.4	173.8	−57.9	173.4			
Cys	−53.1	−88.0	−168.4	170.0				
Ala	−163.4	29.3	180.0					
Arg	−80.2	64.1	174.4	−178.9	−147.4	167.8	−55.3	
Arg	−112.3	−30.1	155.0	−57.2	158.6	−163.8	119.2	
Cys	24.1	−103.2	−167.6	39.4				
Gln	−83.1	−17.0	173.3	−173.4	57.8	−110.9	1.0	
Gln	−70.1	−34.4	177.5	−64.7	70.1	−156.3	−3.9	
His	71.5	39.0	−179.8	−50.2	−43.4			
Model 4,5								
Cys	13.8	161.7	−179.6	−72.1				
Asn	−165.2	135.9	179.5	165.9	14.5			
Cys	−75.1	−51.2	177.5	−52.8				
Lys	−87.7	59.9	−169.4	−176.7	−175.8	178.1	−178.6	179.9
Ala	−97.4	103.2	164.4					
Pro	−70.2	−20.0	−173.4					
Glu	−102.3	−56.1	−163.6	−3.1	70.5	−24.8		
Thr	−89.7	55.0	−168.1	−32.4	71.1			
Ala	−56.0	−33.6	179.8					
Leu	−59.8	−43.3	−177.2	−60.9	175.4			
Cys	−79.0	−43.1	178.1	−52.4				
Ala	−54.6	−38.4	177.4					
Arg	−52.4	−37.0	−178.7	169.2	−81.8	171.1	−109.3	
Arg	−86.1	−53.8	−166.8	−38.1	−177.9	−149.8	100.0	
Cys	−71.2	−14.5	172.4	−146.7				
Gln	−59.0	−22.2	170.6	−129.4	54.6	−143.7	−2.6	
Gln	−72.8	78.1	167.9	−70.9	83.6	102.3	3.2	
His	−130.3	161.7	−179.7	−69.4	−8.1			
Model 6								
Cys	6.7	152.1	178.2	−66.2				
Asn	−80.5	−26.5	−179.7	136.1	−87.0			
Cys	−84.6	−46.5	−154.2	47.4				
Lys	−149.6	−79.8	−160.1	−177.3	−178.8	60.3	−108.6	−172.3
Ala	−95.6	−153.0	−168.8					
Pro	−119.2	72.3	178.8					
Glu	52.3	37.5	−175.7	−74.2	56.7	63.9		
Thr	−111.6	152.5	−179.1	−60.2	42.4			
Ala	−54.8	−26.1	175.8					
Leu	−50.0	−33.0	−175.4	−60.3	177.2			
Cys	−129.1	−54.4	−177.8	36.3				
Ala	−60.7	−21.1	177.8					
Arg	−116.1	25.7	175.7	−163.1	−90.6	164.6	−89.9	
Arg	−144.2	−90.4	−173.9	−58.3	−176.3	−175.1	139.5	
Cys	−68.8	−18.0	177.1	47.3				
Gln	−81.9	−19.0	160.0	−155.2	45.9	−140.5	−4.3	
Gln	−67.5	−50.5	179.3	−85.6	67.0	105.2	3.0	
His	88.7	168.7	−179.7	−55.3	86.7			

The listed dihedral angles conform to the IUPAC IUB convention (1970)

Table 3

Energies and order of minima for proposed apamin structures

Model	Ref.	Energy ($D=1.0r$)	Rank	Energy ($D=2.0r$)	Rank
1	5	−534.5	1	−286.44	2
2	9	−498.9	5	−290.5	1
3	3,4	−499.8	4	−258.6	4
4,5	10,11	−523.3	2	−284.3	3
6	12	−503.2	3	−252.9	5

Energies in kcal/mol. Minimisation was terminated when the rms value of the first derivative was less than 0.01 kcal/mol apamin

relative energies; the order of the minima is different for the different values of this quantity. The change in dielectric constant produces only minor changes in the conformations of the five models. The influence of the dielectric constant on the calculated relative energies is in contrast to the previously observed insensitivity of molecular mechanics calculations to electrostatic contributions for a small peptide [19]. This highlights the essential role played by the electrostatic component of the potential energy parameters in empirical potential energy calculations. However, the value of $2.0r$ for the dielectric constant almost certainly exaggerates the effects of solvent and peptide polarisability on the screening of Coulomb interactions; the use of $1.0r$ probably provides a reasonable if crude description of the electrostatic interactions in polypeptide systems. It is interesting to note that the apamin models 1 and 4 have a markedly similar overall chain fold. However, these structures differ in energy by 11 kcal/mol (for $D=1.0r$). This is an illustration of the complexity of the potential energy surface for this peptide.

In summary, the energy minimisation calculations presented here provide strong support for the structure proposed by Hider and Ragnarsson [5]. Our work shows the usefulness of energy minimisation calculations when used in conjunction with experimental techniques in the study of small polypeptides. Moreover our work highlights the important role that electrostatic contributions may have in influencing the preferred conformation. Having demonstrated the valuable role of energy

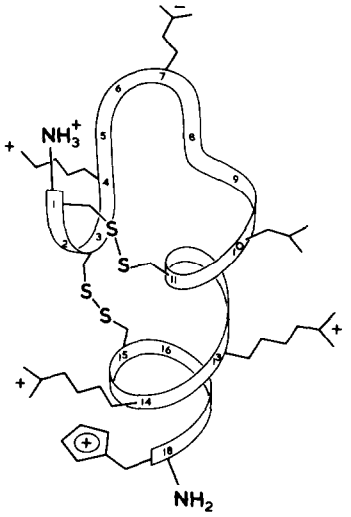

Fig. 1. A schematic representation of model 1.

minimisation techniques in examining the conformation of this peptide, future work will concentrate on the prediction of the way in which changes in the sequence of the polypeptide modify the conformation.

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