Evaluation of the *Bacillus thuringiensis* Cry4Ba Structure by Simulated Annealing

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ABSTRACT

The evaluation of the Cry4Ba toxin in aqueous solution was investigated using the molecular dynamics simulation method (MD). The purpose of this research is compare the quality of Cry4Ba structures obtained from homology modelling, sequences of in vacuo simulated annealing and snap-shot of MD in water.The original atomic coordinates were subsequently treated by 20 cycles of *in vacuo* simulated annealing simulations based on GROMOS96 force field. Each cycle consists of (i) heating phase from 0 to 500 K for 10 ps, (ii) constant-temperature dynamics at 500 K for 10 ps, (iii) cooling phase from 500 to 0 K for 10 ps, and (iv) steepest descent energy minimization. The snap-shot of MD sample from 20 configurations which minimized each structure by a steepest descent algorithm. Each resulting structure was evaluated by WHATCHECK[i] and PROCHECK[ii] programs.

INTRODUCTION

Cry4Ba is a toxin protein which has high specificity to Aedes aegypti mosquito-larvae and efficiency together with their environmental safety and lack of harmful side effect. The mechanism of Cry4Ba toxin are consisted of insertion, aggregation and pore formation (see Fig 1). It behaves as a biological pesticide.

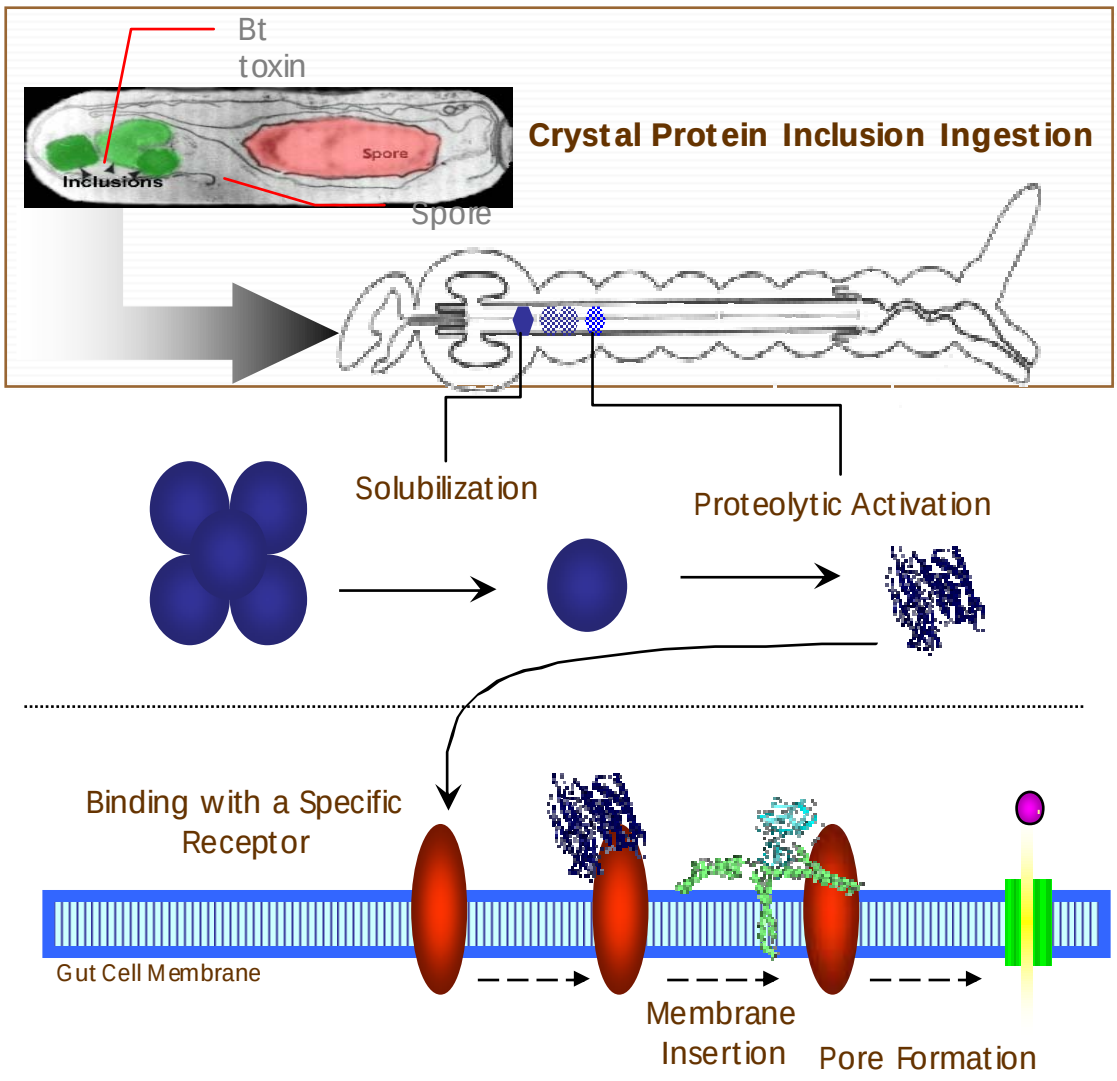

Fig 1. The mechanism of Cry4Ba protein.

In this research, we use the Simulated Annealing method to search for the optimal structures. In Simulated Annealing, control parameters correspond to the temperature. At high temperature, the system passes over high energy barriers and travel through high energy region of conformational space. As the temperature falls, the system accord to the Boltzmann distribution so the lower energy states become more probable. At absolute zero, the system should occupy at the lowest energy state. In this research, we use simulated annealing method to refine the optimal structure of Cry4Ba.

Methodology

The simulated annealing method was using to refine the Cry4Ba homology model structure. In simulated annealing, control parameters correspond to the temperature. The simulated annealing consists of three stages: First, heating up the system from 0 to 500 K. At high temperature, the system passes over high energy barriers and travel through high energy region of conformational space. After that, constant temperature of the system at 500 K (10 ps). Finally, cooling phase from 500 K to 0 K (10 ps), as the temperature falls, the system accord to the Boltzmann distribution so the lower energy states become more probable. After the simulated annealing protocol, we minimized the structure by a steepest descent algorithm with 100 kJ mol^{-1} nm^{-1} of maximum force to converge and evaluated the structure by using WHATCHECK and PROCHECK software. We refined the structure of Cry4Ba in water by sample 20 configurations and minimized each configuration by the a steepest descent algorithm. This method is use to verify the quality of Cry4Ba homology model in water compared with simulated annealing method.

Results ,Discussion and Conclusion

Table1. Quality indices for simulated annealing and water refined.

Performed check	Unrefined	Simulate Annealing Refined	Water Refined
WHATCHECK Z scores			
2^{nd}-generation packing quality (NQUACHK)	-1.612	-1.345 ± 0.100	-2.067 ± 0.123
Ramachandran plot appearance (RAMCHK)	-5.032	-4.733 ± 0.193	-4.180 ± 0.119
χ_1 - χ_2 rotamer normality (C12CHK)	-3.019	-3.227 ± 0.186	-3.002 ± 0.207
Backbone conformation (BBCHK)	-13.724	-12.245 ± 0.691	-13.218 ± 0.623
WHATCHECK RMS Z scores			
Bond lengths (BNDCHK)	0.718	0.709 ± 0.004	0.708 ± 0.003

Table 1. (continue)

Bond angles (ANGCHK)	1.184	1.168 ± 0.009	1.127 ± 0.035
Omega angles (OMECHK)	1.348	1.328 ± 0.039	1.098 ± 0.010
Side-chain planarity (PLNCHK)	1.941	1.982 ± 0.070	1.801 ± 0.109
Improper dihedral distribution (HNDCHK)	1.963	1.969 ± 0.042	1.771 ± 0.033
Inside/outside distribution (INOCHK)	1.137	1.171 ± 0.008	1.129 ± 0.006
PROCHECK			
Most favoured	62.4	63.920 ± 1.248	69.585 ± 1.576
Allowed	30.7	29.670 ± 1.440	25.110 ± 1.692
Generously allowed	3.5	2.630 ± 0.538	2.700 ± 0.399
Disallowed	3.3	3.785 ± 0.339	2.600 ± 0.399

WHATCHECK program was used to evaluate the protein structure. Quality of the obtained model can be judged based on the Z scores of the following parameters: the Ramachandran map (RAMCHK), the packing quality (NQACHK), the χ_1-χ_2 correlation (C12CHK) and the backbone conformation (BBCCHK). The RMSZ are determined for the bond lengths (BNDCHK), bond angles (ANGCHK), omega angles (OMECHK), side-chain planarity (PLNCHK), improper dihedral angles (HNDCHK) and the inside/outside distribution (INOCK). We also analyze the ϕ-ψ values in the most favoured, additional allowed, generously allowed and disallowed regions of the Ramachandran plot as determined with PROCHECK.

It is common practice to regard Z scores close to 0 as good and Z scores larger than 4 or smaller than -4 as outlier. For example, Ramachandran Z scores have a value -0.2 and 0.8 mean that both structures exhibit a Ramachandran plot within one standard deviation of the average in the database. The value of 0.8 is more regular than the one belonging to the value of -0.2. The value -0.2 would be closer to the database-derived average value and it should be better than that of 0.8. However, we considered a

structure as having better validation results if the value gets more positive. Ideal RMS Z scores are close to 1. RMS Z scores smaller than 1.0 mean that the distribution is tighter than expected from high-resolution X-ray crystal structure. On the other hand, for RMS Z scores larger than 1.0 indicate a broader distribution of values than in the database (28).

Evaluation scores for the Cry4Ba model structure refined by gas-phase simulated annealing and simulations in explicit water were shown in Table 1. For Cry4Ba structure refined by simulated annealing, the 2^{nd} generation packing quality (QUACHK) and backbone conformation (BBCCHK) get better whereas the χ_1-χ_2 rotamer normality gets worse when comparing to the unrefined one. In addition, the bond angles and omega angles values decrease (closer to one). In contrast, bond lengths, side-chain planarity, improper dihedral distribution and inside/outside distribution increase. Increment of most favoured regions obtained by PROCHECK analysis indicates that the refined structure of Cry4Ba is averagely better.

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