



ELSEVIER

Journal of Crystal Growth 231 (2001) 273–279

JOURNAL OF **CRYSTAL
GROWTH**

www.elsevier.com/locate/jcrysgro

Optimization of soluble protein crystallization with detergents

Rong-Jin Guan, Miao Wang, Xing-Qi Liu, Da-Cheng Wang*

*Institute of Biophysics and National Laboratory of Microgravity, Chinese Academy of Sciences, Chaoyang District,
Beijing 100101, People's Republic of China*

Accepted 22 March 2001

Communicated by N.E. Chayen

Abstract

Despite the expanding literature describing techniques useful for growing crystals of proteins, successful crystallization remains a largely empirical, operator-dependent science. Detergents, such as β -OG, have been found to be useful additives in the crystallization of both membrane and soluble proteins. We describe the use of detergents to optimize crystallization of four soluble proteins, which resulted in improved crystal quality and reproducibility of results, including promoting the growth of single crystals as opposed to polycrystals and cluster crystals, enhancing the crystal's X-ray diffraction characteristics, and improving the reproducibility of crystal growth. © 2001 Elsevier Science B.V. All rights reserved.

PACS: 81.10; 81.10.a

Keywords: A1. Biocrystallization; A2. Single crystal growth; B1. Biological macromolecules; B1. Detergents; B1. Proteins

1. Introduction

Over 10 000 soluble proteins have been crystallized to obtain their 3-D structures by X-ray crystallography. As a result, crystallization of proteins has developed from a trial-and-error approach to a more systematic technique [1], but preparation of diffraction-quality crystals remains a stumbling block in structure determination [2]. The Hampton Research kits provide a battery of test conditions facilitating the search for initial

conditions of crystal growth [3]. However, finding precise optimal conditions for maximal yield of high quality crystals suitable for X-ray structure analysis is still a challenge. Common problems include multiple or twin crystals, poorly diffracting crystals, and non-reproducibility of crystallization. Techniques used to overcome these problems include controlling the nucleation stage [4–7], growing crystals in microgravity [8,9] and growing crystals in gels [10,11], etc..

Growing crystals in the presence of detergents has also been found to be a useful technique. The use of non-ionic and zwitterionic detergents in the crystallization of membrane proteins is well established [12–15] and has become routine practice. Detergents have also proved useful in

*Corresponding author. Tel.: +86-10-6488547; fax: +86-10-64871293.

E-mail addresses: dawang@sun5.ibp.ac.cn,
wdc@pewdc.ibp.ac.cn (D.-C. Wang).

the crystallization of some soluble proteins [e.g. 16–20]. There is discussion in the current literature about the role of detergent as an additive in the crystallization of soluble proteins (e.g. [21]).

We describe the effect of detergents upon crystallization of four soluble proteins, in terms of improving both crystal quality and reproducibility. These results may be useful when considering how to optimize crystallization of other soluble proteins with detergents.

2. Experiments

2.1. Samples and chemicals

The soluble proteins used in our crystallization experiments were as follows:

(1) ALGP, an analgesic protein from the venom of Chinese *Buthus martensii* Karsch (BmK) scorpion with a molecular mass of 8149.3. The detailed purification procedure has been described previously [22]. The stock solution was 20 mg/ml.

(2) BmK II, an excitatory insect toxin with a molecular weight of 8141.0 isolated from the venom of the BmK scorpion. The purification method was as for ALGP [22]. The stock solution was 20 mg/ml.

(3) BmK dIT-AP, a depressant insect toxin with a molecular mass of 6722.7 also from the venom of the BmK scorpion. Purification was carried out as for ALGP [22], except that the last step was performed using a Resource RPC 3 ml column. The stock solution was 20 mg/ml.

(4) Mabinlin II, a protein isolated from the seeds of *Capparis masaiikai* Lévl, which grows in the south of China [23], and is composed of an A chain of 33 amino acid residues and a B chain of 72 amino acid residues with a total molecular mass of 12.4 kDa. The detailed purification procedure was described in a previous report [23]. The stock solution was 10 mg/ml.

The detergents used in the crystallization, including Zwittergent 3-10, *n*-octanoylsucrose, MEGA-8, C₁₂E₈ and C-HEGA-10, were from Detergent Screen Kit 1 produced by Hampton Research. These detergents were diluted to their critical micelle concentration (CMC, the lowest

concentration above which detergent monomers cluster to form micelles) and stored at -20°C . The buffer reagents Tris(hydroxymethyl)amino-methane (Tris) and *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid] (HEPES) were products of Sigma. PEG 4K was purchased from Fluka and was used without further purification. The stock solution of PEG was 50% (w/v). Other reagents were all analytical grade. All the stock solutions of precipitants and buffers were stored at 4°C and were equilibrated to room temperature before use.

2.2. Crystallization experiments

Initially the Screen and Screen II kits (Hampton Research) were used to search for the crystallizing conditions for the four soluble proteins, BmK II, ALGP, BmK dIT-AP and Mabinlin II. We subsequently tried many other conditions manually based upon the results of the screening experiments. The hanging-drop vapor-diffusion method was chosen to perform the crystallization experiments. The concentration of the samples was later adjusted accordingly. The hanging drop was made up of equal volumes of protein solution and reservoir solution. The trials were observed with a microscope (Olympus) after being incubated at 22°C for 3 days, 1 week, 2 weeks, 1 month, and so on. Trials with otherwise identical conditions were also conducted with an incubation temperature of 4°C .

The detergents from the Detergent Screen Kit 1 (Hampton Research) were tested under those conditions in which proteins, in the absence of detergent, grew in clusters which produced twin or multiple crystals, diffraction-weak crystals and non-reproducible crystals. As the detergents are expensive and they work only in the crystallizing drops, they were only added into the drops. To perform the trials, $1.0\ \mu\text{l}$ protein solution was mixed with $1.0\ \mu\text{l}$ reservoir solution to form the drop, and then 0.3, 0.6 or $0.8\ \mu\text{l}$ detergent at its CMC concentration was added into the drop. The volume of the detergents added into the drops was further adjusted later according to the results of the initial trial. The plates were incubated and observed as described above.

3. Results and discussion

During crystallization of the above four soluble proteins we met a series of problems in growing crystals, which were overcome by the use of detergents to obtain diffraction-quality crystals.

3.1. Changing a polycrystalline state to single crystals

In a recipe using lithium sulfate as precipitant (condition A in Table 1), BmK II appeared in a polycrystalline state (see Fig. 1a). Many different conditions had been tried but none was found to produce single crystals. In the detergent screen, single crystals of BmK II were produced (Fig. 1b) when the detergent Zwittergent 3-10 was added into the crystallizing drop. A decrease in the concentration of lithium sulfate (from 1.2 to 0.8 M) resulted in fewer, larger crystals in the drop. The crystals were found to diffract to a resolution of 2.5 Å. Preliminary X-ray analysis indicates that the crystals belong to space group P6₂22 or P6₄22 with cell parameters $a = b = 65.18$ Å, $c = 172.65$ Å.

3.2. Promoting crystal clusters into single crystals

In the crystallization of ALGP, crystals grown in clusters (Fig. 1c) were found under condition B with PEG 4K as a precipitant (Table 1). Experi-

mentation with many different conditions for crystal growth, other than using detergents, all failed to produce single crystals. Several detergents, such as C₁₂E₉, LDAO, CYMAL-5 and C-HEGA-10 in Screen Kit 1 all improved the yield of single crystals. Comparative studies showed that the detergent MEGA-8 was most effective in improving the yield of perfect single crystals as shown in Fig. 1d. The crystals were found to diffract to 2.8 Å. The space group of the crystals belongs to P222 with cell parameters of $a = 64.35$ Å, $b = 41.11$ Å and $c = 70.04$ Å.

3.3. Enhancing the diffracting ability

Under condition C shown in Table 1, single crystals of ALGP (Fig. 1e) appeared in an ammonium sulfate–Tris system. However, these crystals gave very poor results with X-ray diffraction, which could not be improved despite many attempts to further optimize crystal growth. Addition of the detergent *n*-octanoylsucrose into the crystallizing drop resulted in growth of single tetragonal crystals (Fig. 1f). These resulting crystals were found to diffract to a resolution of 2.5 Å.

3.4. Improving reproducibility of crystal growth

In protein crystal growth, crystals are often seen but then cannot be reproduced later under the same conditions of growth. In our crystallization

Table 1
Roles of detergents in the optimization of crystallization of four soluble proteins

Sample	Condition no.	Basic crystallizing condition	Detergent added to basic condition ^a	Optimization outcome
BmK II	A	1.2 M Lithium Sulfate, 0.1 M HEPES pH 7.5	0.5 μl Zwittergent 3-10	Polycrystalline → Single crystals
ALGP	B	35% PEG 4K, 0.1 M HEPES pH 7.5	0.5 μl MEGA-8	Crystal clusters → Single crystals
ALGP	C	2.8 M Ammonium Sulfate 0.1 M Tris pH 8.5, 5% Dioxane	0.6 μl <i>n</i> -octanoylsucrose	Diffracting inability → Diffracting well
Mabinlin II	D	4.0 M Sodium Chloride, 0.1 M Tris pH 8.5, 5% Dioxane	0.6 μl C ₁₂ E ₈	Non-reproducible → Reproducible
BmK dIT-AP	E	0.8 M Sodium Citrate, 0.2 M Acetate buffer, pH 5.0	0.6 μl C-HEGA-10	Non-reproducible → Reproducible
BmK II	F	0.9 M Sodium Citrate, 0.1 M HEPES pH 7.5	0.5 μl Zwittergent 3-10	Non-reproducible → Reproducible

^aThe volume of detergent added to a 1.0+1.0 μl drop.

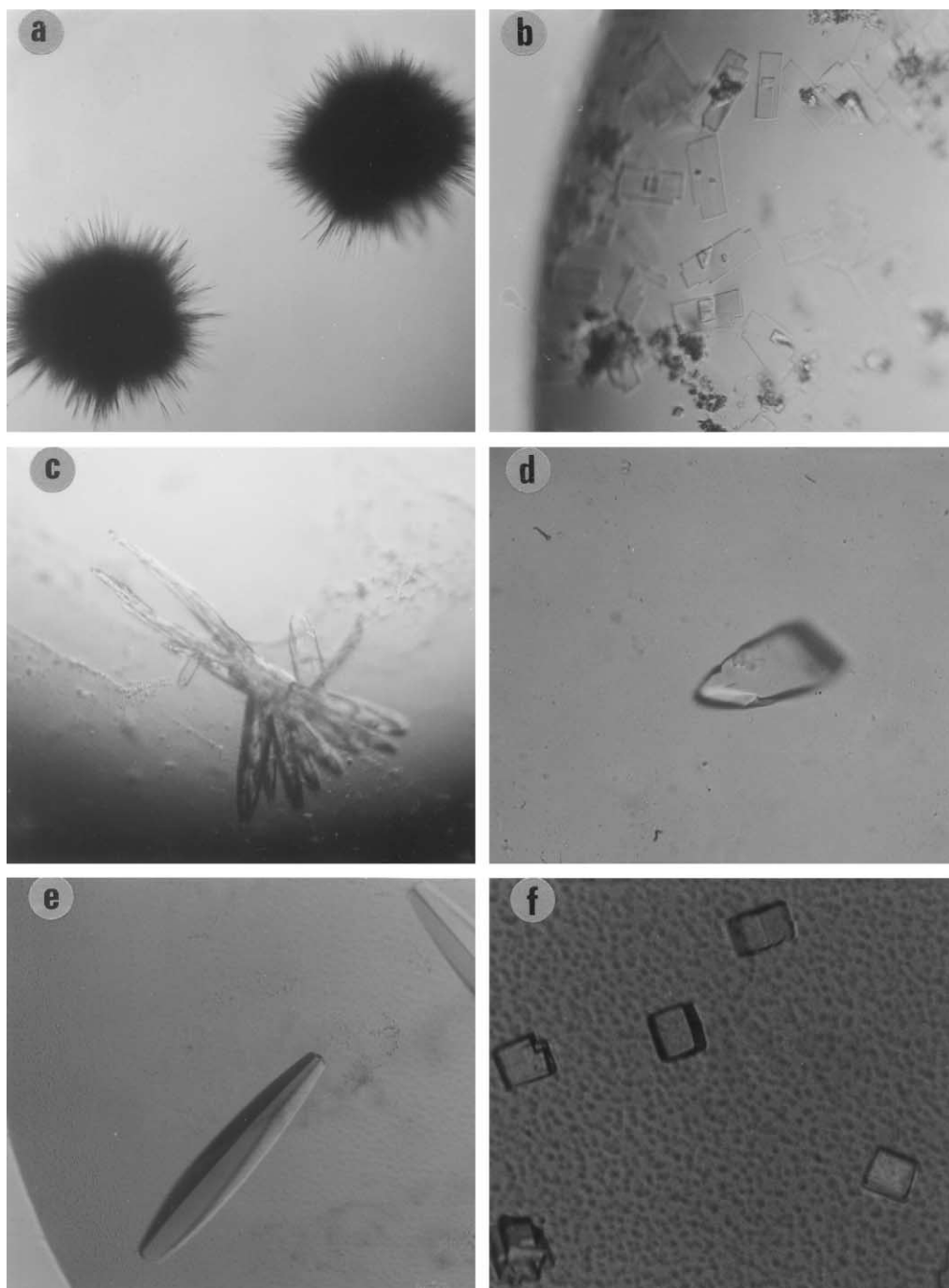


Fig. 1. Comparison of the crystals grown in the absence of detergents (a, c, e) and in the presence of certain detergents (b, d, f) showing the improvement in crystal quality due to the use of detergents. See details in the text. Crystals of BmK II under condition A (Table 1) without detergent (a) and with detergent (b). Crystals of ALGP under condition B (Table 1) without detergent (c) and with detergent (d). Crystals of ALGP under condition C (Table 1) without detergent (e) and with detergent (f).

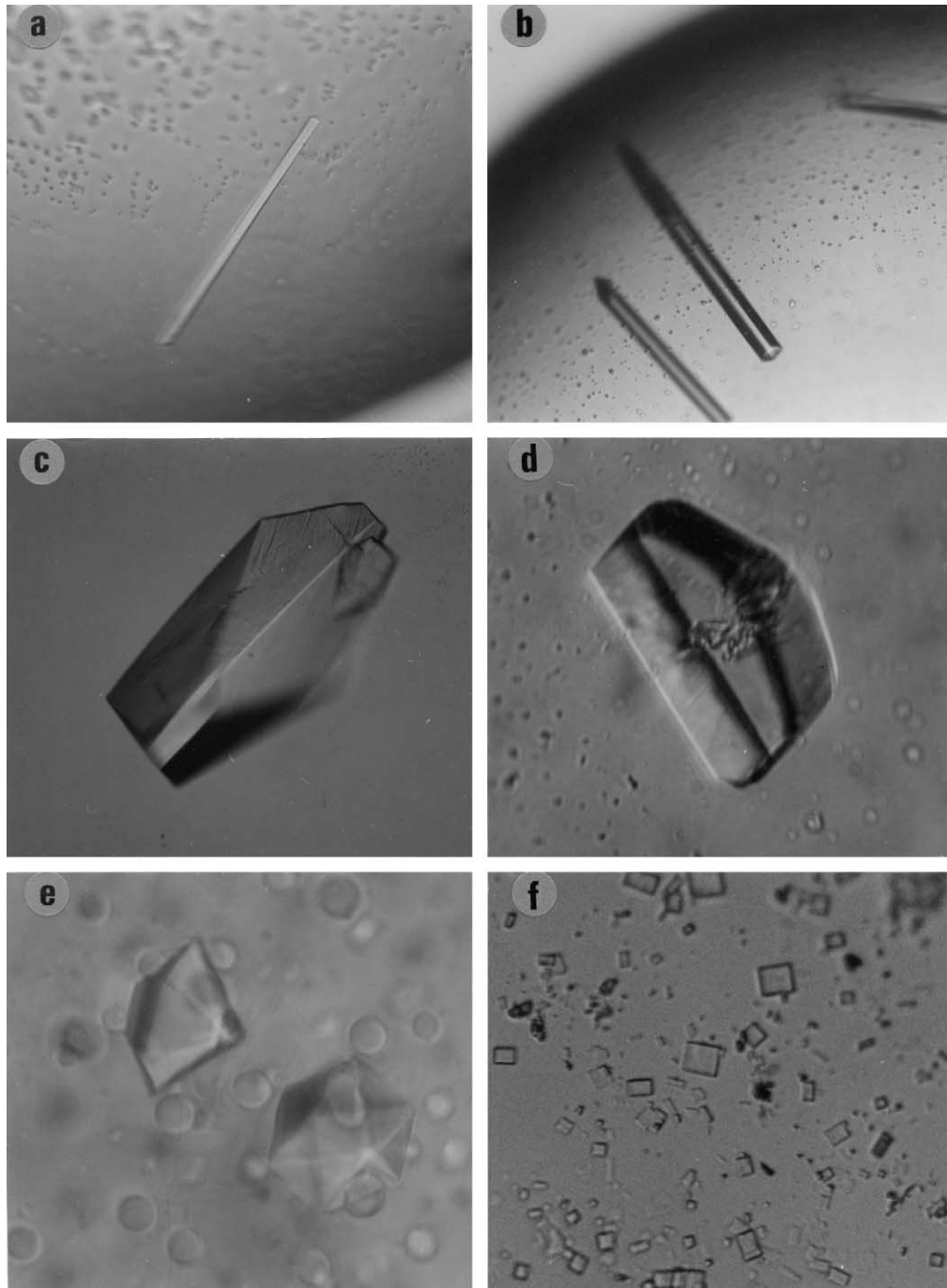


Fig. 2. Comparison of the crystals grown in the absence of detergents (a, c, e) and in the presence of certain detergents (b, d, f) showing the effectiveness of detergents in overcoming non-reproducibility. The crystals grown under conditions without detergents (a, c and e) could not be reproduced later, but those with the detergents (b, d and f) could easily be reproduced. See details in the text. Crystals of Mabinlin II under condition D (Table 1) without detergent (a) and with detergent (b). Crystals of BmK dIT-AP under condition E (Table 1) without detergent (c) and with detergent (d). Crystals of BmK II under condition F (Table 1) without detergent (e) and with detergent (f).

experiments we encountered this problem with three of our proteins, BmK II, Mabinlin II and BmK dIT-AP. In all three cases, the problem was solved by the use of detergents.

During the crystallization of Mabinlin II, the thin-rod-like crystals (Fig. 2a) once appeared under condition D (Table 1), but could not be reproduced later. Screening with the Detergent Screen kit 1 showed that quite a number of detergents, such as CTAB, *n*-hexyl- β -D-glucoside and HECAMEG, could provide reproducible crystals when added to the hanging droplets. Addition of the detergent C₁₂E₈ gave the best reproducible yield of diffraction-quality single crystals of Mabinlin II as shown in Fig. 2b.

In the case of dIT-AP, a crystal (Fig. 2c) was occasionally found under condition E listed in Table 1, which again could not be reproduced reliably despite very careful reduplication of experimental conditions or the use of many similar protocols. Addition of the detergent C-HEGA-10 to condition E yielded diffraction-quality crystals (Fig. 2d), which could easily be reproduced. Interestingly, crystals grown in the presence as well as in the absence of the detergent C-HEGA-10 exhibited the same cell constants in the X-ray analysis (space group R3, $a = b = 65.0 \text{ \AA}$, $c = 176.20 \text{ \AA}$), but crystals grown in the presence of detergent gave a slightly higher resolution (improved from 2.2 to 2.0 \AA).

The same problem was encountered in the crystallization of BmK II. A non-reproducible crystal (Fig. 2e) of BmK II was found under condition F (Table 1). Again, use of methods other than detergents to grow crystals in a reproducible way were unsuccessful. Addition of Zwittergent 3-10 into the hanging droplet gave rise to reproducible growth of crystals, the appearance of which was different from those produced without detergent (Fig. 2f).

The results of our crystallization experiments described above definitely showed that detergents as additional additives could play important roles in promoting the growth of single crystals as opposed to polycrystals and cluster crystals, in enhancing the crystal's X-ray diffraction characteristics, and in improving the reproducibility of crystal growth. At present, the mechanism of these

interesting effects is not understood. It seems that detergents introduced into the crystallizing drops can render protein molecules in solution more hydrophilic so that the homogeneity of protein molecules in solution is increased.

Though our experiments have only involved crystallization of four proteins, the results suggest that detergents may be useful in optimizing the growth of many other soluble protein crystals when problems such as described above are encountered.

Acknowledgements

This work was supported by the National Basic Research Program (G19990756, Yu-95), the Natural Science Foundation and the "921" Research Plan of China. We thank Dr. Andrew Perrett for correcting the English.

References

- [1] R. Giege, *Structure* 7 (1999) R189.
- [2] M. Pusey, N.E. Chayen, *IUCr Newslett.* 8 (2000) 6.
- [3] *Crystallization Research Tools*. Hampton Research catalog.
- [4] D.M. Blow, N.E. Chayen, L.F. Lloyd, E. Saridakis, *Protein Sci.* 3 (1994) 1638.
- [5] J. Hirschler, M.H. Charon, J.C. Fontecilla-Camps, *Protein Sci.* 4 (1995) 2573.
- [6] N.E. Chayen, *Protein Eng.* 9 (1996) 927.
- [7] G.P. Li, Y. Xiang, Y. Zhang, D.C. Wang, *J. Appl. Crystallogr.* 34 (2000), in press.
- [8] N.E. Chayen, E.J. Gordon, P.F. Zagalsky, *Acta Crystallogr. D* 52 (1996) 156.
- [9] E.H. Snell, S. Weisgerber, J.R. Helliwell, E. Weckert, K. Hölzer, K. Schroer, *Acta Crystallogr. D* 51 (1995) 1099.
- [10] B. Cudney, S. Patel, A. McPherson, *Acta Crystallogr. D* 50 (1994) 479.
- [11] K.J. Thiessen, *Acta Crystallogr. D* 50 (1994) 491.
- [12] P.A. Marone, P. Thiyagarajan, A.M. Wagner, D.M. Tiede, *J. Crystal Growth* 207 (1999) 214.
- [13] F. Reiss-Husson, in: A. Ducruix, R. Giege (Eds.), *Crystallization of Nucleic Acids and Proteins*, Oxford University Press, Oxford, 1992, p. 175.
- [14] L. Song, E. Gouaux, *Acta Crystallogr. D* 54 (1998) 276.
- [15] R.M. Garavito, D. Picot, P.J. Loll, *J. Bioenerg. Biomembr.* 28 (1996) 13.
- [16] M. Becker, M.T. Stubbs, R. Huber, *Protein Sci.* 7 (1998) 580.

- [17] Y. Dou, S.J. Admiraal, M. Ikeda-Saito, S. Krzywda, A.J. Wilkinson, T. Li, J.S. Olson, R.C. Prince, I.J. Pickering, G.N. George, *J. Biol. Chem.* 270 (1995) 15993.
- [18] W. Bourguet, M. Ruff, P. Chambon, H. Gronemeyer, D. Moras, *Nature* 375 (1995) 377.
- [19] K.H. Weisgraber, Y.M. Newhouse, A. McPherson, *J. Mol. Biol.* 236 (1994) 382.
- [20] G.A. Bohach, Y. Chi, C.V. Stauffacher, *Proteins* 13 (1992) 152.
- [21] C. Sauter, J.D. Ng, B. Lorber, G. Keith, P. Brion, M.W. Hosseini, J.-M. Lehn, R. Giege, *J. Crystal Growth* 196 (1999) 365.
- [22] Y.M. Xiong, Z.D. Lan, M. Wang, B. Liu, X.Q. Liu, H. Fei, L.G. Xu, Q.C. Xia, C.G. Wang, D.C. Wang, C.W. Chi, *Toxicon* 37 (1999) 1165.
- [23] Z. Hu, L.P. Peng, M. He, *Acta Bot. Yunnanica* 7 (1985) 1.