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A depressant insect toxin with a novel analgesic effect from scorpion *Buthus martensii* Karsch

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Abstract

A new peptide named BmK dITAP3 from scorpion *Buthus martensii* Karsch (BmK) has been identified to possess a dual bioactivity, a depressant neurotoxicity on insects and an analgesic effect on mice. The bioassays also showed that the peptide was definitely devoid of the neurotoxicity on mammals, which indicated that the analgesic effect of BmK dITAP3 could not be ascribed to the syndromic effects of a mammalian neurotoxicity. BmK dITAP3 exhibited 43.0% inhibition efficiency of the analgesic effect on mice at a dose of 5 mg/kg and the FPU value of 0.5 μg/body (~30 mg) on the fly larvae. The p*I* value and the molecular mass determined by MALDI-TOF MS for dITAP3 were 6.5 and 6722.7, respectively. Its first 15 N-terminal residues were determined by Edman degradation, based on which the full amino acid sequence was deduced from the cDNA sequence encoding the peptide with 3′-RACE. Circular dichroism and sequence based prediction analyses showed dITAP3 may have a similar molecular scaffold as the most scorpion toxins but with features of the more β structures and much less of α helix. The details of the purification, characterization and sequencing as well as the sequence comparison with other depressant insect toxins and the correlation between the analgesic effect and the insect toxicity will be reported and discussed, respectively.

Keywords: Scorpion toxin; Depressant insect toxin; Analgesic effect; Purification; Characterization; Sequencing

1. Introduction

Scorpion venoms are well-known sources of peptide neurotoxins that bind to ionic channels at the surface of excitable cells and modify diversely their normal properties. Among others, two groups of neurotoxins, the excitatory and the depressant toxins, named according to the symptomatology developed by injected animals, have been recognized from the scorpion venoms [1]. These toxins specific for insects are characterized as single-chain polypeptides of 60–76 residues cross-linked by four disulfide bridges [2–4]. The excitatory toxins cause a fast contraction paralysis on injected animals and induce repetitive firing in insect nerves, while the depressant toxins cause a slow depressory flaccid paralysis due to depolarization of the nerve membrane and blockage of the sodium conductance in axons [1,5,6]. Due to their great value as tools for studying the pharmacology of insect sodium channels and bright prospect in the design of new insect selective biopesticides [7,8], these insect toxins are widely studied by many research groups. However, it is rare to find a report to describe certain new activity other than neurotoxicity from insect toxins.

The scorpion *Buthus martensii* Karsch (BmK),

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widely distributed in East Asia, is a very mild species. Its venom is not only much less toxic and never causes death of envenomed people, but quite contrarily, is used for disease prevention and therapy in China. Probably with this background, some active peptides with diverse activities other than neurotoxicity have been identified from the BmK venom in recent years, though the mechanism remains to be clarified. For example, a peptide with anti-epilepsy effect was isolated and partially sequenced [9]; the analgesic effect was confirmed in the crude BmK venom [10]; an excitatory insect toxin with an analgesic effect was cloned and sequenced [11]; and a peptide named makatoxin with an effect on nitrergic responses has been discovered [12]. Therefore it will be no surprise if some other peptides with new biological or pharmacological effects could be found in the BmK venom.

Recently, we found a depressant insect toxin, BmK dITAP3, from the venom of scorpion BmK venom. Very interestingly, this insect toxin displays an evident analgesic effect but is devoid of any toxicity on mice, which has not been reported before for scorpion depressant insect toxins. In this paper we will report the purification and characterization of this toxin, including full sequence and secondary structure, which has established a sound basis for the further investigation on its structure and function.

2. Materials and methods

2.1. Sample and chemicals

The venom of BmK scorpion was obtained by electrical stimulation of the post abdomen of scorpions grown in Henan Province in China. All gels used in chromatography, Sephadex G-50, Sephadex G-25 and SP-Sephadex C-25, and in electrophoresis, Ultradex, were products of Pharmacia. The acetonitrile used in reverse-phase chromatography was gradient grade (Merck). The CCA used in mass spectroscopy was from Sigma. The reagents used in N-terminal sequencing were from Perkin Elmer. Enzymes and reagents used in 3′-RACE were obtained from Life Technology. Polymerase chain reaction (PCR) kit was from Promega (USA). DNA sequencing kit was from United States Biochemicals, and [α-^{32}P]dATP was from Amersham (Italy). All other chemicals were of analytical grade.

2.2. Purification

The lyophilized venom (400 mg) of scorpion BmK, having been dissolved in 4 ml 0.05 M NH_4HCO_3 for 5–7 days, was applied to a Sephadex G-50 column (2.6×150 cm) previously equilibrated with 0.05 M NH_4HCO_3, after being centrifuged on a Sigma-202 MK centrifuge at a speed of 6000 rpm for 30 min. Then the column was eluted with the same solution at a flow rate of 18 ml/h and the fractions of 6 ml per tube were collected. The elution was detected by the UV280 absorption with the LKB Bromma UV-detector and recorder. The fraction showing anti-insect toxicity was pooled and lyophilized.

The fraction obtained above was dissolved in 5 ml 0.02 M sodium phosphate buffer at pH 6.5, and was centrifuged at 6000 rpm for 15 min. Then it was subjected to a pre-equilibrated SP-Sephadex C-25 ion-exchange column (1.6×80 cm) which was eluted with the same buffer at a flow rate of 24 ml/h. The flow-through peak containing anti-insect toxicity was collected and concentrated. The desalting was completed on a Sephadex G-25 column (1.6×80 cm) eluted with 5% acetic acid at a flow rate of 60 ml/h.

Further separation was carried out with the preparative isoelectrofocusing electrophoresis on LKB 2117 Multiphor II system with Ultrodex granule gel in the ampholytes pH range from 3.5 to 9.5. The samples focused in the gel were recovered by eluting with 5% acetic acid. After the ampholytes were removed on a desalting Sephadex G-25 column as described above, the Resource RPC 3 ml column (Pharmacia Biotech) was used as the final step in purifying BmK dITAP3, using acetonitrile in a linear gradient.

2.3. Bioassays

2.3.1. Determination of analgesic activity

The analgesic activity was assessed with a mouse-twisting model [13]. ICR mice (male, SPF level, 18–20 g in body weight) taken from the Experimental Animal Center of Beijing were used in the test. Mice were injected intraperitoneally with 0.2 ml 1% acetic acid solution to induce extensive and long-lasting

aches in their internal organs. In response the mouse would keep twisting its body, so the twisting actions could reflect the intensity of the aches. To perform the bioassay, 0.2 ml toxin solutions varying in concentration were injected intravenously from the tail of the mouse, using 0.9% sodium chloride solution as control. Twenty minutes later, 0.2 ml 1% acetic acid solution was then injected intraperitoneally. Five minutes later the mouse-twisting action was counted for 10 min. At each dose including the control, ten mice as a group were injected with the same dose, and the results were analyzed by statistics using a *t*-value test.

2.3.2. Anti-insect toxicity assay

The anti-insect toxicity activity was tested with the larvae of the house fly as described earlier [6]. After culture for 5–6 days, the fly larvae weighing 30 ± 2 mg were selected for the bioassay. The sample was dissolved in 0.9% sodium chloride solution and then injected into the lower part of the larva's abdomen at different doses. Ten fly larvae as a group were injected with the same amount of sample. The amount of the sample injected and the reaction of the larvae after injection were recorded.

2.3.3. Toxicity on mammals

The toxicity of the resulting sample on mammals was tested on ICR mice as described above. Using 0.9% sodium chloride solution as control, ten mice as a group, various doses of the sample dissolved in 0.9% sodium chloride solution were injected into the mouse through its tail vein. The reaction and the doses were recorded.

2.4. Molecular mass determination for BmK dITAP3

The molecular mass of dITAP3 was analyzed by the sensitive and quick MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry). The MALDI mass spectrum was measured with the Biflex III-MS (Bruker, USA). The matrix used was CCA (α-cyano-4-hydroxycinnamic acid), which was dissolved saturated in 1:1 mixture of 1% trifluoroacetic acid and acetonitrile. The mixed solution was used for sample preparation. The sample (200 pmol) was used to perform the experiment. The external mass calibration was accomplished by using cytochrome *c* as peptide standard.

2.5. Sequencing

The full sequence of dITAP3 was deduced from its cDNA. Firstly, 15 N-terminal amino acids were determined by automated Edman degradation with an Applied Biosystem 475 A Protein Sequencer (USA) using the purified dITAP3 sample. The phenylthiohydantoin (PTH) derivatives of amino acids were separated and identified by an on-line PTH analyzer (Model 120 A, Applied Biosystems) on a PTH-C18 column (5 μm, 220×2.1 mm), which was eluted with a linear gradient of acetonitrile.

The cDNA encoding the mature dITAP3 was cloned by 3′-RACE. Five μg total RNAs were taken to be converted into cDNAs using a 3′-RACE kit provided with Superscript II reverse transcriptase and an oligo(dT) containing adapter primer with a *Sal*I restriction site (5′-GGCCACGCGTCGACT-AGTAC$(dT)_{17}$-3′). The partial dITAP3 gene from the 3′ end was then amplified by a pair of PCR primers: gene specific primer of dITAP3 (5′-CGGAATTCGATGGATATATTCGGGGCAGT-3′) corresponding to the N-terminal 1–7 residues (DGYIRGS-) and a universal anchor primer (5′-CUACUACUACUAGGCCACGCGTCGACTAGT-AC-3′). The PCR product was digested with *Eco*RI and cloned into M13mp19. The single-strand DNA of positive clones was extracted and sequenced as described in the protocol of T7 sequenase version 2.0 DNA sequencing kit with labeled nucleotide ([α-^{32}P]dATP). All PCR experiments were performed on a Perkin Elmer GeneAmp PCR system 480.

2.6. Circular dichroism (CD) spectroscopy and prediction of secondary structure

BmK dITAP3 in a concentration of 1.0 mg/ml in 20 mM sodium phosphate buffer at pH 7.0 was used in the CD measurement utilizing a cell with length 0.5 mm. The CD experiment was carried out on a Jasco J-720 system (Japan Spectroscopy, Japan) at room temperature. The spectrum was recorded from 190 to 250 nm at a scan rate of 50 nm/min with a time constant of 1 s. It was measured four times and no significant differences between the mea-

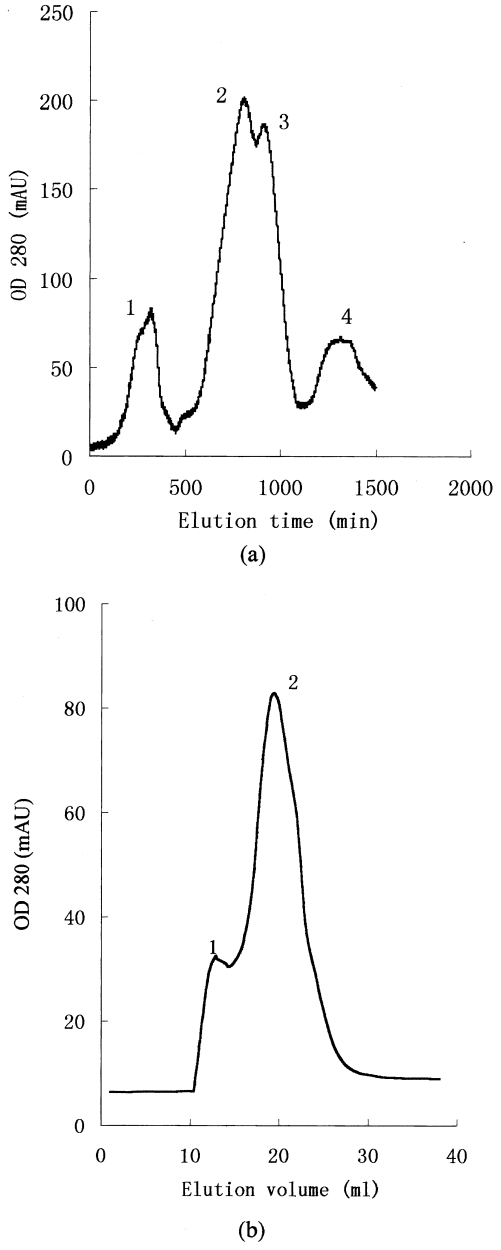

Fig. 1. (a) Elution curve of the venom of scorpion BmK on a Sephadex G-50 column (2.6×150 cm) eluted by 0.05 M NH_4HCO_3 at a flow rate of 18 ml/h. The second fraction was pooled and used in further purification. (b) Elution curve of the flow-through fraction from the SP Sephadex C-25 column on the desalting Sephadex G-25 column. dITAP3 was purified from the second peak.

surements in different time were found. Then the results were averaged. Estimation of the secondary structure from the CD spectra was done with the estimation program provided with the system by the company (J-700 for Windows Secondary Structure Estimation).

The secondary structure of dITAP3 was also predicted from the sequence by using the PSSP method provided on the internet (web site: http://imtech.ernet.in/raghava/pssp/).

3. Results

3.1. Purification

The pure BmK dITAP3 was obtained by the successful combination of chromatography methods and preparative isoelectrofocusing electrophoresis. The second fraction eluted out from the Sephadex G-50 column (Fig. 1a) containing the anti-insect toxicity was applied to a SP-Sephadex C-25 column after being lyophilized to powder. The flow-through fraction was pooled according to its anti-insect toxicity and was then desalted on a Sephadex G-25 column, on which two protein peaks were obtained (Fig. 1b). The second peak was pooled and then used in a preparative isoelectrofocusing electrophoresis. The fraction focused at pH 6.5 (Fig. 2) was recovered by being eluted with 0.05 M NH_4HCO_3. The concentrated sample was further subjected to a Resource RPC column (3 ml) as the final step (Fig. 3). The purified dITAP3 was analyzed on a C8 reverse-phase column (5 μm, 4.6/250) on the ÄKTA Purifier system (Pharmacia Biotech.), which showed that it possessed purity higher than 90% (Fig. 4).

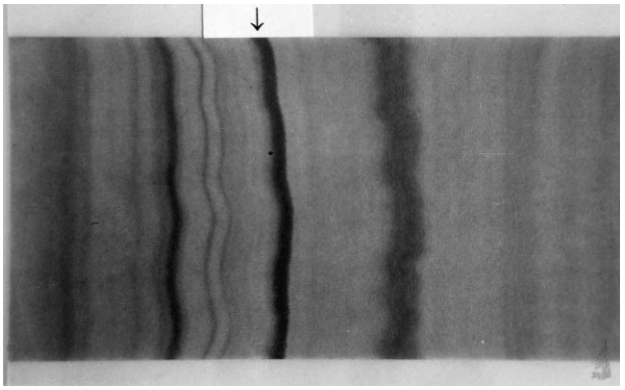

Fig. 2. Photograph of the preparative electrophoresis gel, in which BmK dITAP3 was focused at the marked position, pH 6.5.

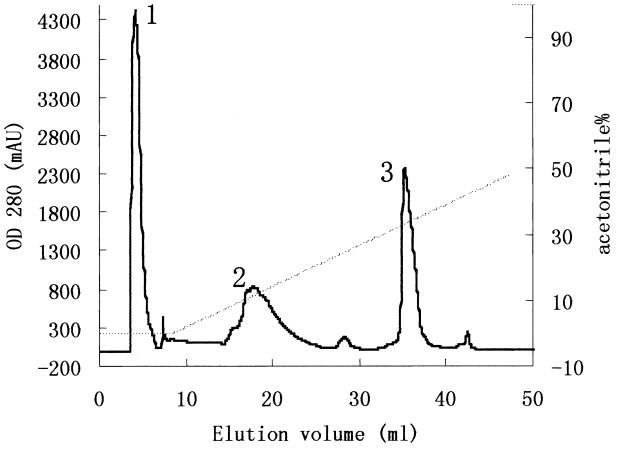

Fig. 3. The purification of BmK dITAP3 in the last step on a Resource RPC 3-ml column with a linear gradient of acetonitrile (the dashed line indicates the gradient). The sample in the third peak was pooled and named BmK dITAP3.

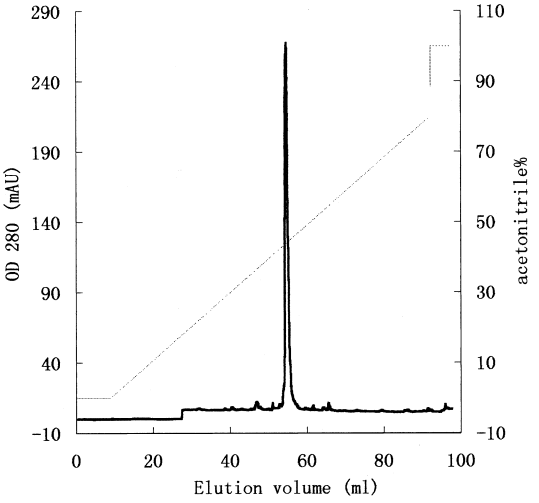

Fig. 4. Reverse-phase analysis of BmK dITAP3 on a C8 column (the dashed line indicates the gradient of acetonitrile), which showed that dITAP3 had a purity higher than 90%.

3.2. Bioassay

3.2.1. Analgesic activity

Tested with the mouse-twisting model, BmK dITAP3 showed obvious analgesic activity. The experimental data and data processing are listed in Table 1. The inhibition curve is shown in Fig. 5. Because the t values from the statistics (Table 1) at each dose were all greater than $t_{(18)\,0.01}$ (2.878), all P values were less than 0.01, which indicated a high statistical reliability of the results. It exhibited 43.0% inhibition efficiency at a dose of 5 μg/g (0.699 in Log form at the horizontal axis in Fig. 5).

Table 1
Statistics of analgesic effect test for BmK dITAP3

$\bar{x}$	Dose (mg/kg)	Log (dose)	Count of twisting (x)	Inhibition efficiency (%)	S	S_c^2	$S(\bar{x}_1-\bar{x}_2)$	t	P
Control			56, 65, 55, 56, 60, 64, 60.1 60, 58, 65, 62		3.81				
dITAP3	1	0	50, 49, 48, 51, 46, 52, 49.4 48, 47, 51, 52	17.8	2.12	9.50	1.38	7.76	<0.01
	2	0.301	42, 42, 48, 43, 43, 46, 44.1 41, 44, 47, 45	26.6	2.33	9.97	1.41	11.33	<0.01
	3	0.477	41, 38, 41, 39, 42, 40, 40.5 42, 37, 44, 41	32.6	2.07	9.40	1.37	14.29	<0.01
	4	0.602	38, 36, 35, 37, 41, 35, 37.4 37, 35, 41, 39	37.8	2.32	9.95	1.41	16.09	<0.01
	5	0.699	37, 31, 34, 34, 33, 32, 33.2 34, 32, 36, 29	44.8	2.35	10.02	1.42	19.00	<0.01
	7	0.845	31, 26, 27, 33, 31, 29, 30.3 30, 30, 32, 34	49.6	2.50	10.38	1.44	20.68	<0.01

The control is 0.9% sodium chloride solution; the number (x) is the twisting times of tested mouse counted for 10 min. The inhibition efficiency is defined as the ratio of $(N_0-N)/N_0$, in which N_0 is the mean twisting times of control group counted for 10 min, and N is the mean twisting times of experimental group with different dosage of dITAP3. The formulas used are as follows: (Here $n_1=n_2=10$).

$$\bar{x}=\sum_{i=1}^{n}x_i \quad S=\sqrt{\frac{\sum(x-\bar{x})^2}{n-1}} \quad t=\frac{|\bar{x}_1-\bar{x}_2|}{S_{(\bar{x}_1-\bar{x}_2)}} \quad S_{(\bar{x}_1-\bar{x}_2)}=\sqrt{S_c^2\left(\frac{n_1+n_2}{n_1n_2}\right)} \quad S_c^2=\frac{(n_1-1)S_2^1+(n_2-1)S_2^2}{(n_1-1)+(n_2-1)}$$

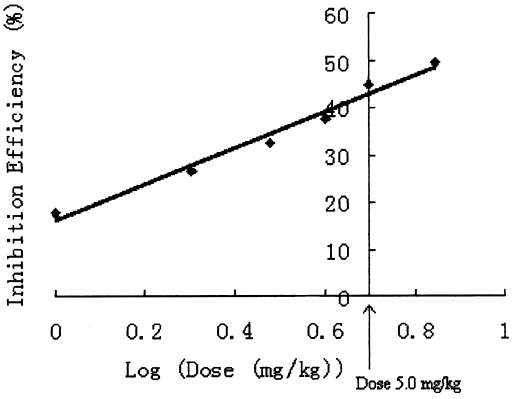

Fig. 5. Analgesic activity curve of BmK dITAP3. The inhibition efficiency at a dose of 5.0 mg/kg (0.699 in Log form in the figure) is 43.0%.

3.2.2. Anti-insect toxicity

The insect toxicity of BmK dITAP3 was tested with blow-fly larvae. Injected into the larva, dITAP3 caused a fast contraction and then a delayed, slow and progressive depressant paralysis. The FPU (flaccid paralysis unit, defined as the amount of toxin that causes half of the recipient larvae flaccid within 5 min of injection) value for dITAP3 was 0.5 μg/body (~30 mg), which showed the depressant insect toxicity of dITAP3 was relatively weak.

3.2.3. Toxicity on mammals

In order to know whether the analgesic effect of dITAP3 is relevant with the syndrome of the mammal-directed toxicity, the toxicity on mammals of dITAP3 was tested with ICR mice. After dITAP3 samples were injected into the mouse through the tail vein, no paralytic symptom was observed even at a dose of 10 mg/kg which is 15 times higher than the LD_{50} (the dose that causes death of half of the mice tested) of BmK M1, a representative mammal toxin from the scorpion BmK, determined with the same method and procedure [14]. This is also the highest dose which could induce the detectable mam-

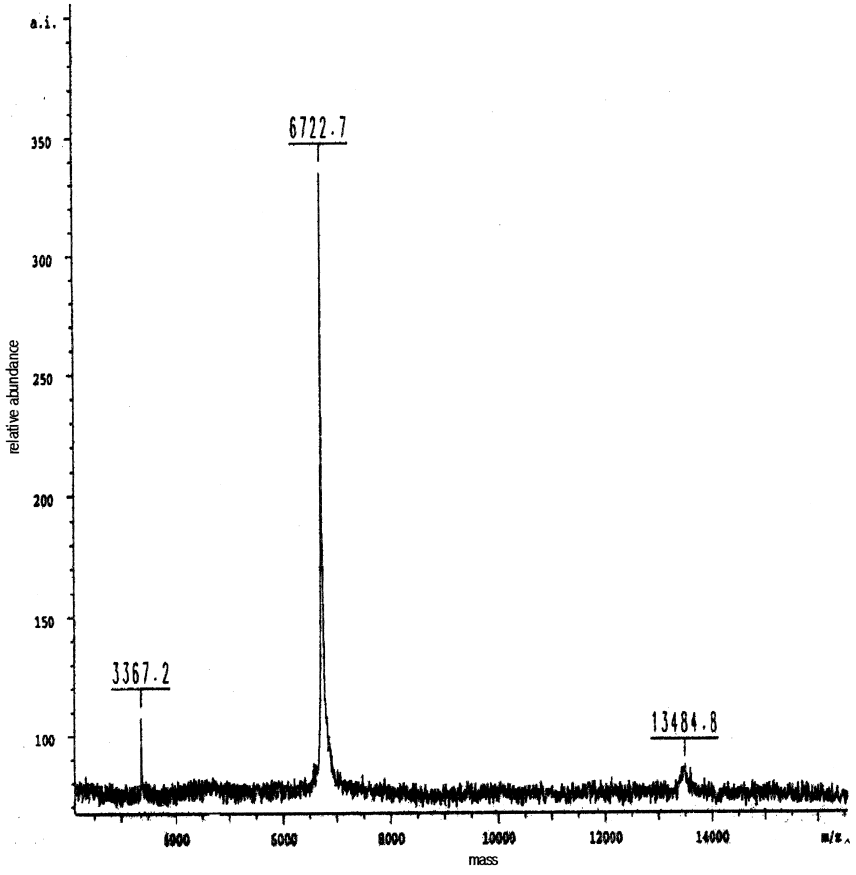

Fig. 6. MALDI-TOF mass spectrum of BmK dITAP3 determined on Biflex III-MS.

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1                                   10
GAT GGA TAT ATA AGA GGA AGT AAC GGA TGC AAG GTT TCA TGC TTA TGG
 D   G   Y   I   R   G   S   N   G   C   K   V   S   C   L   W

              20                                  30
GGA AAT GAA GGT TGC AAT AAA GAA TGC AGA GCG TAC GGT GCC TCT TAT
 G   N   E   G   C   N   K   E   C   R   A   Y   G   A   S   Y

                          40
GGT TAT TGC TGG ACC TGG GGA CTT GCA TGC TGG TGT CAA GGC CTT CCT
 G   Y   C   W   T   W   G   L   A   C   W   C   E   G   L   P

     50                                  60
GAT GAT AAG ACA TGG AAA TCT GAA AGT AAT ACA TGC GGT GGA AAA AAG
 D   D   K   T   W   K   S   E   S   N   T   C   G   G   K   K

TAA TTTTGTTTAAAAGTACTAAAAGAATATGTATGTTAAAATAA CAAGTAAATACATATA
End

AATAAAGAACCTTCATAATpolyA
```

Fig. 7. Nucleotide sequence of the partial cDNA encoding the mature dITAP3 and the deduced amino acid sequence of dITAP3. The sequence was determined from 3′-RACE products and from cDNA amplification by reverse PCR. The primers are underlined. The deduced amino acid sequence corresponding to mature dITAP3 is in boldface. The last three residues GKK are the recognition site and donor of the amidation of the C-terminus and will be removed in the post-translational modification process.

mal-directed toxicity in all Bmk toxins identified so far [14]. The result indicated that dITAP3 was devoid of mammalian neurotoxicity.

3.3. Molecular mass

The molecular mass of BmK dITAP3 measured from the MALDI-TOF mass spectrometry on Biflex III-MS (Bruker) was 6722.7, as shown in Fig. 6. Considering most scorpion depressant toxins discovered so far consist of 61 amino acid residues, the measurement further supports that dITAP3 belongs to the depressant insect toxin group.

3.4. Amino acid sequence

The first 15 N-terminal amino acid residues of BmK dITAP3 were determined by Edman degradation to be DGYIRGSNGCKVSCL. Based on the first seven residues, a primer was synthesized for cDNA cloning. 3′-RACE produced one major DNA fragment about 300 bp. Sequencing of this cDNA showed that it encoded a mature peptide of 61 amino acids (see Fig. 7). The first 15 amino acid residues deduced from this partial cDNA clone was identical with that determined by protein sequencing shown above. The estimated value of the molecular mass (6732.4) from the deduced sequence is basically coincident with that determined by mass spectroscopy from the purified protein sample (6722.7). It is noticed that there is a 9.7 difference between these two values. Considering the accuracy for mass determination by the MALDI-TOF machine (in a linear configuration) is 0.15% (see PAN Facility, http://cmgm.stanford.edu/pan/voyager.html), this difference falls into the error range (±10.8) of mass determination in the case of dITAP3.

3.5. Secondary structure

The CD spectrum of BmK dITAP3 in the UV range of 190–250 nm is shown in Fig. 8. The secondary structure estimation showed that the dITAP3 molecule is rich in β structures while it has little

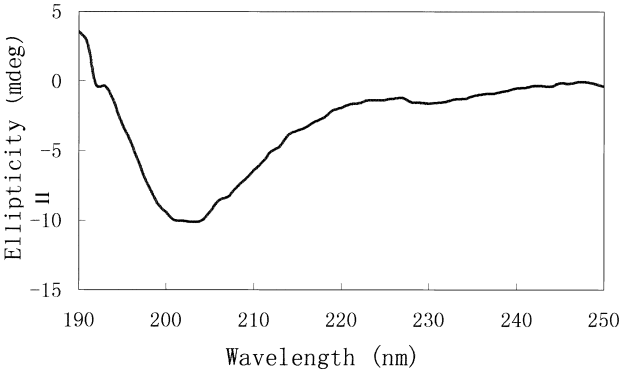

Fig. 8. CD spectrum of BmK dITAP3 in the UV range 190–250 nm analyzed on a Jasco 720 system at pH 7.0 and a concentration of 1.0 mg/ml at room temperature.

Table 2
Secondary structure of dITAP3 from CD analysis and prediction in comparison with that from the crystal structure of a representative mammalian toxin, BmK M1

Sample	α Helix (%)	β Strand (%)	Coil (%)	Method
BmK dITAP3	4.2	52.7	43.1	CD analysis[a]
	3.3	41.0	55.7	PSSP prediction[b]
Bmk M1	15.6	26.5	57.9	Crystal structure [15]

[a]J-700 for Windows Secondary Structure Estimation, Version 1.10.00.
[b]http://imtech.ernet.in/raghava/pssp/.

α helix. The secondary structure prediction from sequence performed with the PSSP method provided at the http://imtech.ernet.in/raghava/pssp/ gave similar results. The detailed data are listed in Table 2. As of now there is no report on three-dimensional structure of a depressant insect toxin. The detailed structural information is expected from X-ray or NMR structural analysis.

4. Discussion

4.1. Sequence comparison

Sequence similarity between dITAP3 and known proteins were searched using the NCBI BLAST electronic mail servers. The results showed about 80% identity and the same cysteine motif between dITAP3 and the known depressant insect toxins, but very low identity (44.3%) between dITAP3 and the mammal-directed toxin (see Fig. 9), except a common cysteine motif. In association with the result of insect toxicity test shown in this report, dITAP3 should be classified as a depressant insect toxin. The sequence alignment also shows a remarkable difference appearing in position 6–7 which are neutral residues in dITAP3, but basic ones in other toxins (Fig. 9). The implication of this distinctness cannot be elucidated right now. The identity between dITAP3 and M1 is very low (39%), in fact random, though they have the same cysteine motif, which is in agreement with the lack of the mammalian neurotoxicity in dITAP3.

4.2. Correlation of analgesic effect with insect toxicity

The bioassay shown in this report displays the definite analgesic effect of dITAP3. In fact, it is much stronger than those analgesic chemicals in common use. For example, Tranquilizer, a pain killer used commonly in the clinic, could only display 62.4% inhibition activity even at a dose of 80 mg/kg shown in the parallel experiment [11]. However, dITAP3 has no detectable toxicity on mice even at an extremely high dose. Therefore the analgesic effect of dITAP3 cannot be ascribed to the syndromic effects of the mammalian neurotoxicity that dITAP3 may possess. It seems to have other special molecular properties relevant to this analgesic effect, though the mechanism is unknown at present.

Sequence comparison (Fig. 9) and bioassay (see Section 3.2) indicate that dITAP3 belongs to the depressant insect toxins. So far several depressant insect toxins have been isolated from venoms of other scorpion species (see Fig. 9). However, apart from BmK dITAP3, no one was reported to exhibit the analgesic effects to mice. The sequence alignment

```
                    10         20         30         40         50         60   Identity
BmK dITAP3 DGYIRGSNGC KVSCLWGNEG CNKECRAYGA SYGYCWTWGL ACWCEGLPDD KTWKSESNTC G*   100%
 Ba  IT2    -----RRD-- -----F---- -D---K---G ---------- ---------- ------T--- -   86.9 [16]
 Bot IT4    -----RRD-- -----F---- -D---K---G ---------- ---------- ------T--- -*  86.9 [17]
 Bot IT5    -----KRD-- -----F---- -D---K---G ---------- ---------- ------T--- -*  86.9 [17]
 Lqh IT2    ----KRRD-- --A--I---- -D---K---G ---------- ---------- ------Y--- -*  83.6 [3]
 Lqq IT2    -----KRD-- -L---F---- -----KS--G ---------- ---------E ------T--- -   83.6 [18]
 Bj IT2     -----KKD-- ----II---- -R---V-H-G -F-------- -----N---A V----ST--- -   75.4 [19]

                   10         20         30         40         50         60         70  Identity
BmK dITAP3 ..DGYIRGSN GCKVS.CLWG NEGCNKECRA YGA.SYGYC. WTWGL...AC WC.EGLPDDK TWKSESNTCG*   100%
BmK M1     VR-A--AKPH NC.-YEC.AR --YC-DLCTK D--K-.--CQ -.V-KYGNGC -CI-.---NV .PIRVPGKCH   44.3% [15]
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Fig. 9. Sequence comparison of dITAP3 with other depressant insect toxins and a representative mammal-directed toxin, BmK M1. All sequences have been aligned forcing the matching of cysteine residues which paired with the same pattern. Those residues different from dITAP3 are shown by name in compared toxins. Asterisk indicates an amidated C-terminal. The major residues of dITAP3 different from that of compared depressant toxins appeared at positions 6–7 which are highlighted with boldface. (From Refs. [3,16–19]).

(Fig. 9) shows that they are highly homologous. A general question was therefore raised: do all depressant insect toxins from scorpion venoms possess the analgesic effect? This is open to further investigation.

Besides this, two excitatory insect toxins, BmK ITAP [11] and AngP1 (unpublished) have also been identified to possess a similar analgesic effect with distinct potency on mice. It seems that this analgesic effect is correlative to the general molecular feature of the insect toxins. However, the excitatory and depressant insect toxins belong to different groups, which have distinct mode to interact with the receptor. Thus one can infer that the analgesic effect of these peptides may have a molecular mode and mechanism different from that of insect toxicity, but a structure–function determinant simultaneously existing in a molecular framework shared by both of these activities. Therefore it would be interesting to elucidate the three-dimensional structure of dITAP3.

4.3. Possible structural features

The secondary structure of dITAP3 deduced from the CD analysis and the sequence-based prediction is basically consistent (Table 2). Although these two methods have certain errors in quantitative analysis, the outcome from them can give the general secondary structural feature of a protein molecule. The basic coincidence of the results from these methods for dITAP3 shows an essential reliability. Compared with a representative mammalian toxin, BmK M1 [15], dITAP3 may have 3 times less a helix and 2 times more β strand than that in BmK M1. It is well known that the general folds of all scorpion toxins are similar. It is composed of a dense core of secondary structure elements, including an α helix and an anti-parallel β sheet connected by three/four disulfide bonds to form a βαββ motif [15,20,21]. The secondary structure analyses of dITAP3 show that this small protein with a dual bioactivity may possess a similar scaffold to that in general scorpion toxins but features evidently in more β structures and less α helix. The further identification of the structural feature of dITAP3 is now waiting for the three-dimensional structure determination, which is already under way in our laboratory.

Note added in proof

The molecular mass of dITAP3 was more accurately determined recently by using a Finigan LCQ ion trap mass spectrometer (ThermoQuest) equipped with an electrospray ionization source. The result showed that the molecular mass of dITAP3 is 6732.0, which is in good agreement with that deduced from the sequence (6732.4).

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