A SENSITIVE RADIOIMMUNOASSAY OF A SCORPION NEUROTOXIN

Toxin I of *Androctonus australis Hector*

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1. Introduction

Scorpion neurotoxins form a family of homologous proteins which are basic and have approx. mol. wt 7000 [1]. They consist of a single peptide chain crosslinked by four disulfide bridges [2]. The complete amino acid sequences of some of them [3,4], as well as the N-terminal of others, have been determined: their comparison has led to a classification into four groups [5]. They have been shown to affect the conduction of ions through membrane channels [6–10], and are thus good tools for the study of these structures on the molecular level. Toxins I and II of *Androctonus australis Hector* have been ^{125}I-labeled: specific radioactivities up to 2000 Ci/mmol have been obtained [11,12]. We report here the setting up of a radioimmunoassay allowing a sensitive and specific detection of toxin I of *Androctonus australis Hector*.

2. Materials and methods

Toxins I, II and III of *Androctonus australis Hector* (Aah toxin I, AaH toxin II and AaH toxin III) and toxin I of *Buthus occitanus tunetanus* (Bot toxin I) were purified according [2].

AaH toxin I was ^{125}I-labeled and isolated from the reaction medium by precipitation with its monospecific antiserum as described [11,12]. Ten different preparations were performed in the course of this work: the mean specific radioactivity was 1750 ± 400 Ci/mmol, i.e., 0.87 ± 0.17 ^{125}I atom incorporated/mol toxin.

Serum against AaH toxin I has been prepared from rabbit, its neutralizing capacity tested on mice (C_{57}/BL/6) was 157 μg toxin/ml serum [13].

Anti-rabbit serum prepared in donkey was purchased from Wellcome, Beckenham, England (RD 17) and normal rabbit serum was obtained in our laboratory.

2.1. *Quantitative analysis*

Two successive experiments have been performed in order to check the behaviour of both ^{125}I-labeled and native toxins in the presence of the AaH-toxin I antiserum.

A first quantitative precipitin test allowed to obtain the conditions of maximum precipitation for each toxin and to know the amount of ^{125}I-labeled toxin present in the precipitate (see fig.1). The maximum amount of native toxin precipitated was determined in a second experiment by toxicity assay on the solubilized pellet. A single precipitation was carried out on 20 μg native AaH toxin I applying the same incubation conditions. The antigen–antibody complex was dissociated with 10 M acetic acid* (80 μl). The acid was blown out under a gentle stream of nitrogen and the toxin solubilized in 0.05 M sodium phosphate buffer pH 7.4, containing bovine serum albumin 5°/₀₀ (w/w) (PBA buffer). The insoluble γ globulins were centrifuged off and the toxicity of the supernatant was determined.

From the first experiment it was clear that native

* Toxicity of AaH toxin I was found to be unaltered by this acidic treatment [12]

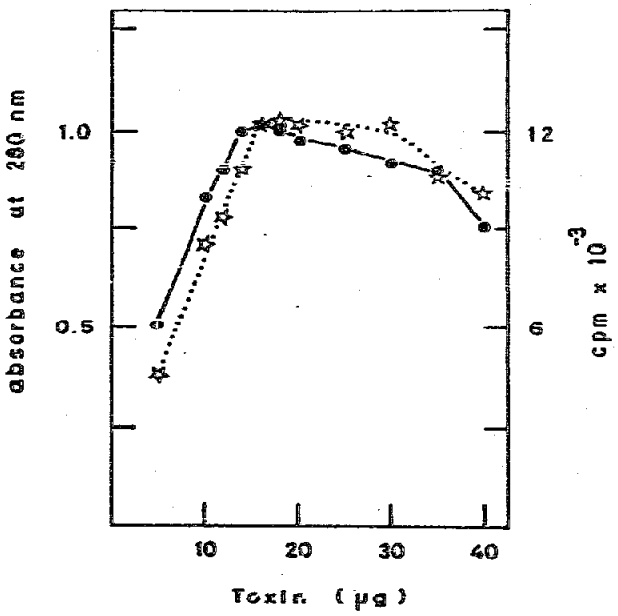

Fig.1. Quantitative precipitation by the serum prepared against AaH toxin I, of a mixture of native and ^{125}I-labeled AaH toxin I. Different volumes of a 0.15 M NaCl solution containing the ^{125}I-labeled and native toxins in a ratio of 1 : 6250 were added to 0.2 ml specific antiserum in a series of test tubes. Volumes were further made up to 1 ml with 0.15 M NaCl. After incubation first at 37°C (1.5 h), then at 4°C (15 h), solutions were centrifuged at 3000 rev/min for 15 min and the supernatants counted for radioactivity. Pellets were washed 3 times in cold saline, then dissolved in 1 ml 0.5 N NaOH for measurements of the A_{280} (●——●) and radioactivity (☆——☆).

and iodinated toxins behave similarly with respect to the antiserum (fig.1). This observation could be extended further: 75% ^{125}I-labeled toxin and 70% native toxin were recovered in the precipitate. This indicated that both toxins behave identically.

2.2. *Radioimmunoassay procedure*

All samples were analyzed in duplicate. All dilutions and final volume adjustments throughout the experiments were achieved with PBA buffer. In test tubes, the diluted reactants were added successively: PBA buffer (0.2 ml), standard toxin or unknown (0.1 ml), ^{125}I-labeled AaH toxin I (0.1 ml), serum against AaH toxin I (0.1 ml). The tubes were counted for total radioactivity, then incubated either for 1 day at 37°C or for 6 days at 4°C. After incubation, the separation of bound from free antigen was carried out by a double antibody system: 0.2 ml normal rabbit serum diluted 300 times (carrier) and 0.2 ml donkey anti-rabbit serum diluted 16 times were added to each tube. The precipitation was allowed to proceed at 4°C during 42 h. The resulting precipitates were centrifuged (12 000 × *g*, 5 min) and counted for bound radioactivity. Results were expressed as the ratio bound : total radioactivity percent (B/T × 100). Corrections have been introduced for ^{125}I decay. The non-specific counts were numbered: for each standard curve one additional sample was incubated with a large excess of native toxin (10^4 times more AaH toxin I than the highest concentration of the scale).

3. Results and discussion

Three standard curves have been established with native AaH toxin I. Conditions and results are given in table 1 and fig.2. Each point stands for the average of closely-related duplicates. These standard curves have been obtained repeatedly with freshly prepared ^{125}I-labeled antigen. In each case, the amount of native AaH toxin I leading to the half-maximum effect was calculated and was found to be 2840, 230 and 7.2 pg for the standard curves I, II and III, respectively. Accurate quantification of the toxin is possible: see standard curves I and II. When dealing with high concentrations of toxin, the reference to standard curve I allows a quicker measure of the toxin. A greater sensitivity is achieved by dilution of the serum and increase of incubation time: standard curves II and III. In the case of standard curve III, very small amounts of toxin (10^{-15} mol) can be detected. These results emphasize the strong avidity of the serum against AaH toxin I towards the ^{125}I-labeled antigen: 50% radioactivity still remains bound even when the antiserum is diluted 1 250 000 times. Taking into account the specific radioactivity of ^{125}I-labeled toxin, we commonly work with 1000 cpm which, up to now, set the limits of the test. In the case of standard curves I and II, it is even possible to carry out the experiment with a two-weeks old ^{125}I-labeled toxin, providing that new reference curves are worked out. On the contrary, standard curve III has to be set up for each new assay with a freshly-prepared ^{125}I-labeled toxin.

Table 1

Standard curve	^{125}I-labeled AaH toxin I	Assay antiserum dilution	Incubation conditions
I	20 000 cpm 0.118 ng	1/25 000	24 h, 37°C
II	5000 cpm 0.029 ng	1/250 000	6 days, 4°C
III	1000 cpm[a] 0.0059 ng	1/1 250 000	6 days, 4°C

[a] Radioactivity countings were made over 10 min

Experimental conditions for the setting up of the standard curves I, II and III shown on fig.2

Three other pure toxins: AaH toxin III, AaH toxin II and Bot toxin I, which belong to the three different groups of toxins (groups 1, 2 and 3, respectively) found in the venoms of North African scorpions, have been studied for competition with ^{125}I-labeled AaH toxin I. Experimental conditions were those of standard curve I. Results are shown also on fig.2. Half-maximum effect was obtained with 30-times more AaH toxin III and 4300-times more AaH toxin II than AaH toxin I. In the case of Bot toxin I, no effect was obtained even with a 4300-times excess of toxin. Considering the venom of *Androctonus australis Hector*, toxin III has been classified first by N-terminal [5], then by the whole sequence determination [14], in the same group as toxin I. It is thus not surprising to find some cross reactivity of both toxins with the serum prepared against AaH toxin I. This result could be expected since toxin III was even precipitated in agarose gel and neutralized by this serum [13]. However, the quantification of toxin I in this venom by radioimmunoassay still remains possible, since it contains less toxin III than toxin I [2]. This method may be also useful to detect, in other scorpion venoms, toxins related to this group. On the contrary, results obtained with Bot toxin I and AaH toxin II do not show any significant cross reactivity between toxins belonging to group 1 and toxins of groups 2 or 3: the half-maximum effect obtained with 12 μg AaH toxin II might be explained rather by a 0.02% contamination by AaH toxin I, than by a very weak reactivity of AaH toxin II with the antiserum. Moreover, the possibility to disclose the AaH toxin I at concentration of about 5×10^{-12} M gives a new tool for studies on the mechanism of action of scorpion toxins. Radioimmunoassay of AaH toxin II and Bot toxin I is now in progress.

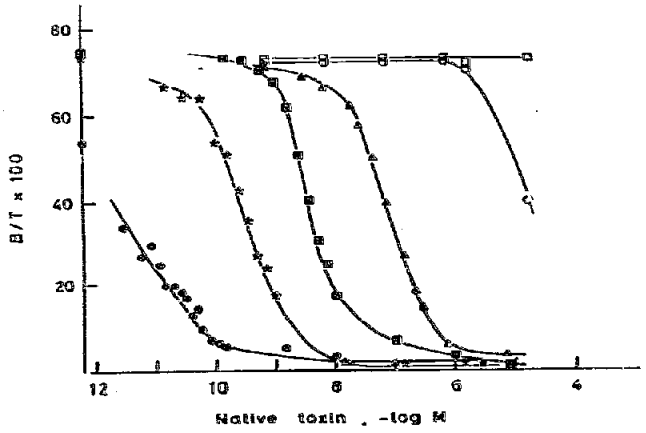

Fig.2. Effect of various scorpion toxins on the binding of ^{125}I-labeled AaH toxin I to the serum prepared against AaH toxin I. Standard curves achieved with AaH toxin I: (■——■) standard curve I; (★——★) standard curve II; (●——●) standard curve III (experimental conditions are described in table 1). For AaH toxin III (▲——▲); AaH toxin II (○——○); and Bot toxin I (□——□). Incubation conditions were the same as for standard curve I. The abscissa is the logarithm of the molar concentration of native toxin in the solution tested. The ordinate is the ratio bound : total radioactivity percent.

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