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Research Article

Characterization of Libyan Cobra (*Naja haje*) Venom Using Fluorescence and UV-Visible Spectroscopy

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Abstract

Snake venoms act as a preparative to defend animals against predators and helps in immobilizing and digestion of prey. Venoms consist of a combination of enzymes and toxins, such as metalloproteases, phospholipase A2, L-amino acid oxidase and toxins, including cytotoxins and neurotoxins. In addition to their toxicity, venom components exhibit several pharmacological activities and can be used as templates for drug design. The Libyan cobra venom was studied using UV-visible and fluorescence spectroscopic techniques. The cobra protein main chain absorbs light in the region of 240 - 340 nm. The aromatic side-chains of cobra venom contain tyrosine, tryptophan and phenylalanine which are responsible for the absorbance in this region. Cobra venom provides intrinsic fluorescence emissions due to excitation of tryptophan residues, with some contribution from phenylalanine and tyrosine emissions. In addition, disulphide bridges contribute considerable absorption in this wavelength range. The main fluorescence obtained is due to tryptophan which has a wavelength of maximum absorption at 280 nm and an emission peak ranging from 310 to 350 nm. UV-visible absorption and fluorescence spectroscopic techniques are sensitive and rapid to study cobra venom in order to better comprehend the performance of this venom.

Keywords: *Snakebite; Envenomation; Libyan Cobra; Protein Fluorescence*

Introduction

Snake bites are crucial public health problem among many African countries including Libya, Tunisia, Egypt and Algeria [1,2]. Africa inhabits more than 400 snake species of which about 30 are extremely poisonous. These species which belong to four different families namely: *Viperidae, Colubridae, Atractaspididae and Elapidae,* were found to be responsible for most human fatalities, as reported by the World Health Organization (WHO) [3]. There are eight species of cobra snakes including *Naja mossambica, Naja haje, Naja nigricollis, Naja siamensis, Naja kaouthia, Naja melanoleuca, Naja sputatrix* and *Pseudechis australis.* The first seven species are cobras from the genus *Naja* and are found throughout Africa including Libya and Asia, while the king brown/mulga snake (*P. australis*) is indigenous to Australia (Cobras (*Naja* spp.)) [4-7].

Cobra and other snake venoms usually show absorption maxima between 275 and 280 nm which are caused by the absorbance of the two aromatic amino acids tryptophan (Trp) and tyrosine (Tyr) and to a small extent, by the absorbance of cystine (i.e. of disulphide

bonds). The absorbances of Trp and Tyr depend on the enviromental changes of their chromophores. They are faintly red-shifted when relocated from a polar to a non-polar surroundings [8-10]. Cobra venom contains three amino acids with intrinsic fluorescence properties, tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) but only tyrosine and tryptophan respond experimentally since their quantum yields are high sufficient to provide a considerable fluorescence signal. The three residues might be used to trail protein folding since their fluorescence characteristics (quantum yields) are responsive to their surroundings which alters when the protein folding or unfolding [11-13].

Aim of the Study

The main aim of our study was to further characterize the Libyan cobra venom using spectroscopy techniques.

Materials and Methods

All experiments were conducted in Tris buffer (0.01M Tris, 0.1M NaCl at pH 7.4). Glass-distilled deionized water and analytical score reagents were used during experiments. pH values were measured for all solutions with a calibrated pH-meter (Jenway model 3510, Staffordshire, UK). All buffer solutions were filtered throughout Millipore filters (Millipore, UK) of 0.45 mm pore diameter.

Absorbance spectra: Absorbance spectra were measured with Analytic Jena Specord 200 Plus (1.4nm band width, scanning, dual beam, single cell holder spectrometer, London, UK) using quartz cells of 1.00 cm path length. UV-vis absorbance spectra were recorded in the two hundreds to five hundreds nanometer range and a spectral bandwidth of three nanometers. For the final spectrum the baseline was subtracted from the buffer solution. The protein concentration of the venom samples was assayed spectrophotometrically using Markwell., *et al*. technique [14]. Bovine serum albumin obtained from Sigma-Aldrich was utilized for standard assays.

Fluorescence spectra: Fluorescence excitation and emission spectra were measured using spectrofluorometer (Jasco FP-6200, Tokyo, Japan) utilizing fluorescence four-sided quartz cuvettes of one centimeter path length. The mechanical shutter-on task was used to diminish photo bleaching of the sample. The selected wavelength chosen provided aggregate excitation of tyrosine and tryptophan residues. The excitation and emission spectrum was corrected for background fluorescence of the buffer.

Venoms: Libyan Cobra (*Naja haje*) venom was extracted by physical stimulation of the animal and acquired in semisolid or liquid form, respectively, from the Department of Zoology, Faculty of Science, University of Tripoli (Libya) and kept at -20°C until utilized. Venoms were added to two milliliter of 0.01M Tris, 0.1M NaCl at pH 7.4.

Results and Discussion

Absorption spectra

Absorption spectra of cobra venom (Figure 1) exhibit absorption maxima between 275 and 280 nm which consequence from the absorbance of the 2 aromatic amino acids tyrosine (Tyr) and tryptophan (Trp) and to a little degree by the absorbance of disulphide bonds of cystine. The absorbances of Tyr and Trp were measured in 0.01 M Tris, 0.1M NaCl at pH 7.4 and therefore the spectra are explicit to this microenvironment. Recently, it has been reported that absorption spectra of proteins are not primarily characterized by the ultraviolet region (185 - 320 nm) of the electromagnetic spectrum but the peptide aggregates revealed absorption beyond 350 nm, caused by monomeric proteins lacking aromatic amino acids, disulphide bonds, and active site prosthetic groups which were expected to remain optically silent beyond 250 nm [15,16]. It was also reported that UV-vis absorption profiles for monomeric proteins rich in charged amino acids spanning 250 - 800 nm have opened up a new label-free optical spectral window for probing biomolecular structure and interactions. By combining experimental and computational studies the authors suggested that the broad absorption profiles of these proteins arise from

photo excited charge transfer (CT) transitions of spatially proximal charged amino acids such as lysine (Lys) and glutamate (Glu). The studies revealed that the tuned Lys-Glu dimer spectrum spans 150 - 650 nm exhibiting five specific types of CT excitations with diverse and large spatial charge separation length scales of 2 - 10 Å. These include inter-/intra-residue peptide backbone to peptide backbone (BB-CT) excitations spanning 160 - 210 nm, inter-/intra-residue peptide backbone to side chain (BS-CT) excitations spanning 160 - 260 nm, and side chain to side chain (SS-CT) excitations, which show the broadest absorption range spanning 260 - 650 nm [17].

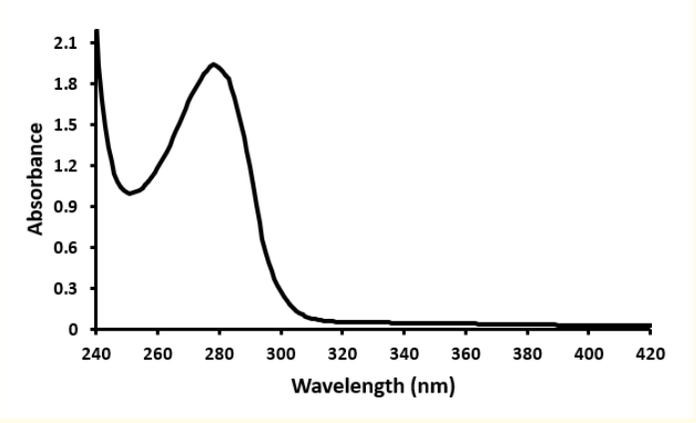

Figure 1: *Ultraviolet-visible absorption spectrum of cobra venom (30 µg/ml) vs wavelength from 240 - 420 nm in 0.01M Tris, 0.1M NaCl at pH 7.4. The spectrum was corrected for small background fluorescence contributions from the buffer solution and was scaled to visualize the pure spectrum of the venom.*

Fluorescence spectra

The fluorescence spectrum shows fluorescence intensity *versus* wavelength (Figure 2) of the Libyan cobra venom. The obtained fluorescence results from molecular rearrangements, ground state, excited-state reactions complex formation, clashing quenching and energy relocation. The fluorescence emission intensity as shown in figure 2 exhibits a maximum emission at 350 nm suggesting that Trp residues activated by the excitation light moved from the buried hydrophobic environment into a relatively polar environment which is consistent with earlier reports [18].

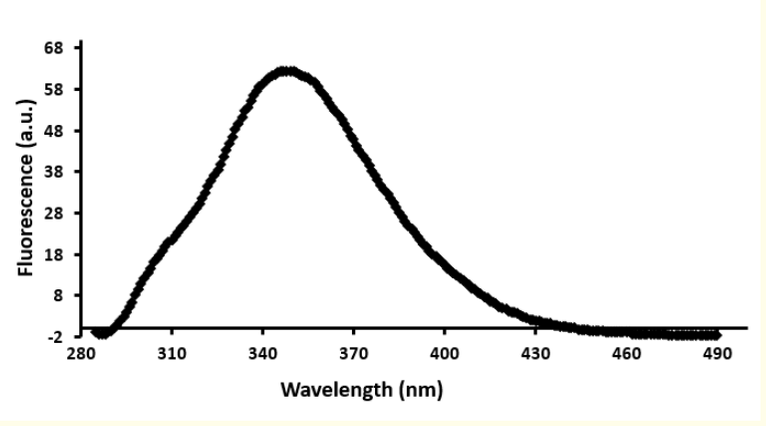

Figure 2: *Plot of fluorescence emission of Libyan Cobra (Naja haje) venom (30 µg/ml) vs wavelength from 285-490 nm using excitation of λ280 nm in 0.01M Tris, 0.1M NaCl at pH 7.4. Spectra were corrected for small background fluorescence contributions from the buffer solution and were scaled to visualize the shift (a.u.: Arbitrary Units).*

Related results indicate an increase of intrinsic tryptophan fluorescence and a blue change of the maximum emission wavelength on addition of MgATP to ArsA ATPase (catalytic subunit of the pump protein), indicating the shifting of tryptophan one hundred fifty nine into a less polar surroundings [19]. The fluorescence of Phospholipases A_2 if excited at two hundreds eighty nanometer is mostly due to the existence of a single tryptophan residue (Trp3) which is situated on the outside of the enzyme molecule exposed to the surroundings [20].

As indicated in the literature the solvent polarity and the local environment have profound effects on the spectral emission properties of fluorophores. The effect of solvent polarity is an important determinant of the Stokes shift, which was clearly observed in our experiments. Emission spectra are simply measured resulting in several publications on emission spectra of fluorophores in many solvents or attached to proteins, membranes, RNA or DNA. One main utilize of solvent effects is to establish the polarity of the probe binding location at the macromolecule. This is accomplished by comparison of emission spectra and/or quantum yields if the fluorophore bound to the macromolecule or dissolved in solvents of different polarity. The consequences of the surroundings on quantum yields and fluorescence spectra are complex and are owing to numerous factors including: probe conformational changes, viscosity, solvent polarity, rate of solvent relaxation, rigidity of the local environment, internal charge transfer, excited state reactions, proton transfer, probe–probe interactions or changes in non-radiative and radiative decay rates. These numerous effects might offer chances to probe the local environment surrounding of the fluorophore. Nevertheless, surroundings consequences are frequently complex and even solvent polarity cannot be described using a solitary theory. The Lippert-Mataga equation partly explicated the consequence of solvent polarity, but does not explain for additional effects for instance hydrogen bonding to the fluorophore or internal charge transfer that depends on the polarity of the solvents [21,22].

Conclusion

The UV-visible absorption and fluorescence spectroscope approaches are sensitive and fast techniques to help the possibilities in searching for natural or synthetic inhibitors for cobra snake venoms for therapeutic purposes. In future studies to investigate interactions and type of venom components considering candidates for these interactions could be performed. We expect that Libyan cobra venom will play an important part in the advancement of understanding snake poisoning (in the near future).

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