Research paper

Structural and functional characterization of complex formation between two Kunitz-type serine protease inhibitors from Russell's Viper venom

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a b s t r a c t

Snake venom Kunitz-type serine protease inhibitors (KSPIs) exhibit various biological functions including anticoagulant activity. This study elucidates the occurrence and subunit stoichiometry of a putative complex formed between two KSPIs (Rusvikunin and Rusvikunin-II) puri

complex was insuf ciently characterized in structural terms, and little evidence was presented to support a structural association of the components of the complex. Furthermore, our studies also suggested that the Rusvikunin complex contributes to the overall toxicity of RV bites [9], thus warranting further characterization. Therefore, in this study we identify the protein components of the Rusvikunin complex by LC-MS/MS analysis. Furthermore, spectro- uorometric, classical gel-

3. Results and discussion

Fractionation of Rusvikunin complex (see Supplementary Fig. S1) on a RP- C_{18} HPLC column resulted in separation into two protein peaks - one large peak, named Rusvikunin-II [9], and a small peak, named Rusvikunin [8]

a_2e macroglobulin also inhibited the brinogenolytic activity of Rusvikunin complex (supplementary Table SII

ltration column which was equilibrated with buffer containing Rusvikunin-II resulted in appearance of three major protein peaks with retention times of 11.7 min, 12.1 min and 12.3 min with corresponding molecular mass of ~21 kDa, ~14 kDa and ~7 kDa, respectively (Fig. 4A). The intensity of these equilibrium gel-ltration peaks was dependent on the injected Rusvikunin: Rusvikunin-II ratio (Fig. 4A). The highest intensity of the two major peaks eluted from the GF column with a retention time of 11.7 min

Additionally, this technique is quite ef cient for studying protein-protein interactions in solution [25,26], and measurement of CD in the far UV region (178 e 260 nm) can provide valuable information on changes in the conformations of proteins when they interact to form a complex [26]. The CD spectra of Rusvikunin and Rusvikunin-II demonstrated predominance of b-sheet structure (~60%) which is in accordance to our previous observations [8,9]. Interaction of Rusvikunin with Rusvikunin-II at 1:1 and at 1:2 molar ratio did not show any signi cant change in the secondary structure of interacting proteins (Fig. 5). These data support the earlier observations that protein-protein interactions may result in only

3.6. Determination of interaction between Rusvikunins in solution

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biochi.2016.08.005 .

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