

Ancordin, the major rhizome protein of madeira-vine, with trypsin inhibitory and stimulatory activities in nitric oxide productions

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Abstract

Anredera cordifolia (Ten.) Steenis, or the synonymous name of *Boussingaultia baselloides* or *Boussingaultia gracilis* var. *pseudobaselloides*, is a South American species of ornamental succulent vine, commonly known as the madeira-vine. The fresh leaves of madeira-vine are frequently used as vegetables. *A. cordifolia* is an evergreen climber that grows from fleshy rhizomes. The rhizome contained one major (23 kDa) protein band under non-reducing condition in the SDS-PAGE. The first 15 amino acids in the N-terminal region of the major protein band (23 kDa), named tentatively ancordin, were KDDLVLVDIGGNPVV which were highly homologous to sequences of winged bean seed protein ws-1, *Medicago truncatula* proteinase inhibitor, soybean trypsin inhibitor, and sporamin. By using activity stains, the ancordin showed trypsin inhibitory activity in the SDS-PAGE gel which was found not only in rhizomes but also in aerial tubers, but few in fresh leaves. The crude extracts from rhizomes of madeira-vine were directly loaded onto trypsin-Sepharose 4B affinity column. After washing with 100 mM Tris-HCl buffer (pH 7.9) containing 100 mM NaCl, the ancordin was eluted directly by 0.2 M KCl-HCl buffer (pH 2.0). In calculation, the purified protein exhibited 0.0428 mg trypsin inhibition/mg ancordin (corresponding to 0.53 unit of TPCK-treated trypsin inhibited/mg ancordin). The purified ancordin was used to evaluate the nitric oxide productions in RAW264.7 cells in the presence of polymyxin B (poly B, 50 mg/ml) to eliminate the lipopolysaccharide (LPS) contaminations. It was found that ancordin (1.25–5 mg/ml) could dose-dependently ($R = 0.954$) stimulate the nitric oxide (NO) productions (expressed as nitrite concentrations) in RAW264.7 cells without significant cytotoxicity, and kept the similar effects in NO production in 6.25 mg/ml ancordin.