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湖北钉螺凝集素分离纯化及相对分子质量的测定

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Isolation and Purification of *Oncomelania hupensis* Agglutinin and Determination of its Molecular Weight

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摘要 目的 分离、纯化湖北钉螺（*Oncomelania hupensis*）体内凝集素，测定其相对分子质量。 方法 用饱和度为0～40%的硫酸铵对钉螺腹足部软体组织匀浆液进行分离，对其沉淀物先后采用Sephadex G-75凝胶过滤层析和Sepharose 4B亲和层析进行纯化。采用Brandford法测定纯化后凝集素的蛋白质含量，红细胞凝集试验测定其凝集活性，并计算比活力。用十二烷基硫酸钠?聚丙烯酰胺凝胶电泳（SDS-PAGE）检测凝集素的纯化效果，并测定其亚基相对分子质量。利用Sephadex G-75凝胶层析测定钉螺凝集素的相对分子质量。 结果 饱和度为0～40%硫酸铵对钉螺凝集素组织匀浆液进行分离，比活力从组织匀浆液的21.74 titer/mg提高到了61.93 titer/mg。经Sephadex G-75凝胶层析和Sepharose 4B亲和层析，钉螺凝集素的比活力分别提高到75.89 titer/mg和963.86 titer/mg。对凝胶过滤层析和亲和层析纯化后的凝集素进行SDS-PAGE分析，可见单一清晰条带，凝集素亚基的相对分子质量约为M_r 53 000。凝胶层析测定钉螺凝集素的相对分子质量约为M_r 78 000。 结论 盐析法、凝胶层析和亲和层析联合应用可对湖北钉螺凝集素进行有效的分离纯化。湖北钉螺凝集素为单亚基蛋白。

关键词： 湖北钉螺 凝集素 分离 纯化 相对分子质量

Abstract： Objective To isolate and purify agglutinin from *Oncomelania hupensis* snail and determine its molecular weight. Methods Agglutinin was preliminarily isolated from snail tissue homogenate by 0%-40% saturated ammonium sulfate，and then successively purified with Sephadex G-75 gel filtration and Sepharose 4B affinity chromatography. Bradford assay was used to determine the protein content. The agglutination activity was determined by rabbit erythrocytes. The purity of agglutinin preparations was assessed by SDS-PAGE. The molecular weight of agglutinin subunit was determined by Sephadex G-75 gel filtration. Results The specific activity of snail tissue homogenate was 21.74 titer/mg. After ammonium sulfate precipitation， Sephadex G-75 gel filtration and Sepharose 4B affinity chromatography， the specific activity of snail agglutinin from the homogenate solution increased to 61.93 titer/mg，75.89 titer/mg and 963.86 titer/mg， respectively. SDS-PAGE analysis indicated that snail agglutinin （M_r 53 000） was purified by Sephadex G-75 gel filtration and Sepharose 4B chromatography. The molecular weight of the snail agglutinin produced by Sephadex G-75 gel filtration was M_r 78 000. Conclusion Combined use of salt fractionation，gel filtration and affinity chromatography can be efficient for extraction and purification of agglutinin from *Oncomelania hupensis* species. The snail agglutinin is characterized as monosubunit protein with a molecular weight of M_r 78 000.

Keywords： *Oncomelania hupensis* Agglutinin Extraction Purification Molecular weight

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