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Isolation and purification of staphylococcus aureus hibernationpromoting factor inactivating of the ribosome

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Abstract

© 2016, International Journal of Pharmacy and Technology. All rights reserved.In Staphylococcus aureus hibernation-promoting factor (SaHPF) binds to ribosomes depleting translation and turning metabolism of this pathogenic bacteria in energy saving mode. This phenomenon can be recognized as unspecific resistance mechanism, which is exploited by staphylococci to persist in presence of large spectrum of antibiotics. Investigation of structure of SaHPF and its interaction mechanisms with ribosome can aid to design new drugs, rendering S. aureus sensitive to antibiotics. We developed techniques for SaHPF purification, which provide this protein in appropriate quantity and quality for further structural analysis. Gene Sahpf has been cloned in expression vector pGS21a. To facilitate purification process His-tag coding sequence was introduced into Sahpf gene to generate SaHPF protein with six histidine residues on C-terminus. Eschercha coli strain BL21star (DE3) harboring Sahpf::pGS21a was used for the protein expression. The protein purification was conducted using affinity chromatography with Ni-NTA resin followed by size-exclusion chromatography in Superdex-75. High purification rate and homogeneity of SaHPF preparations obtained allows further study of this protein using nuclear magnetic resonance and development of crystallization procedure for X-ray analysis.

Keywords

Hibernation-promoting factor Staphylococcus aureus, Isolation and purification protein, NMR, Ribosome, Structure