Inhibitory Effects of Novel Immucillin Analogues on *Borrelia burgdorferi* Bgp Nucleosidase

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Recombinant Bgp was expressed and purified. Enzyme purity was confirmed by Spectrophotometric analysis of Bgp activity were run using Initial and delayed onset velocities (V_0', V_0) using B. burgdorferi ranging from 6pM-analogues are potent inhibitors of binding protein (SAM) and are kept at low micromolar concentrations due to their inhibitory activity.

This project examined the effect of transition state inhibitors on the surface binding B. burgdorferi glycosaminoglycan-binding protein (Bgp) nucleosidase using recombinant Bgp and whole-cell B. burgdorferi activity assays. The transition state analogues are potent inhibitors of Bgp activity with K_i values ranging from 6pM-6nM. Bgp on the surface of live B. burgdorferi was also inhibited by treatment with low nanomolar concentrations of transition state analogues.

Immucillin transition state analogues will be potent inhibitors of Bgp nucleosidase and antibiotics to treat Lyme disease.

The pathogenic spirochaete B. burgdorferi causes Lyme disease and is transmitted by deer ticks when they feed. Lyme disease is multisystemic—it adversely affects the heart, joints, and skin. Recent studies demonstrate that B. burgdorferi possesses three methylthioadenosine/S-adenosylhomocysteine (MTA/SAH) nucleosidases essential for the catabolic breakdown of both MTA and SAH. Both MTA and SAH are by-products of major pathways involving S-adenosylmethionine (SAM) and are kept at low micromolar concentrations due to their inhibitory activity.

Methods

- Recombinant Bgp was expressed and purified
- Enzyme purity was confirmed by SDS-Polyacrylamide Gel Electrophoresis
- Spectrophotometric analyses of Bgp activity were run using Cary 50 and 100 UV-Visible Spectrophotometers
- Using Beer’s Law (A=εcL), ε = 1.6mM⁻¹cm⁻¹ determined concentration of nucleosidase substrate in assay
- Initial and delayed onset velocities (V_0, V_0', V*) were calculated with varying concentrations of inhibitor
- Using IgorPro, inhibition constants (K_i) were calculated by fitting data to the equation for competitive inhibition: V_0'/V_0 = (K_m + [S])/((K_m+[S]+K_i)/K_i)

Results

- Bgp is an active MTA nucleosidase found on the surface of B. burgdorferi
- Immucillin analogues were potent inhibitors of recombinant Bgp with subnanomolar to low nanomolar K_i values
- Bgp was readily inhibited in whole cell assays using low nanomolar concentrations of Immucillin analogues

Conclusion

The transition state analogues inhibit Bgp and live B. burgdorferi whole cells. Further studies can test inhibitors against other MTA/SAH nucleosidases and lead to the development of new drugs that combat Lyme disease.

References


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