Title: Structure-function studies of intrinsically disordered outer surface proteins of Lyme spirochetes.

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Abstract:

Predicted outer surface proteins of Borrelia burgdorferi, the causative agent for Lyme disease, were evaluated for the presence and absence of intrinsically disordered regions (IDRs).Based on these results, borrelial outer surface proteins were grouped into four classifications: a) IDP-Foldon b) Foldon-IDR c) IDR-foldon-IDR and d) Foldon-IDR-Foldon. We hypothesize that the intrinsic disorder in these borrelial outer surface proteins are essential for interaction with host proteins. As a result we have chosen to study Mlp (multi copy lipoproteins) of the first category which have disordered N-terminus and folded C-terminus. Mlp's are a paralogous family of proteins with nine members MlpA, MlpB, MlpC, MlpD, MlpF, MlpG, MlpH, MlpI and MlpJ. Little is known about the structure and function of Mlp proteins, however, they are expressed during infection in the mammalian host. In order to seek potential host interaction partners and to test our hypothesis that IDPs are signatures for eukaryotic protein interactions, we have cloned Mlp's and have recombinantly expressed them in E.coli. We then aim to 1) screen for potential interactions with host ligands present in human blood and or in the extracellular matrix in order to test our hypothesis that IDRs are a necessary feature of these proteins for interacting with host molecules, 2) and solve high-resolution three-dimensional crystal structures of selected Mlp proteins. We believe that these studies will underpin how bacterial IDRs physically interact with eukaryotic proteins and provide a platform to quantitatively study a potentially fundamental aspect of host-pathogen interaction at the molecular level.

Sequence Alignment :

Sequence Alignment of Mlp proteins reveal two distinct classes with MlpB and MlpJ (sharing 80% identity) and the other class of MlpA, MlpC, MlpD, MlpH, MlpI, MlpF, MlpG (sharing 65-80%) identity.

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MlpB      MKIINILFCLLLIVLNSCNANDNDTFNNNSVQQTKSRKKRDLSQKELLQQEKITLTSDEE  60
MlpJ      MKIINILFCISLLLLNSCNSNDNDTLKNN-AQQTKSRKKRDLSQEELPQQEKITLTSDEE  59
          *********: *::*****:*****::** .*************:** ************

MlpB      KMFTSLVTAFKYTVEKLSGDTNGCNNENKNKCTGFFDWLSEDIQKQKELAGAFTKVYNFL  120
MlpJ      KMFTSLINVFKYTIEKLNNEIQGCMNGNKSKCNDFFDWLSEDIQKQKELAGAFTKVYNFL  119
          ******:..****:***..: :** * **.**..*************************

MlpB      KSKAQNEAFDTYIKGAIDCKKTLHKIVIIITNM-EKVRTKRAYFRGVAGSIFTDNNDNDG  179
MlpJ      KSKAQNETFDTYIKGAIDCKKNTPQDCNKNNKYGDGDNLIEQYFRGVANDMS-NRNSNEE  178
          *******:*************.  :     .:  :  .  . ******..:  :.*.*:

MlpB      IYKCLKDELLNDTSNHYEGLTSDWDN    205
MlpJ      IYQYLKDELLKE-DNHYAGLTANWQN    203
          **: ******:: .*** ***::*:*

MlpG      MKIINILFCLFLLMLNGCNSNDTN-----TKQTKSRQKRDLTQKEATQEKPKSKSKEDLL  55
MlpH      MKIINILFCLFLLMLNGCNSNDNDTLKNNAQQTKSRRKRDLTQKEVTQEKPKS--KEELL  58
MlpC      MKIINILFCLFLLMLNGCNSNDNDTLKNNAQQTKRRGKRDLTQKETTQEKPKS--KEELL  58
MlpF      MKIINILFCLFLLLLLNSCNSNDNDTLKNNAQQTKSRGKRDLTQKEATPEKPKS--KEELL  58
MlpA      MKIINILFCLFLLLLNSCNSNDNDTLKNNAQQTKSRGKRDLTQKEATPEKPKS--KEELL  58
MlpI      MKIINILFCLFLLMLNSCNSNDTN-----TSQTKSRQKRDLTQKEATQEKPKS--KEDLL  53
MlpD      MKIINILFCLFLLMLNGCNSNDTN-----NSQTKSRQKRDLTQKEATQEKPKS--KEELL  53
          *************:**.*****.:      .*** * ********.* *****   **:**

MlpG      REKLSDDQKTQLDWLKTALTGVGKFDKFLENDEGKIKSALEHIKTELDKCNGND---EGK  112
MlpH      REKLNDDQKTQLDWLKTALTDAGEFDKFLENNEDKIKSALDHIKSELDKCNGKENGDVQK  118
MlpC      REKLSDDQKTHLDWLKPALTGAGEFDKFLENDDDKIKSALDHIKTQLDSCNGDQ-AEQQK  117
MlpF      REKLSEDQKTHLDWLKEALGNDGEFDKFLGYDESKIKSALNHIKSELDKCTGDN-SEQQK  117
MlpA      REKLSEDQKTHLDWLKEALGNDGEFDKFLGYDESKIKTALDHIKSELDKCNGND-ADQQK  117
MlpI      REKLSEDQKTHLDWLKTALTGAGEFDKFLGYDEDKIKGALNHIKSELDKCTGDN-SEQQK  112
MlpD      REKLNDNQKTHLDWLKEALGNDGEFNKFLGYDESKIKSALDHIKSELDSCTGDK-VE-NK  111
          ****.::***:***** ** . *:*:***  ::.*** **:***:**.*.*..     *

MlpG      NTFKTTVQGFFSGGNIDNFADQATATCN----      140
MlpH      NTFKQVVQGALKGGIDGFGASNATTTCNGS--      148
MlpC      TTFKTVVTEFFKNGDIDNFATGAVSNCNNGG-      148
MlpF      STFKQTVQGFFSGGNIDNFANNAVSNCNNGGS      149
MlpA      TTFKQTVQGALSGGIDGFGSNNAVTTCGNGS-      148
MlpI      STFKEVVKGALGGGIDSFA-TSASSTCQAQQ-      142
MlpD      NTFKQVVQEALKGGIDGFE-NTASSTCKNS--      140
          .*** .*   : .*  .     * :.*
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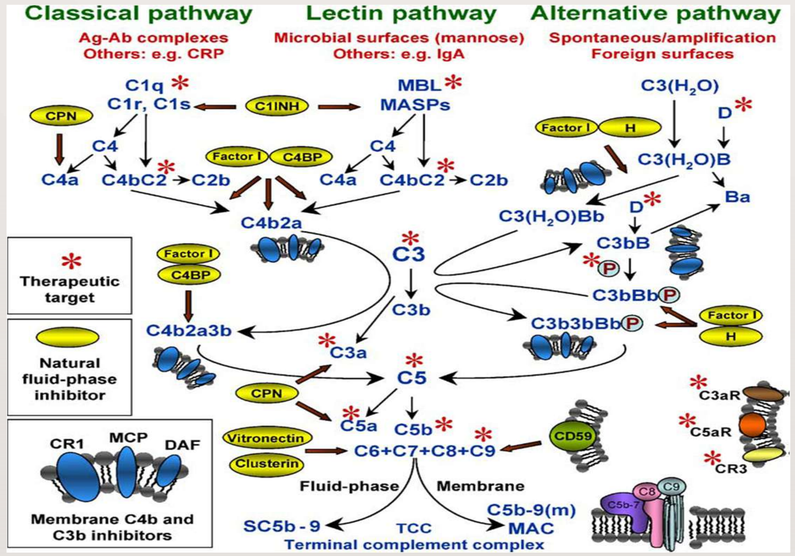
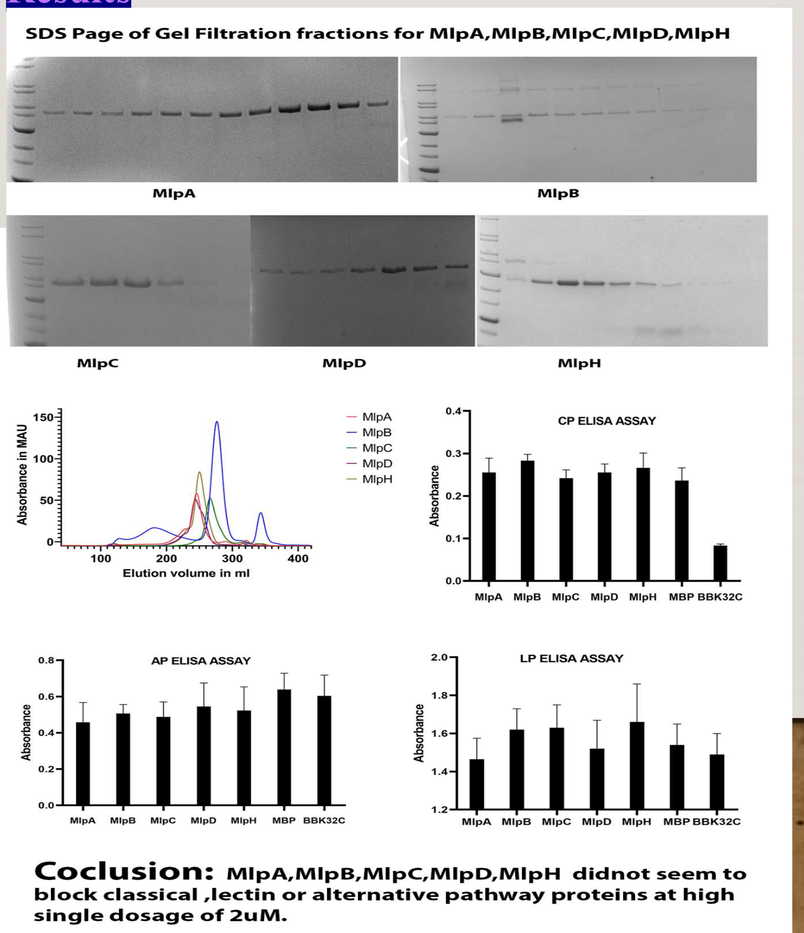
Complement System of Innate Immunity



Results



Coclusion: MlpA,MlpB,MlpC,MlpD,MlpH didnot seem to block classical ,lectin or alternative pathway proteins at high single dosage of 2uM.

Future Work:

1) Developing Functional Screens for Mlp and other IDP proteins for elucidating functions hitherto unknown.

2) Crystallization and structure solution of Mlp proteins for understanding structural features of these IDP proteins.

3) Identifying more outer surface proteins that are involved in pathogenesis of Lyme disease.

4) Solving crystal structure of these newly identified proteins.

5) Elucidating functions of these newly identified proteins and identifying structure function relationships.

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