53rd Annual Meeting of SBBq

53rd Annual Meeting of the Brazilian Society for Biochemistry and Molecular Biology (SBBq)
Águas de Lindóia, SP, Brazil - May 18th to 21st, 2024

SBBq

D.11- Development of Aminoquinolines as Fluorescent DNA Minor Groove Probes

Bertozo, L.C.1, Ximenes, V.F.1
1Química, Faculdade de Ciências, Universidade Estadual Paulista (São Paulo, Brasil)

Fluorescent probes are commonly used to measure DNA and examine the interaction with newly developed ligands. Aminoquinolines are fluorescent compounds that are sensitive to medium hydrophobicity. This study aimed to investigate the interaction of aminoquinolines with DNA and to develop new fluorescent probes that can be used to investigate DNA-ligand interactions. The binding between DNA and aminoquinolines was evaluated based on fluorescence enhancement, from which the binding constant, the effect of ionic strength, and competition with DNA ligands were assessed. The study found that when a newly developed amino-substituted aminoquinoline (AQ) interacts with DNA, it results in a unique fluorescent band centered at 590 nm. This red-shifted fluorescent band is associated with a new absorption band (490 nm), showing that a ground-state complex has formed. The formation of the complex was also demonstrated through circular dichroism, anisotropy, and fluorescence lifetime. The complex was pH-dependent and optimized at an acidic medium. This preference was due to the interaction with a protonated form of AQ, AQ(H+), and the role of electrostatic forces. This was confirmed by the strong dependence on the ionic strength of the medium, specifically on magnesium ions. The study also found that the complex was specific to the DNA minor groove, compared to established DNA probes such as Hoechst stain, acridine orange, and ethidium bromide. The studies revealed the advantages of aminoquinolines over the commercial DNA minor groove stain, the Hoechst dye. Hence, we propose its further application in cell-based assays.
Keywords: Fluorescent Probe, DNA, Minor Groove

D.12- Interaction studies between Plasmodium falciparum Activator of the Hsp90 ATPase activity 4 (PfAha4) cochaperone with the Hsp90 Middle Domain (Hsp90M).

Rocha, A.M.F.1, Seraphim, T.V.1, Dau, M.J.F.1, Lopes, I.E.S1, Borges, J.C.1
1Instituto de Química de São Carlos, Universidade de São Paulo (São paulo, Brasil)

Hsp90, a 90 kDa Heat shock proteins, function as a molecular chaperone crucial for maintaining proteostasis. They interact with misfolded proteins, helping in their correct folding process, in addition to preventing aggregation. They assist in protein transport throughout the cell and are related to marking proteins for degradation. Therefore, Hsp90s are pivotal for the survival of various protozoan parasites like Plasmodium falciparum and Leishmania braziliensis. Consequently, inhibition or alteration in the cycles of these proteins represent potential therapeutic targets. Preventing the correct development of microorganisms that cause diseases such as malaria and leishmaniasis in humans. Cochaperones are a group of proteins that directly modulate and assist the HSP90 activity. Among them, the Activator of HSP90 ATPase activity (Aha) stands out. They bind to Hsp90 during its catalytic cycle at the N-terminal and middle domain, thereby enhancing the chaperone's stability and stimulating its ATPase activity. Currently, four distinct Aha isoforms have been identified, labeled 1-4 herein. Here, we will focus on P. falciparum Aha4 (PfAha4) isoform. This work aims to help understand the interaction between the recombinant construction of the Middle domain of PfHsp90 (PfHsp90M) and the recombinant PfAha4. The recombinant PfHsp90M and PfAha4 proteins were expressed in Escherichia coli (BL21DE3) strain by the pET28a expression vector. Both were purified by using two chromatographic steps: Ni+2 affinity followed by size exclusion chromatography. Circular dichroism, intrinsic tryptophan fluorescence and SDS-PAGE electrophoresis were used to investigate the stability of the proteins at different pHs. To evaluate the interaction, the isothermal titration calorimetry was used, together with the analytical size exclusion chromatography technique. The proteins were expressed and purified folded with a high degree of purity and were shown to be stable under the different pHs conditions analyzed. The PfAha4 interacted with PfHsp90M construction driven by both enthalpy and entropy. It is concluded, therefore, that under the pH conditions tested, the interaction between the PfAha4 and PfHSP90M proteins occurred, and it was also observed that the protein maintained its structure even when the pH varied. Keywords: Aha, Hsp90, P. falciparum