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A new functional model for prediction of chaperone activity of the recombinant *M. tb* Acr (α-Crystallin) using insulin as substrate

Mycobacterium tuberculosis Acr is an important protein expressed in latent tuberculosis which is active as an oligomer in preventing misfolding of cellular proteins. In this study, *Mycobacterium alpha crystallin (Acr)* gene was cloned and expressed in *E. coli*. The recombinant Acr protein was purified by Nickel-NTA resin. The oligomeric state of Acr was confirmed by gel filtration chromatography using Sephacryl S-200 and Native-PAGE. The activity of recombinant Acr was checked by preventing thermal aggregation of citrate synthase at 45 ºC and the chaperone activity against insulin B chain aggregation at 60 ºC and 37 ºC. Chaperone activity studies were performed with insulin at different mole ratios of Acr with two types of samples, His tag elutes (H) and His tag elutes with gel filtration (G). Polynomial graphs were plotted which could be used to predict activity. It was observed that ratio of different sizes of oligomers (9 to 24 mers) had a significant effect on chaperone activity. Using mole ratio of Acr for both (H) and (G) samples to insulin substrate and ratio of oligomers, we determined number of Acr molecules binding to insulin as a model substrate. We found that if 1.54% of the insulin chain is covered completely by the (G) samples, aggregation is completely inhibited as compared to 6% with (H) samples. Pre-heat treatment studies were carried out at 37 ºC, 60 ºC and 70 ºC. Far-ultraviolet circular dichroism (UV-CD) analysis provided fresh insights into the role of β sheets and α helices in activity, especially in (H) samples suggesting a reversible transition from α helices to ß sheets. This enabled us to formulate a functional model for binding of Acr to insulin B chain which incorporated four types of secondary structure molecules. This is a useful tool to analyze *in vitro* preparations of recombinant Acr and build more consensuses on the structure activity relationship especially in terms of oligomeric ratios.

Biography

Gautam Krishnan has worked at Astra Zeneca India Pvt. Limited, Bangalore, Biocon, India Pvt., Ltd., Bangalore, Goodwin Biotechnology, Goa, India. At Astrazeneca, he worked on drug discovery programs for malaria and antibiotics with a team of 5 personnel, facilitated screening of 20,000 compounds for an anti-malarial drug discovery program and in developing enzymatic assays for anti-malarial drug discovery program and anti-bacterial program. At Biocon, he was part of an R&D team that launched insulin and is credited with 2 international and 2 Indian patents. At Goodwin Biotechnology India, Goa, he helped establish a new lab in Goa, India, the subsidiary of Florida-based, Goodwin Biotechnology Inc., undertook two and half-month training at Goodwin Biotechnology USA in 2006 to acquire experience in c-GMP. He has two publications to his credit.

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