Poster I-66

Crystal structure of hemoglobin from Cerdocyon thous at 2.2 Å resolution using syncrotron radiation.

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Short Abstract: The blood of Cerdocyon thous was purified, and crystals were grown by the vapor-diffusion .The structure has been determined to a resolution of 2.2 Å. The R and Rfree factor are 0.232 and 0.168. Final quality was checked by PROCHECK. Thanks to EMBRAPA, LNLS,CNPq and RENABIME.

Long Abstract:

Crystal structure of hemoglobin from Cerdocyon thous at 2.2 Å resolution using syncrotron radiation.

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Cerdocyon thous is an important reservoir of the Latin America zoonotic visceral leishmaniasis and it is responsible for the endemic nature at the rural and peri-urban areas. C. thous is found naturally infected by Leishmania chagasi and the Lutzomya longipalpis is a natural vector of this protozoose. The blood of the C. thous was collected with EDTA and washed three times with 150 mM NaCl. Red blood cells were collected by centrifugation and the hemolysates were prepared by lyses of the washed erythrocytes with 4 volumes of water, followed by a second centrifugation step. The extract was fractionated with 80% ammonium sulfate to precipitate the hemoglobin and dialyzed. The conventional preparative size exclusion chromatography with a Sephadex G-100 column was also performed in order to obtain large amount of protein.

Crystals were grown by the vapor-diffusion hanging-drop technique using the screening kits for protein crystallization. The crystals were grown in the presence of PEG 4000 and PEG 8000 as a precipitant at pH range from 6.5 to 8.5. Crystals were mounted in loops and data was collected at the CPR-DB03 beam line of the LNLS with a MarCCD 165 mm detector. Processing was performed with HKL2000 with data from 40.00 to 2.20. Indexing showed an orthorhombic crystal lattice and space group P212121 with cell parameters a = 52.73, b = 84.24 and c = 130.28. The crystal structure has been determined to a resolution of 2.2 Å. The structure was solved by molecular replacement using the structure of Chrysocyon brachyurus (PDB entry 1FHJ) as a starting model. Simulated annealing was performed with the CNS software in the first cycle or refinement. Each refinement run with CCP4 was followed by manual intervention using the molecular graphic program O. The R and Rfree

factor are 0.232 and 0.168, respectively. The final quality of the model was checked by PROCHECK.

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