ESRF

Experiment title:

MAD Phasing for T5 exonuclease

Experiment number:

LS326

Beamline:

BL19

Date of Experiment:

from: 9/12/95 to: 13/12/95

Date of' Report:

8/8/1996

Shifts:

9

Local contact(s):

Andy Thompson

Received at ESRF :

27 AUG 1996

Names und affiliations of applicants (*indicatesexperimentalists):

Dr. D. Suck
EMf3L
Postfach 10.2209
69012 Heidelberg
Germany

*Dr. T. Ceska

Report:

A successful MAD experiment was carried out on BL19 on SeMet labelled protein crystals of T5 5-exonuclease." The paper describing the structure has been published in Nature 382 90-93 (1996); Ahelical arch allowing single-stranded DNA to thread through T5 5-exonuclease; T.A. Ceska, J.R. Sayers, G.Stier & D. Suck.

General comments on ESRF Facilities.

The local contact for my experiment Andy Thompson .(EMBL, Grenoble) was extremely helpful in setting up the experiment and in starting data collection and processing.
The support from the EMBL-Grenoble in, for example, providing a MAR imaging plate scanner was very important to the quality and ease of the data collection.

ABSTRACT

5’-Exonucleases are essential enzymes involved in both DNA replica. tion and repair[1]. Apart from their obvious exonucleolytic action removing nucleotides from the 5’-end of nucleic acid molecules such as Okazaki fragments[2] many 5’-3’ exonucleases have been shown to possess intriguing endonucleolytic activities[3,4]. T5 5’-3’ exonuclease shares many similarities with LN-termini of eubacterial DLNA polymerases[5], however, unlike eubacteria. phages such as T5. T-4 and T7 express polymerase and 5’-cxonuclease proteins from separate genes. The 2.54 Å crystal structure of the phage T5 5'-exonuclease reveals a novel motif for binding DNA, the helical arch. A model consistent with a threading mechanism is proposed where single-stranded DNA could slide through the arch. formed by two helices containing positively charged and hydrophobic residues respectively. The active site is at the base of the arch and contains two metal binding sites.