Staphylococcal Nuclease Expression and Purification

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ABSTRACT

During my time in the 2004 REU program in Tallahassee, I worked as a research assistant in Piotr Fajer's lab at the IMB and NHMFL; I learned the methodology and equipment used to express and purify staphylococcal nuclease for use in experiments that aim to demonstrate the viability of W-band EPR (95GHz; later studies will work at frequencies as high as 220GHz) as a tool to identify structural information about proteins [2], as well as to determine the amount of motion MSL and MTSSL labels undergo in relation to the proteins they are attached to. Staphylococcal Nuclease, a DNA-cleaving enzyme from the bacterium *Staphylococcal Aureus*, has been extensively studied since 1966 [1]. Its structure and amino acid sequence are well known, and methodology has long been in place for its controlled mutation. My focus has been on the microbiology and biochemistry involved making the W-band study possible.

INTRODUCTION

There are no actual results addressing the experimental questions thus far [3], and an adequate description of the full significance of the obtained EPR spectra are beyond the scope of my experience and understanding. Therefore I will present the methodology used in preparation of mutants of staphylococcal nuclease. The T22C variant (meaning that the amino acid *T*hreonine at the 22 position was mutated to the amino acid *C*ystein; see figure 1) was the most successfully expressed mutant; T13C and T44C had minimal expression by late July. Below I will outline the method used to express and purify these mutants.

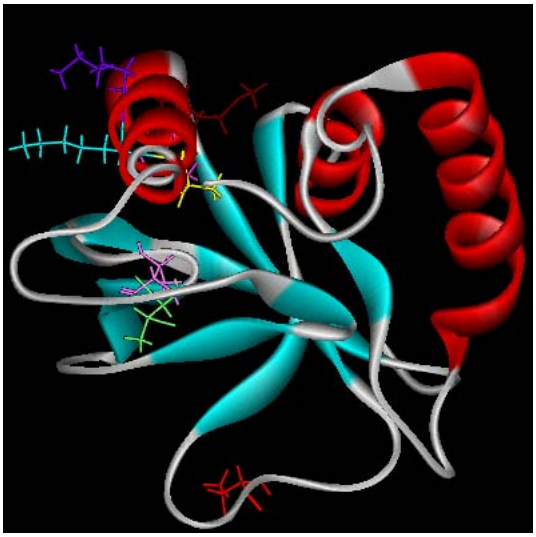

Figure 1. Diagram of staphylococcal nuclease. MTSSL label at T22C shown in green; label at T44C shown in purple. T13C not visible.

METHOD

Lambda Expression System in *E. Coli* used. Mutant strains are taken from glycerol stocks stored at -80°C. This sample is suspended in 1-2 ml of LB broth at 100µg/ml ampicillin and grown at 30°C for 3-5 hours. Once this small grow-up is complete, 1ml is used to inoculate 250ml of TB media, also made 100µg/ml in ampicillin. This is grown in 37°C for 12-18 hours.

Once the 250ml culture is complete, it should be tested for successful growth with the spectrophotometer. The OD_{600} of this culture should be between 15-30.

The culture is then centrifuged at 3500-4000 rpm at 4°C for 10 minutes. Bacterial cells will have collected in the pellet, which is resuspended in 50ml of extraction buffer. Once the cells are resuspended, they are swirled on ice for 30 minutes. The suspended cells are centrifuged again at 3500-4000 rpm at 4°C for 10 minutes. The supernatant is discarded, leaving a loose pellet. Much of the contaminating material should have been removed by this stage, without appreciable loss of nuclease. However, gel electrophoresis of a 10µg sample against a sample of nuclease is advised, to make sure that the rest of the process is worth continuing! Assuming a workable protein content, the cell pellet is resuspended in 50 ml of extraction buffer #2, and swirled on ice for 30 minutes. The sample is now centrifuged at 8000 rpm at 4°C for 15 minutes, to remove cellular material, leaving only protein in the supernatant.

The nuclease is now separated from other protein by a series of ethanol precipitations. The supernatant is poured into a new centrifuge bottle, excluding the pellet. An equal volume (approximately 50ml) of cold 200 proof ethanol is added and the whole sample is incubated undisturbed at -20°C for 3-5 hours. A large precipitate of unwanted protein should have formed. This is discarded and the supernatant is poured into a new bottle, with the addition of 100 ml of cold 200-proof ethanol. The sample is then incubated undisturbed at -20°C for 12 hours. After centrifuging the sample at 5000 rpm at 4°C for 10 minutes, the supernatant can be discarded. The pellet should contain mostly nuclease at this point, and is resuspended in column load buffer (as little as possible) for the final stage of its purification. Another gel electrophoresis test is advisable at this point.

The extract containing the protein is loaded into a cation exchange column, eluted with 4 bed volumes (1ml Pharmacia Fast Flow S-Sepharose) of column load buffer. After the extract has passed the column, it should be rinsed with 10 bed volumes of load buffer. The protein is then eluted with 3 bed volumes of load buffer made 250mM in NaCl. 1/20th volume 1 M TrisHCl pH 7.0 is added to the eluate, and the protein is precipitated by adding 3 volumes of cold 200 proof ethanol and incubating at -20°C for 30 minutes. After centrifuging at 1500-200 RCF at 4°C for 10 minutes and discarding the supernatant, the protein should be about 95% pure. Higher purity can be obtained by running additional columns. 5-15mg of protein is a typical product.

The protein is now suspended in labeling buffer; MTSSL labels will bind with cystein on the protein and the sample will be ready for EPR study.

REFERENCES

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