Expression and purification, Biophysical characteristic verification of recombinant PDZ3 protein (組換 PDZ3 タンパク質の発現・精製と物性検証)		
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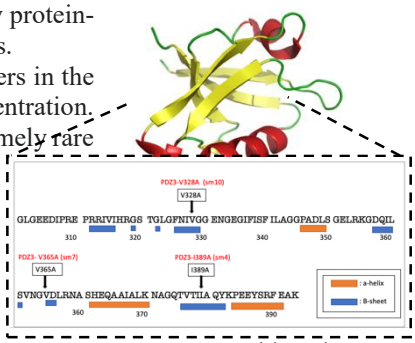
Introduction (背景)

PSD95-PDZ3 the third PDZ domain　that constitutes a Postsynaptic density protein-95 (PSD95), and plays an important role in neurotransmission via synaptic cells.

In previous PSD95-PDZ3 studies, the second peak corresponding to oligomers in the DSC thermogram, and the peak shifted depending on the pH and protein concentration. Although heat-denatured proteins often irreversibly heat-aggregation, it is extremely rare to form reversible oligomer (RO) at high temperatures in this way. Because of this phenomenon, PDZ3 is considered.

In this study, I attempted to inhibit RO formation at high temperature by substituted one residue with Alanine (Ala) in PDZ3-. I used three variants of PDZ domain such as PDZ-I389A, PDZ3-V365A, PDZ3-V328A.



(Figure1: PDZ domain structure and three variants amino acid structure)

By using various experimental and computational tools such as CD, DLS, SLS spectroscopy, we could analyse and manipulate the structure and biophysical properties of proteins. Moreover, we could determine the formation of small, soluble aggregates or oligomers that might related to full aggregation.

1. Materials and Methods (実験方法)

In this study, I expressed and purified PDZ-I389A, PDZ3-V365A, PDZ3-V328A. In the E. coli BL21 (DE3) was transformed by the heat shock method then pre-cultured in LB medium (100 ml) (37 ° C, 250 pm, 12-15 hr). 0.2 mM IPTG was added to LB medium 1L for main culture and the cells were cultured 37 ° C, 120 rpm for another 4 hours.

Then sonication, and centrifuging (4 ° C, 8000 rpm, 20 min), and add about 1 ml of 1M HCI to the supernatant fraction to adjust the pH to pH3.

Next, purified by using Reversed phase HPLC protein concentration of sample was adjusted by NanoDrop 2000, acetic acid was added at a final concentration of 10% (v / v), and the mixture was concentrated by a filtering a pore size of 0.2 µm just before injected to the HPLC machine.

After that, determined from the experimental molecular weight (Exp. Mw) by MALDI-TOF MS, and compared with theoretical molecular weight (Cal MW) that calculated with Protparam software. Relative errors in percentages (IExp. Mw -The. Mw | / The .Mw x 100.) was calculated.

Finally, CD spectra of 0.2 mg/mL of PDZ3 variants were performed in 50mM potassium phosphate pH 7.5 at 25,40,60,80. For DLS and SLS measurements, 1mg/mL of PDZ3 variant was measured at 25C and 37C.

2. Results and Discussion　(結果と考察)

Characterization with both Analytical and Large Reversed phase HPLC showed a sharp single peak around in the ratio of 40%. Which means succeeded to theoretical values purified protein. Then MALDI-TOF MS measurement result differed from theoretical values less than 2Da and relative error in percentage was calculated.

In the CD spectra protein denaturation occurs in 80 degree. It indicates that protein denaturation occurring at high temperature. DLS and SLS spectroscopy showed small size aggregate at pH 7.5.

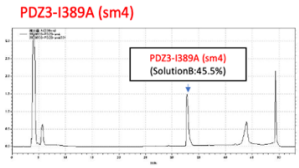
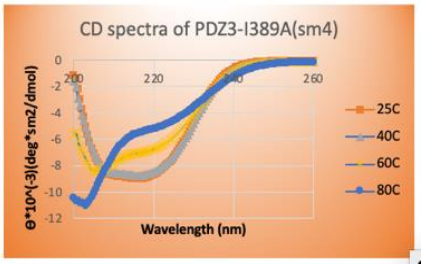


Figure 2. HPLC result

Name	Exp. Mw (Da)	Cal.Mw (Da)	Relative error (%)
PDZ3-I389A (sm4)	10962.456	10961.27	$1.08*10^{-2}$
PDZ3-V365A (sm7)	10975.740	10975.29	$4.1*10^{-3}$
PDZ3-V328A (sm10)	10975.740	10975.29	$4.1*10^{-3}$

Figure 3. MALDI-MS result



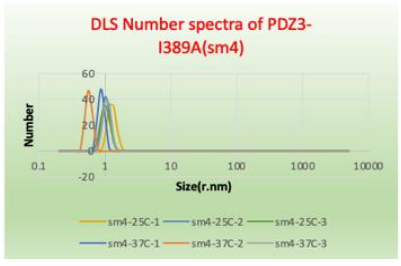


Figure4: CD Figure5: DLS result

result