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CATEGORY：Biochemistry

PROJECT TITLE ：Expression and Purification of Soluble CD 157

SCHOOL：Anglo Chinese Junior College

FINALISTS：Gabriel Cheong Kah Kay

COUNTRY：Republic of Singapore

Expression and Purification of Soluble CD157

Gabriel Cheong Kah Kay

Background:

CD157 is a 31kDa ectoenzyme that is expressed at elevated levels in patients with rheumatoid arthritis (RA). The full function of this protein has yet to be determined, and the correlation between CD157 and RA is not well understood.

Objective of Project:

I sought to devise a method to purify a sufficient quantity of soluble CD157 for generating antibodies via injection into mice, to allow further research into the function of the protein.

Procedure Used:

Usually, if *E. Coli* with a plasmid carrying the CD157 gene is incubated at 37°C and allowed to express the protein, all the CD157 produced is insoluble and trapped in inclusion bodies. It is hypothesised that the high temperature causes rapid movement of the protein resulting in haphazard folding of the protein, making it insoluble. As such, I lowered the incubation temperature of CD157 to 30°C and 25°C to see if it would yield more soluble CD157. I then lysed the cells by the freeze-thaw method and centrifuged the suspension, extracting the supernatant for analysis of soluble CD157 and analysing the results using SDS-PAGE and subsequent Western Blotting to detect if the protein was present.

Following that, I purified the soluble protein expressed at the incubation temperature that yielded the most protein, which happened to be 25°C, using Talon beads which would bind to the 6×Histidine tag that had been genetically incorporated into the CD157 gene in the plasmid. I quantified the results using Coomasie Blue staining.

Results:

Even though incubation at 25°C yielded the most soluble CD157, a mere 30μg of CD157 per litre of bacteria culture could be extracted by purification. This showed that lowering temperature does have an effect on increasing the yield of soluble protein, but it is insufficient for extraction to be used in inducing production of antibodies for further research.

Of academic interest, the proteins were chiefly found in oligomers of 2 to 4 units and hence, CD157 could exist as a dimer in the membrane. If this is the case, then the other CD157 unit could have obscured the histidine tag, making purification by this method an ineffective one.

Conclusion:
The results show that the hypothesis of lowering the temperature to allow the protein to fold into the correct confirmation to remain soluble is rather justified. Therefore, lowering the incubation temperature of bacteria for expression of other proteins would probably allow more protein to remain soluble in the cytosol.

The hypothesis that the histidine tag interferes with the purification process can also be investigated by using other protein purification methods to confirm whether or not the expression of soluble CD157 is in fact higher than experimentally determined by this method.

The present method of denaturing the inclusion bodies of *E. Coli* with 6M urea to yield sufficient CD157 can continue to be used to generate antibodies for further research. Hopefully, the relationship between RA and CD157 will be established in the near future so that more effective cures may be developed.