Production and properties of cross-linked recombinant pro-resilin: an insect rubber-like biomaterial

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Introduction

The design and synthesis of novel biomolecular materials, based on mimicking the properties of molecules found in nature, is providing materials with unusual properties. Resilin serves as an energy storage material in insects and facilitates flight, jumping (in fleas, froghoppers etc) and sound production (cicadas, etc). Resilin is initially produced as a soluble protein and in its mature form is crosslinked through formation of dityrosine units into a very large insoluble polymer. In the present study, we have synthesized a recombinant form of resilin that can be photochemically cross-linked into a resilient, rubber-like biomaterial that may be suitable for spinal disc implants (1). This material is almost perfectly elastic and its fatigue lifetime in insects must be >500 million cycles. A High yield lactose induced fermentation method has been developed for producing soluble recombinant proteins which led to 20 - fold increase in volumetric productivity at a level of up to 300 mgl^{-1}, relative to that obtained from conventional IPTG induction. In addition, a facile "salting-out and heat" purification method allowed rapid and efficient downstream processing of a large quantity of soluble recombinant resilin-like proteins.

Experimental

***Synthesis of crosslinked recombinant resilin*:** A portion of exon 1 from the *Drosophila melanogaster* resilin gene (CG15920) (2) was cloned and expressed in *E. coli* (1). A solution of the purified recombinant protein was photochemically crosslinked in the presence of 1mM [Ru(bpy)3]Cl and 20mM ammonium sulfate.

Fermentation procedures

Overnight culture (A_{600} = 2) was diluted (1: 100) to fermenter medium containing glycerol as a carbon source in a fermenter (Brunswick) with 2 - 3 L working volume. The pH was maintained at 7.0, and air was supplied at a rate of 2 – 3 lm^{-1} at 37 °C for initial cultivation. The cells were grown to the depletion of carbon source. Primary induction was carried out by the addition of IPTG and lactose, and the incubation was maintained for further 6 h. At the end of this primary induction, 200 ml of cell culture was re-grown in 5 L fresh medium containing lactose only as a carbon source and inducer in the same condition as above at 37 °C, or at 27 °C for 18 h. When cell density did not change much for 1 h, the cells were collected by centrifugation (10,000 g, 30 min at 4 °C).

Soluble Protein Purification

Cells were resuspended in lysis buffer and sonicated. Following centrifugation, cleared lysate was treated with PEI (polyethyleneimine). A soluble fraction of recombinant protein was recovered to near purity by heating and salting out with ammonium sulfate. Resilin-like proteins coacervated when cooled, leading to formation of a high concentration (up to 300 mg/ml) of recombinant protein in the lower liquid phase obtained. Further concentration steps were not needed.

Results and discussion

Figure 1 shows the result of purification steps of rec1-resilin.

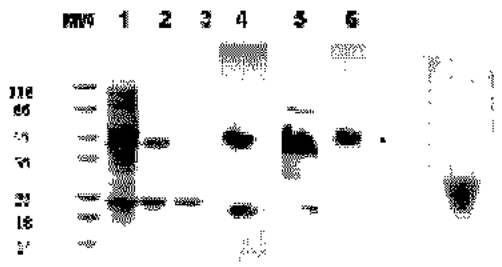

Fig 1. Analysis of purification of rec1-resilin on SDS-PAGE and its coacervate.
Lanes; MW, Molecular weight standards (kDa); 1, Clear cell lysate; 2, Supernatant of heated clear lysate; 3, Supernatant of ammonium sulfate precipitate (to 20 %); 4, Resuspended ammonium sulfate precipitate; 5, Supernatant of lane 4 after heat; 6, Supernatant of reheated suspension of lane 5 pellet.

A soluble fraction of recombinant protein was produced to near purity by "heat and salting out" method (arrow in Fig 1). It was found that lactose induced fermentation method yielded almost 20 times of that from IPTG induction in LB (Table 1). Two factors, primary IPTG induction and fresh medium in the following lactose induction fermentation were found to attribute to this high yield of recombinant resilin production.

Table 1. Protein yields under various fermentation conditions

Sample No in Fig 2	Induction and medium	Specific yield (mgg^{-1} cells)[a]	Volumetric protein yield (mgl^{-1})[b]
1-1	Primary IPTG induction	0.2	60
1-2	Primary IPTG and lactose induction in fresh medium at 37°C	9.6	300
1-3	Primary IPTG and lactose induction in fresh medium at 27°C	10	300
2-1	lactose induction in fresh medium at 37°C	6	120
2-2	lactose induction in fresh medium at 27°C	3.3	120

Cell specific yield[a], and volumetric productivity[b] by auto-induction of rec1-resilin in a shake-flask were ca 3 mgg^{-1} and 30-35 mgl^{-1}, respectively.

The results on the analysis of mechanical properties of cross-linked rec1-resilin showed that ca 92 - 98% resilience and around 20% of dityrosine formation (1), measured by a scanning probe microscope (SPM) operated in force mode and by amino acid analysis by HPLC separation after acid hydrolysis, respectively.

New products in research pipe line

In addition to rec1-resilin, new resilin constructs from different origins (buffalo fly and cicada) have been cloned and expressed in *E.Coli* in a same manner as above. Preliminary result of Western analysis indicated the different activity against anti-rec1- resilin (*Drosophila*) antibody, particularly in buffalo fly resilin encoding only exon1 and full length without exon2. This may imply the structural moiety unavailable in those recombinant proteins. Despite the similarity of the structure of exons and the existence of resilin motif (eg. **YGAP**) between *Drosophila* and buffalo fly, buffalo fly resilin protein sequences are not well aligned with the one from *Drosophila*, which may imply different protein properties. Investigation of mechanical properties of additional recombinant resilin like proteins including synthetic ones is in progress.

Conclusions

The lactose induction fermentation method was found to be a very efficient method in small scale production and should be amenable to large to industrial scale with overall low operating cost. Together with a facile purification method, it offers a facile and low-cost method to improve the production and purification of highly resilient resilin-like biomaterials. These methods will be of immense value as we begin to dissect the structure/function relationships of this new family of remarkable bipolymers.

References

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2. Ardell DH and Andersen SO. (2001) *Tentative identification of a resilin gene in Drosophila melanogaster.* Insect Biochem. Mol. Biol., **31**: 965-970.