Charge Transfer of Recombinant Proteins

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Abstract and Introduction:

In nature, cytochrome *c* (Cyt *c*) serves a role in the synthesis of adenosine triphosphate (ATP) [1]. Cyt *c*, a mitochondrial protein [1,2], passes single electrons by oxidation and reduction of its redox center [2]. Researchers employ Cyt *c* for charge transfer research due to its solubility [1] and "accessible redox center" [3].

For charge transfer, Cyt *c* must form stable contact with electrodes and must also have a "defined orientation" in a two terminal electrode junction [3]. To increase immobilization, Schröper *et al*. placed cysteine or hexahistidine tags at either the N or C terminus of Cyt *c*, by genetically altering the pJRhrsN2 construct [4]. Immobilization improved by electro-static interactions of C-cys with "carboxy-terminated self-assembled monolayers (SAM)" [3] and by affinity binding of N-his_6 with nickel-nitrilotriacetic acid (NiNTA) SAM.

The motivation to obtain recombinant proteins arises from the desire to conduct further research on their integration into bioelectronic devices (cross bars and break junctions) and to learn more about charge transfer [3]. The focus of this work was to obtain high-yields and to characterize C-cys, C-his, N-his, and NC-his Cyt *c*.

Experimental Procedure:

To obtain Cyt *c*, first, a mixture of 1.0 μL plasmid deoxyribonucleic acid (DNA) and 50 μL [5] E.coli BL21 cells was iced for 30 minutes [6]. Then cells were heat-shocked at 42ºC [5] for 45 seconds before plating on ampicillin plates.

Since initial yields had decreased, several culturing methods were investigated to determine which modifications would increase expression, modeled after [4] and [6]. First, yields were evaluated for cultures with and without precultures (methods 1-5 [4]) as shown in Figure 1. Second, aeration (shaker speed) and medium [4] were varied (methods 1, 6, and 7). Third, larger volumes were tried (methods 8 and 9). For precultures and cultures, the incubation temperature was 37ºC overnight, and 37ºC for 2 hrs and then 30ºC for 50 hrs, respectively. For glycerol stocks [6], individual colonies were precultured in LB, screened, combined with glycerol, and stored at -80ºC. Cyt *c* was expressed from these solutions by method 4.

To purify Cyt *c*, cells were pelleted, re-suspended in protease inhibitor, lysed, and centrifuged. Next, cation exchange and affinity chromatography columns were prepared for wildtype and C-cys and for C-his and NC-his Cyt c, respectively. Wild-type and C-cys while C-his and NC-his were eluted with 0.5M NaCl buffer and 250 mM imidazole buffer, respectively.

For cyclic voltammetry (CV) of complex Cyt *c* and Cyt *c* reductase, electrodes were placed into 1 mM 4-mercatophenol (MPhOH) [3]. Also, 0.8 mg/ml of Cyt *c* reductase was combined with C-cys Cyt *c* for 2 hrs [3]. Afterwards, electrodes were placed in the complex for another 2 hrs. To measure the reaction in NADH, with a 2 mV/s scan rate, the buffer was changed to 7.5 mM NADH buffer [3]. For current-voltage characterization of C-cys Cyt *c* in a break junction, first 10 mM 11-mercaptoundecanoic acid solution (MUA) and then 10 μM C-cys Cyt *c* were applied to electrodes for at least 5 minutes each.

Method	Preculture Medium	Preculture Volume (ml)	Culture Medium	Culture Volume (ml)	Shaker Speed (rpm)	Cyt *c* Expression (Red Cultures/Total Cultures)
1	LB	5	TB	200-250	165	0/8
2	TB	5	TB	200-250	200	2/4
3	TB	25	TB	200-250	200	6/10
4	-	-	TB	200-250	200	11/18
5	-	-	LB	200-250	200	2/14
6	LB	5	TB	200-250	180	2/24
7	LB	5	LB	200-250	180	0/2
8	-	-	TB	500	200	1/9
9	TB	25	TB	500	200	0/5

Figure 1: E.coli preculture and culture methods and results.

Results and Conclusions:

Higher expression was obtained from direct culturing. As shown in Figure 1, 11/18 cultures (method 4) and 2/4 cultures (method 8) had high expression or appeared red (Cyt *c*). Also, preculturing 5 or 25 ml TB [4] (method 2 and 3) improved expression; 2/4 and 6/10

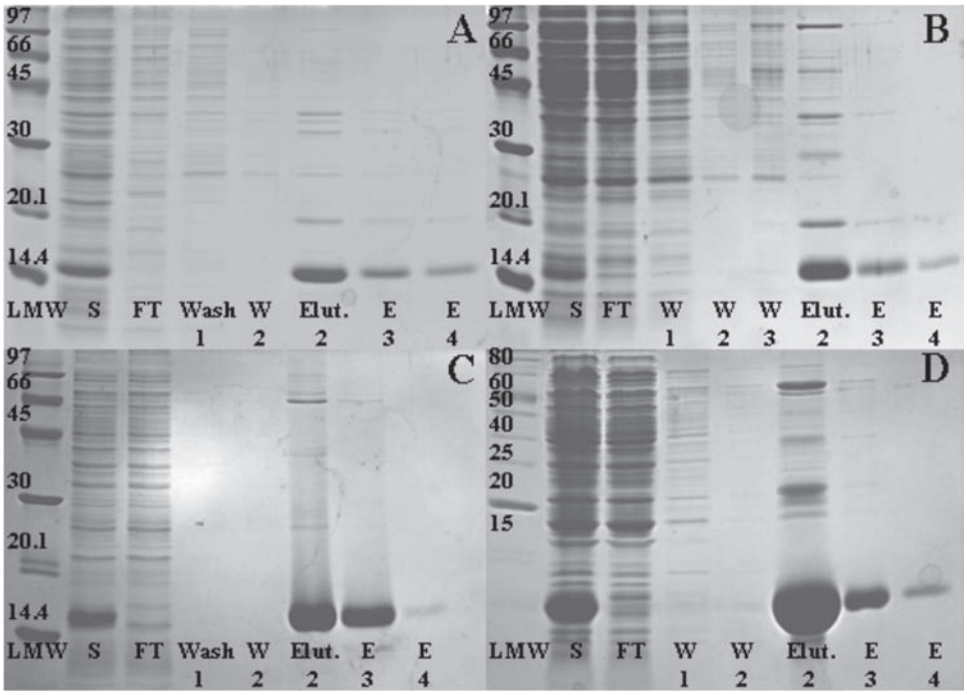

Figure 2: SDS PAGE of wildtype; (A), C-cys (B), C-his (C), and NC-his (D) Cyt c.

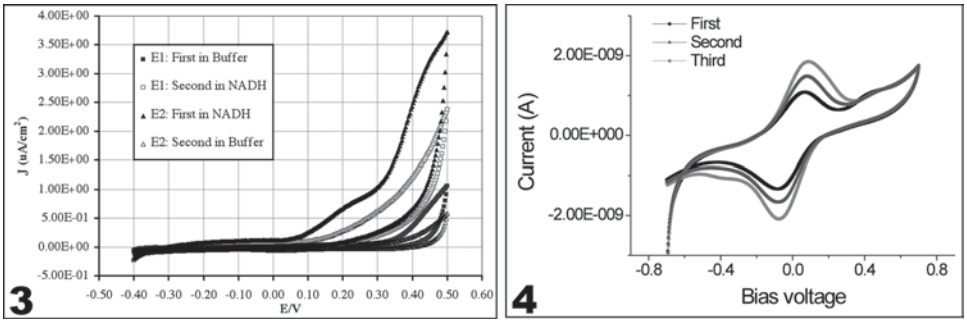

Figure 3: Electro-enzymatic reaction of C-cys Cyt c and Cyt c reductase in NADH [8].

Figure 4: Current-voltage characteristics of C-cys Cyt c integrated into a break junction [9].

red cultures. However, larger volumes (method 8 and 9) showed lower expression of 1/9 and 0/5 red cultures. Higher expression from direct culturing may be caused by an optimal 250 ml culture volume [7]. The expression of recombinant proteins from frozen glycerol stocks was consistent; 6/6 red cultures.

SDS PAGE (Figure 2) reveals distinct bands at 14.4 kilo-Daltons (kDa) [3], especially in second elution fractions. Lanes S and FT represent supernatant and flow through. UV-Vis shows absorbance peaks: 415, 520, and 550 nm, demonstrating a properly "incorporated heme group" [3]. Surface plasmon resonance (SPR) indicates "homogenous" immobilization [3]. Also, CV of C-cys Cyt *c* shows single peaks for oxidation and reduction of the redox center.

Figure 3 shows a strong peak 3.70 μA/cm^2 near 0.5V (Ag/AgCl) for the complex of C-cys Cyt *c* and Cyt *c* reductase in NADH (electrode 2, E2), compared to 1.05 μA/cm^2 near 0.5V in buffer. Since the complex was incubated before its immobilization, this may have increased its immobilization because the native binding site of C-cys Cyt *c* heme group would be more "accessible" to Cyt *c* reductase. Last, break junction data of C-cys Cyt *c* (Figure 4) show single oxidation and reduction peaks.

In conclusion, higher yields of wildtype, C-cys, C-his, and NC-his Cyt *c* were obtained through TB direct culturing or by 25 ml TB preculturing [4]. Also, glycerol stocks consistently expressed proteins.

Future Work:

The next steps are; further study of the integration of C-cys Cyt *c* into bioelectronic devices, and the expression and characterization of bifunctional N-his/C-cys Cyt *c*.

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