Low Temperature Culture Effects on sCR1 Productivity by rCHO Cells

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Abstract: Recombinant CHO, *r*CHO, cells are usually cultured at 37 ºC. Unlike the specific growth rate, which decreases at low temperature, effects of low temperature culture on specific productivity rate are not so clear. We studied low temperature culture effects on *s*CR1 productivity in *r*CHO cells and reported here. The specific growth rate of *r*CHO increased according to increase of culture temperature. The specific rate of *s*CR1 at 37 ºC was similar to that at 35 ºC, however that at 33 ºC increased by 1.3 compared with that at 37 ºC. These results suggest that the productivity of *s*CR1 could become higher by using the shift of culture temperature.

Key words: *r*CHO, CHO, sCR1, CR1, complement receptor, cell cycle, specific growth rate, specific production rate, low temperature culture, batch culture, serum-free medium, yield, glucose, lactate, viable cells

1. INTRODUCTION

The human complement receptor type 1, CR1, is a polymorphic membrane glycoprotein expressed on human erythrocytes, monocytes, macrophages, granulocytes, follicular dendritic cells, glomerular podocytes, and B- and T-lymphocytes. Since CR1 can serve several regulatory functions such as co-factor activity with factor I, inactivation of bursting oxidization of neutrophil cells, hemolytic reaction with complement, and processing of C3 to C3a and C5a, it is widely used for clinic application. The CR1 plasmid was transfected to CHO cells, and 220 kD of soluble CR1, *s*CR1, could be expressed and secreted by CHO cells. The purified *s*CR1 is confirmed to

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retain its well-known functions similar to those of cell-bound receptor CR1 in vitro. On the other hand, temperature is a key environmental parameter that influences cell growth and recombinant protein production. Most mammalian cells are cultured at 37 ºC. Lowering culture temperature below 37 ºC decrease specific growth rate. In many cases, the specific production rate, q, of CHO cells was not enhanced by lowering the culture temperature. On the other hand, some reports say that the q of CHO cells increase at lower temperature (Yoon *et al*. (2003), Hendrick *et al*. (2001)). In the present study, we investigated the effect of low temperature culture on CHO cell growth and *s*CR1 production rates.

2. MATERIALS AND METHODS

The cell line of CHO (CRL-10052) was used. It was originally an adherent cell, however it was changed to a floating one. Cells were cultured in a 2 L fermentor with a 1.2 L working volume at various temperatures. pH and DO were maintained at 7.2 and 40% of air saturation by CO_2 and O_2, respectively. Agitation speed was 100 rpm. A serum-free medium on the basis of IMDM with 1% Penicillin-Streptomycin-Neomycin antibiotics mixture was used. Concentrations of glucose, glutamine, glutamate, lactate, and ammonia were measured by Bioprofile 400 (NOVA). *s*CR1 concentration was measured by using the modified method by Wang *et al*. (Kato *et al*. (2002)). Briefly the supernatant of medium sample by centrifuge was dialyzed by using a seamless cellulose tube and then injected to HPLC gel filtration column chromatography (TSK gel G3000SWXL, TOSOH). As elution buffer, the Tris buffer (pH=7.4) containing 0.05% CHAPS was used. To analyze the cell cycle distribution, flow cytometric analysis was carried out by using a FACSAria flow cytometer (BD).

3. RESULTS AND DISCUSSION

To determine the effect of low culture temperature on *s*CR1 productivity, batch cultures of *r*CHO were carried out at 4-different temperatures such as 37, 35, 33 and 30 ºC (Figure 1). The specific growth rate of *r*CHO increased according to the culture temperature (Table 1). Figure 2 shows the relationship between *s*CR1 concentration and time integrated cell concentration. Since the relationship had linearity, the slope becomes to the q_{sCR1}. Other specific rates were obtained in the same way and shown in Table 1. The q_{sCR1} at 37 ºC is similar to that at 35 ºC. However the q_{sCR1} at 33 ºC increased by 1.3 compared with that at 37 ºC

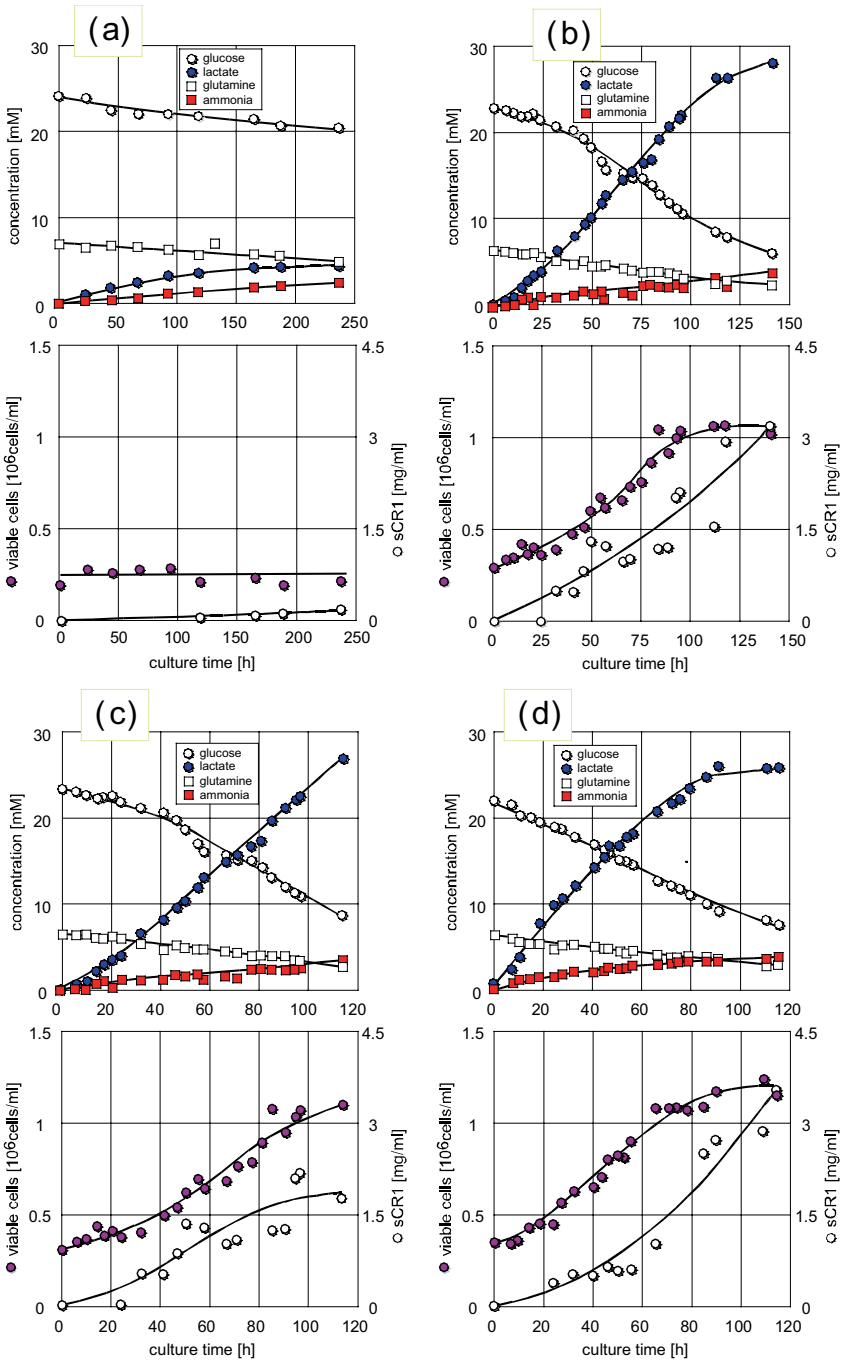

Figure 1. Time courses of rCHO culture at various temperatures.

Table 1. Effect of culture temperature on specific rates and yields.

Temp [ºC]	*μ* [h^{-1}]	*Specific rate (q)* [μ mol 10^6cells^{-1} h^{-1}]					*Yield* [-]	
		Gluc	*Lact*	*Gln*	*Amm*	*sCR1*	*Lac/Gluc*	*Amm/Gln*
37	0.0174	0.150	0.252	0.031	0.032	1.50e-4	1.68	1.03
35	0.0106	0.180	0.302	0.041	0.047	1.53e-4	1.68	1.13
33	0.0082	0.170	0.253	0.035	0.036	1.90e-4	1.48	1.03
30	0.0000	0.060	0.060	0.095	0.037	1.77e-4	1.58	1.15

The q_{Gluc} and q_{Lac} were almost same without influence of temperature difference except at 30 ºC. The q_{Gluc} and q_{Lac} were almost same in all temperature range. However the yields of glucose to lactate change a little in all temperature range as well as the yields of glutamine to ammonia does.

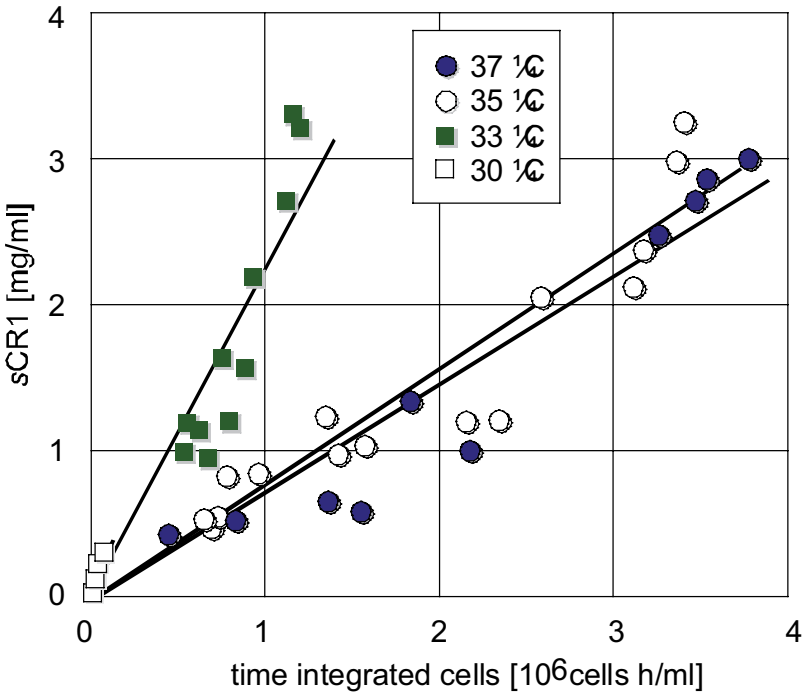

Figure 2. Relationship between sCR1 concentration and time integrated cell concentration at various temperatures.

Many reports have pointed out that enhancement of specific production rate is observed by arresting cells in G0/G1 phase and the accumulation of cells in G0/G1 phase could be achieved by lowering culture temperature. In this experiment the ratio of G0/G1 phase and the specific production rate of *s*CR1 increased in lower temperature culture.

Table 2. Distribution of cells in different phases on the cell cycle of rCHO cells.

***Temp* [°C]**	***Culture time* [h]**	***G0/G1* [%]**	***S* [%]**	***G2/M* [%]**
37	55	62.3	15.7	22.3
	74	63.9	17.3	18.8
	90	67.3	15.1	17.6
35	57	66.6	11.5	21.9
	71	61.6	13.9	24.7
	114	58.9	17.0	24.1
33	115	81.5	2.7	14.7
	144	81.0	4.3	15.8
30	237	78.3	5.6	16.1

4. CONCLUSION

Lower temperature culture decreases the specific growth rate of *r*CHO, however it significantly increases the productivity of *s*CR1. It was suggested that the productivity of *s*CR1 could become higher by using the shift of culture temperature.

ACKNOWLEDGMENTS

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