Silk Protein Sericin Improves Mammalian Cell Culture

Sericin As a Mitogenic Supplement to Culture Media

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Abstract: Mammalian cell cultures generally require supplementation with fetal bovine serum (FBS), or its replacement, into the culture media. Sera contain various unidentified and unknown factors and the risk of infections, including bovine spongiform encephalopathy (BSE), is of serious concern. Therefore, the supplementation of sera into culture media is a major obstacle for purification to recover cell products and this limits pharmaceutical acceptance of products. In this study, we examined whether the sericin protein, derived from the silkworm cocoon, can be effectively used as a substitute for FBS in mammalian cell culture. Together with fibroin, sericin is a major component of raw-silk and is removed from raw-silk by a treatment called degumming to make the silk lustrous and semitransparent. In order to investigate the effect of sericin on the proliferation and the productivity of mammalian cells, sericin was added to cultures of various mammalian cell lines, such as murine hybridoma 2E3-O. Sericin successfully accelerated the proliferation of the cells. Moreover, the production of MoAb by the hybridoma cells was also improved in the presence of sericin. Although heat easily denatures and inactivates most proteins, sericin maintained mitogenic activity after conventional autoclaving (20 minutes) and longer (60 minutes) autoclaving.

Key words: sericin, hybridoma, monoclonal antibody, serum-free medium

R. Smith (ed.), Cell Technology for Cell Products, 397–401.

1. INTRODUCTION

A variety of mammalian cells, including CHO and BHK, are industrially cultured to produce biomaterials such as proteins and gene therapy vectors and are used as transplants for cell therapy. Most mammalian cells need serum, or a replacement, in the culture medium, and fetal bovine serum (FBS) is used most frequently. However, FBS is frequently contaminated with viruses, and even the risk of bovine spongiform encephalopathy (BSE) is of major concern and serum also contains numerous factors outside the operator's control. Thus, the supplementation of serum to culture medium is a serious obstacle in the purification of products. Therefore, an alternative to FBS as a supplement to mammalian cell culture is eagerly desired.

We focused on sericin as an alternative to FBS and we reported that sericin accelerated the proliferation of various mammalian cells (Terada *et al*., 2002). In this study, we examined the optimal concentration of sericin when supplemented to hybridoma cultures and the condition of sterilization of sericin.

Sericin and fibroin are the two major components of raw silk with fibroin being the predominant component. Sericin, a gummy coating on raw-silk filaments, is removed by a treatment called degumming to make the silk lustrous and semitransparent. The degumming treatment is essentially an alkaline scouring operation and is carried out at boiling temperatures. During this treatment, sericin is degraded and solubilized in water and abolished from silk. Various functions of sericin have been revealed and novel applications have been proposed. Sericin inhibits tyrosinase and lipid peroxidation (Kato *et al*., 1998), and so sericin is utilized in cosmetics. Dietary supplementation of sericin in mice successfully suppressed colon carcinogenesis induced by 1,2-dimethylhydrazine (Sasaki *et al*., 2000a). Enhancement of the bioavailability of several metal ions during consumption of sericin was also indicated in rat (Sasaki *et al*., 2000b). A recombinant sericin peptide protected E. coli from freezing stress (Tsujimoto *et al*., 2001). Sericin also improved mammalian cell survival during cryopreservation (Sasaki *et al*., in press)

2. MATERIALS AND METHODS

The hybridoma cell line 2E3-O was cultured in serum-free ASF104 medium (Ajinomoto, Japan) in 24 well plates (Sumitomo Bakelite, Japan) at 37˚C in humidified air containing 5% CO2.

The numbers of viable and dead cells were determined by counting in a hemocytometer under a phase contrast microscope using trypan blue

exclusion. MoAb concentration in the culture supernatant was determined by ELISA.

3. RESULTS

In order to determine the optimum concentration of sericin, the proliferation of the hybridoma cells in the presence of 0.05%, 0.10% and 0.15% sericin was measured and shown in Fig. 1. At day 2, the viable cell number of the cultures with 0.15% sericin was the highest, but at day 3, viability with 0.1% sericin was the highest. Further experiments were performed to determine the optimal concentration of sericin for hybridoma cultures.

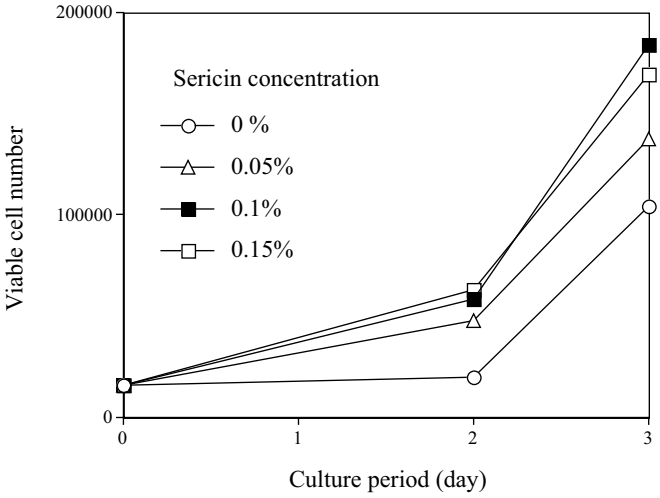

Figure 1. Growth curve of hybridoma 2E3-0 in the presence of sericin.

As shown in Fig. 2-a, the highest cell number was found in cells that were cultured in the presence of 0.1% sericin. Figure 2-b shows that the culture with 0.075% sericin produced the highest amount of MoAb, and the cultures with 0.1% produced the second highest amount. These results indicate that 0.075 - 0.1% is the optimum concentration of sericin to be supplemented to the hybridoma cultures.

Furthermore, we investigated the effect of sterilization on the mitogenic activity of sericin. Sericin was sterilized under various conditions and supplemented to the hybridoma cultures. As shown in Fig. 3, the mitogenic activity of sericin was not compromised, even after longer (60 minutes) autoclaving. These results indicate that sericin is suitable as a supplement for mammalian cell culture.

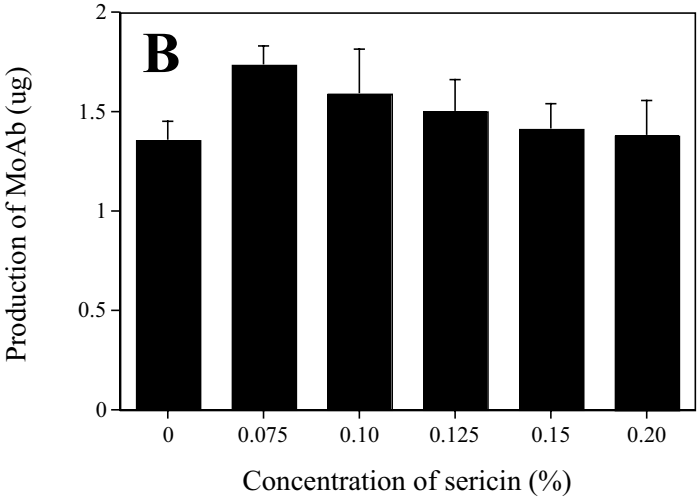

Figure 2. Effect of sericin on the proliferation (A) and MoAb, and (B) production of hybridoma 33000 cells were seeded and cultured for 24 hours (N=4).

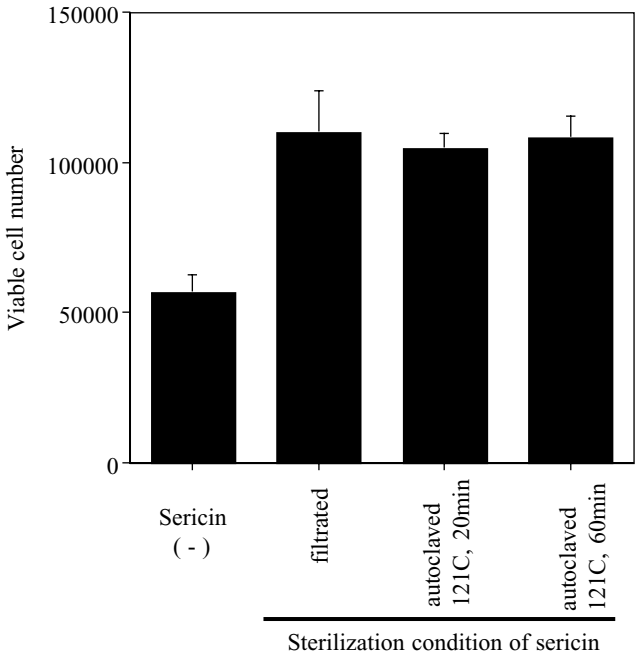

Figure 3. Mitogenic effect of sericin sterilized by different conditions on the hybridoma 29,000 hybridoma cells were seeded and cultured for 2 days (N=4).

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