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# Essential Role of $Mg^{2+}$ in Flocculation of *Saccharomyces diastaticus*

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(Key words : flocculation, *Saccharomyces diastaticus*, $Mg^{2+}$-deficient)

## Abstract

Flocculation of *Saccharomyces diastaticus* IFO1958 was studied. Cells of IFO 1958 did not flocculate even in the stationary phase without $Mg^{2+}$ although they began to flocculate strongly 18h after inoculation in the presence of $Mg^{2+}$. Cycloheximide completely inhibited induction of floc-forming ability of $Mg^{2+}$-deficient cells. Co-flocculation between flocculent cells and $Mg^{2+}$-deficient cells was investigated by treatment by proteolytic enzymes and chemical modification. Treatment of $Mg^{2+}$-deficient cells by proteolytic enzymes did not affect the co-flocculation with flocculent cells. Photo-oxidation or mercaptoethanol-reduction of $Mg^{2+}$-deficient cells failed to diminish the co-flocculation with flocculent cells while treatment of $Mg^{2+}$-deficient cells by periodate caused a considerable loss of the co-flocculation. On the other hand, flocculent cells deflocculated by proteolysis or chemical modification of protein component failed to co-flocculate with $Mg^{2+}$-deficient cells. These findigs suggest that $Mg^{2+}$-deficient cells are non-flocculent because of lack of protein component essential for flocculation of cells of IFO 1958.

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# INTRODUCTION

Flocculation of brewer's yeast cells is important and interesting from both biochemical and industrial standpoints. It is defined as the phenomenon wherein yeast cells adhere in clumps and sediment rapidly from the medium in which they are suspended[1]. It is described that flocculation is caused by interaction between cell surface protein and mannan[2, 3]. It was also reported that $Mg^{2+}$ plays an essential part in flocculation of beer yeast IFO 2018, a flocculent strain of S. *cerevisiae*[4-6]. Although *S. diastaticus* is now taxonomically regarded as *S. cerevisiae, S. diastaticus* is able to ferment starch to produce ethanol because it has ability to secret glucoamylase.

This paper describes an essential role of $Mg^{2+}$ in flocculation of *S. diastaticus*.

# MATERIALS AND METHODS

## Yeast strain

*S. diastaticus* IFO 1958 was used throughout. The strain was obtained from Institute for Fermentation, Osaka.

## Cultivation

The yeast cells, cultivated in the semi-synthetic medium described in the previous paper[4], were washed three times with sterilized deionized water and inoculated at a cell concentration of 1 $\mu$g/ml into fresh medium of the same composition or fresh medium deficient in $Mg^{2+}$. Cultivation was carried out at 28℃ with shaking on a rotatory shaker. Yeast cells cultivated for appropriate time were harvested and washed three times with deionized water.

## Estimation of flocculation

The degree of flocculation (D.F. value) of cells of a single strain and that of co-flocculation observed when flocculent cells and non-flocculent cells were mixed was estimated as described before[3].

## Addition of cycloheximide into growing culture

After 1 $\mu$g/ml of cycloheximide and $Mg^{2+}$ was added into $Mg^{2+}$-deficient cell culture grown for 18h, cells grown for 21h after inoculation were harvested and D.F. values were determined.

## Treatment of cells with proteolytic enzymes and chemical modification of cell surface protein and carbohydrate components

### Proteolytic enzemes

10mg of cells was incubated with trypsin or chymotrypsin as described previously[7].

### Mercaptoethanol-reduction

10mg of cells was treated with 0.1M mercaptoethnol in the presence of 8M urea, as described before[8].

### Photo-oxidation

10mg of cells was photo-irradiated in the presence of methylene blue and 8M urea at the room temperature, as described before[8].

### $NaIO_4$

10mg of cells was treated with 20mM $NaIO_4$ at 0℃ for 30min in the dark, as described previouly[3].

After appropriate treatments described above, cells were washed three times with deionized water and then used in the flocculation and co-flocculation experiments.

## RESULTS AND DISCUSSION

### Time-course of flocculation of cells of *S. diastaticus* IFO 1958

Figure 1 shows a time course of flocculation of cells of *S. diastaticus* IFO 1958 in the presence and absence of $Mg^{2+}$. Cells cultivated in the presence of $Mg^{2+}$ began to flocculate 18h after inoculation while cells grown in the absence of $Mg^{2+}$ did not flocculate until 24h after inoculation. Cells cultivated for 21h in the presence of $Mg^{2+}$ and in the absence of $Mg^{2+}$ were designated as flocculent cells and $Mg^{2+}$-deficient cells, respectively.

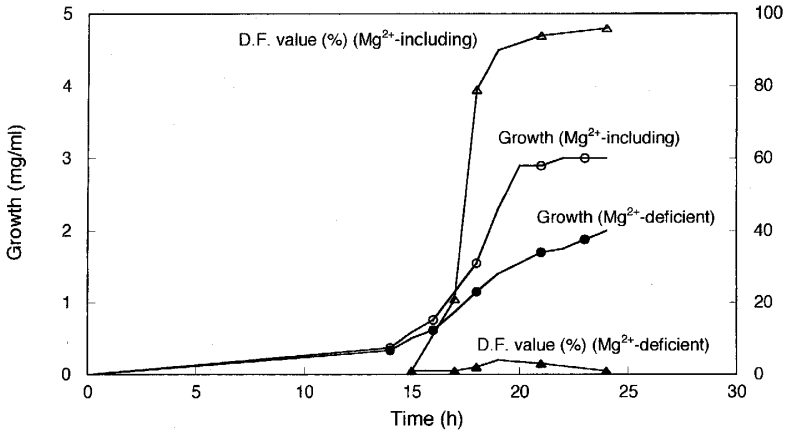


Figure 1. Time-course of Growth and Flocculation of Cells of *S. diastaticus* IFO 1958 Grown with and without $Mg^{2+}$

**Effect of cycloheximide on induction of floc-forming ability of $Mg^{2+}$-deficient cells**

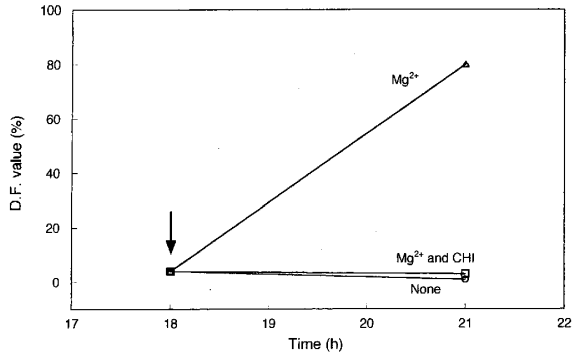


Figure 2. Effect of Cycloheximide on Induction of Floc-forming Ability of $Mg^{2+}$-deficient Cells.

Figure 2 shows effect of cycloheximide on induction of floc-forming ability of growing non-flocculent $Mg^{2+}$-deficient cells. When $Mg^{2+}$ was added to $Mg^{2+}$-deficient cell culture grown for 18h, cells flocculated strongly after 3 h. Cycloheximide strongly inhibited the induction of floc-forming ability of $Mg^{2+}$-deficient cells by $Mg^{2+}$, suggesting that *de novo* protein synthesis at ribosomes is necessary for the induction of floc-forming ability of $Mg^{2+}$-deficient cells by $Mg^{2+}$.

**Effect of treatment with proteolytic enzymes and chemical modification of cell surface protein and carbohydrate components on co-flocculation**

As shown in Figure 3, strong co-flocculation was observed when non-flocculent $Mg^{2+}$-deficient cells and flocculent cells were mixed.

Figure 3 shows also the effect of treatment with proteolytic enzymes of cell surface protein of non-flocculent $Mg^{2+}$-deficient cells on co-flocculation between $Mg^{2+}$-deficient cells and flocculent cells. Treatment of $Mg^{2+}$-deficient cells with trypsin or chymotrypsin failed to affect the co-flocculation significantly.

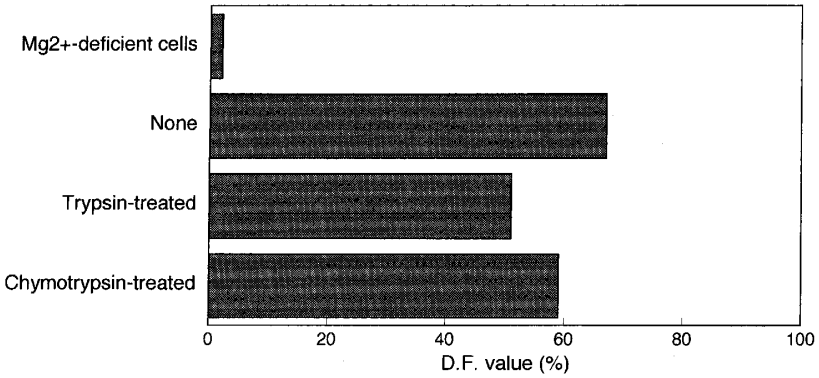


Figure 3. Effect of Treatment with Proteolytic Enzymes of Non-flocculent $Mg^{2+}$-deficient Cells on Co-flocculation with Flocculent Cells.

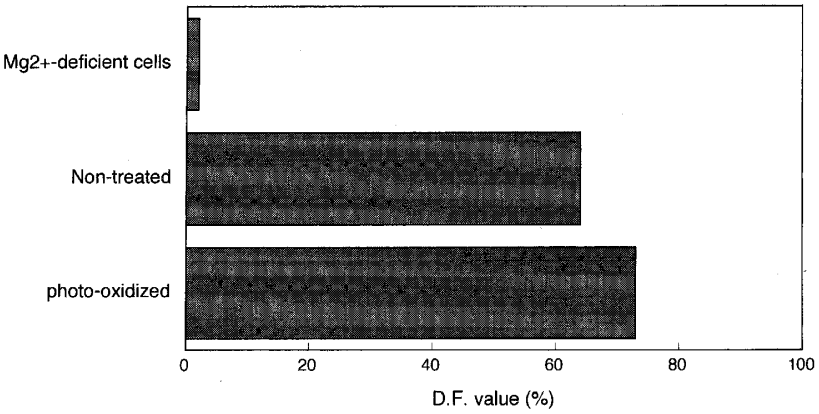


Figure 4. Effect of Photo-oxidation of Non-flocculent $Mg^{2+}$-deficient Cells on Co-flocculation with Flocculent Cells.

Figure 4 illustrates effect of photo-irradiation of $Mg^{2+}$-deficient cells in the presence of methylene blue on the co-flocculation between $Mg^{2+}$-deficient cells and flocculent cells. It is known that photo-irradiation in the presence of methylene blue preferentially brings about modification of imidazole groups of histidyl residues in proteins[9]. It has also been described that floc-forming ability of flocculent cells of *S. cerevisiae* IFO 2018 is lost by photo-irradiation in the presence of a photo-sensitizer because of the destruction of the steric structure of a surface protein component essential for flocculation[8]. Photo-irradiation did not bring about a loss of the co-flocculation but somewhat enhanced the co-flocculation.

Figure 5 shows effect of reduction with mercaptoethanol of cell surface protein of non-flocculent $Mg^{2+}$-deficient cells on co-flocculation between $Mg^{2+}$-deficient cells and flocculent cells. Mercapto-ethanol-reduction of the $Mg^{2+}$-deficient cells, in particular, in the presence of 8M urea stimulated co-flocculation with flocculent cells strongly.

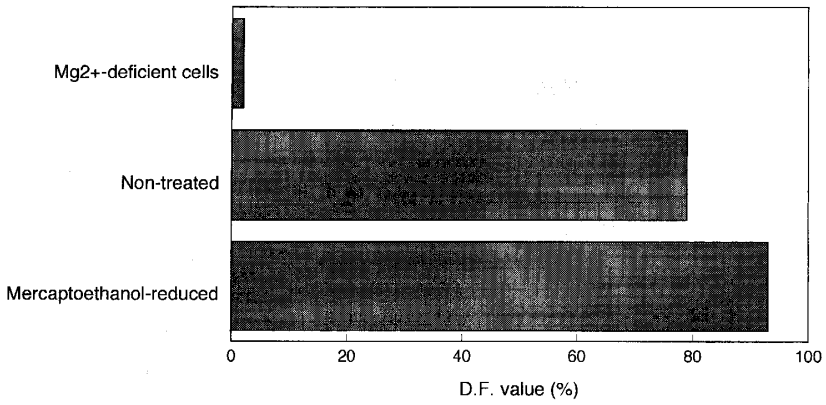


Figure 5. Effect of Reduction with Mercaptoethanol of Non-flocculent $Mg^{2+}$-deficient Cells on Co-flocculation with Flocculent Cells.

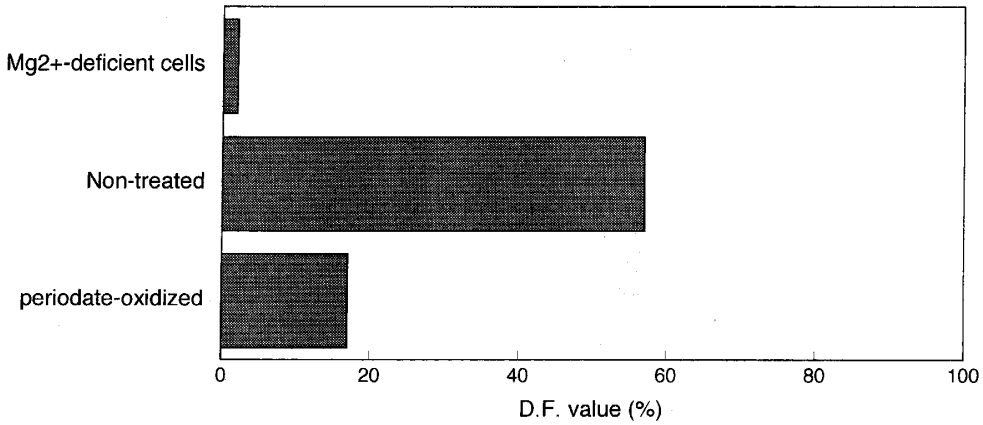


Figure 6. Effect of Oxidation with Periodate of Non-flocculent $Mg^{2+}$-deficient Cells on Co-flocculation with Flocculent Cells.

Treatment with periodate is known to result in the C-C bond cleavage of vicinal dihydroxy compounds including carbohydrates. As shown in Figure 6, periodate-oxidation of $Mg^{2+}$-deficient cells diminished considerably co-flocculation with flocculent cells.

These results suggest strongly that not surface protein components but surface carbohydrate components of $Mg^{2+}$-deficient cells are essential for co-flocculation with flocculent cells.

Next, effect of proteolytic treatment and chemical modification of flocculent cell surface components on co-flocculation between $Mg^{2+}$-deficient cells and flocculent cells was investigated.

As shown in Figure 7, flocculent cells lost the floc-forming ability by treatment with protelytic enzymes. Cells deflocculated by the treatment with proteolytic enzymes failed to co-flocculate with non-flocculent $Mg^{2+}$-deficient cells.

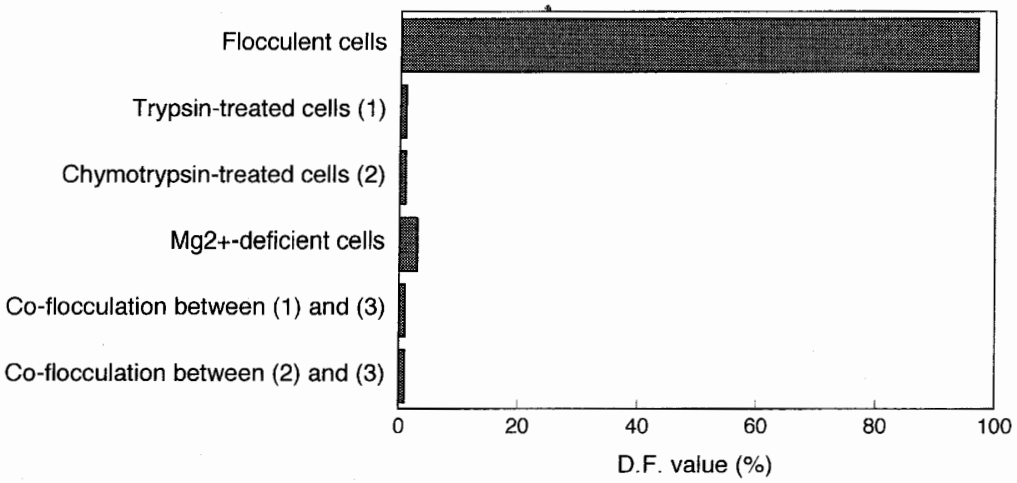


Figure 7. Effect of Treatment with Proteolytic Enzymes of Flocculent Cells on Co-flocculation with $Mg^{2+}$-deficient Cells.

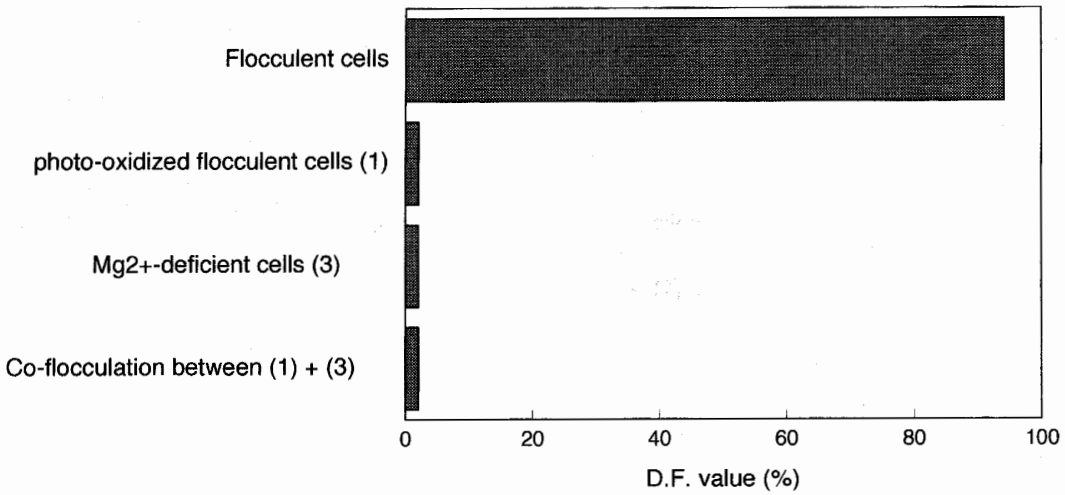


Figure 8. Effect of of Photo-oxidation of Flocculent Cells on Co-flocculation with $Mg^{2+}$-deficient Cells.

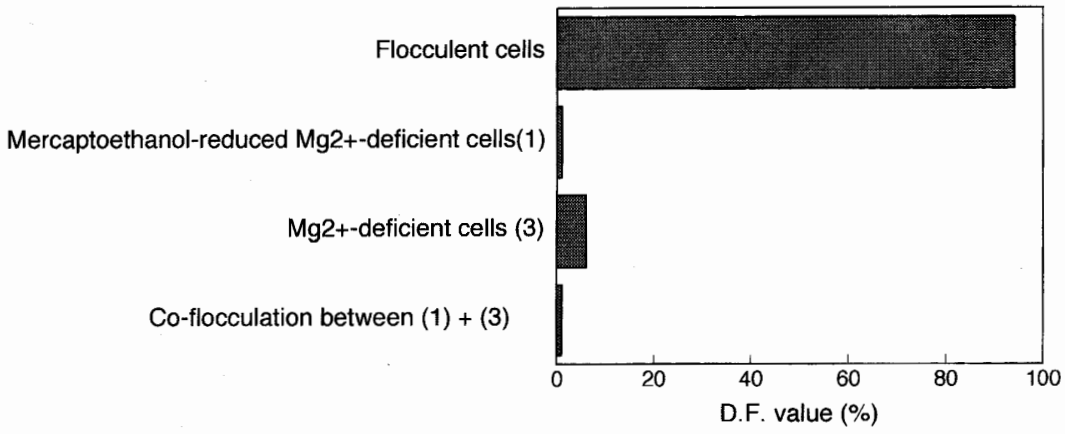


Figure 9. Effect of Reduction with Mercaptoethanol of Flocculent Cells on Co-flocculation with $Mg^{2+}$-deficient Cells.

As shown in Figure 8 and Figure 9, flocculent cells lost the floc-forming ability by photo-oxidation and mercaptoethanol reduction. Both cells deflocculated by photo-oxidation and mercaptoethanol-reduction did not co-flocculate with non-flocculent $Mg^{2+}$-deficient cells. These results suggest that surface protein components of flocculent cells are essential for self-flocculation of flocculent cells and co-flocculation with $Mg^{2+}$-deficient cells.

Figure 10 shows effect of periodate oxidation of flocculent cells on self-flocculation of flocculent cells and co-flocculation with $Mg^{2+}$-deficient cells. Flocculent cells lost the floc-forming ability by periodate oxidation, suggesting that carbohydrate components (mannan) on the flocculent cell surface also play an important part in the floc-forming ability of flocculent cells. Flocculent cells deflocculated

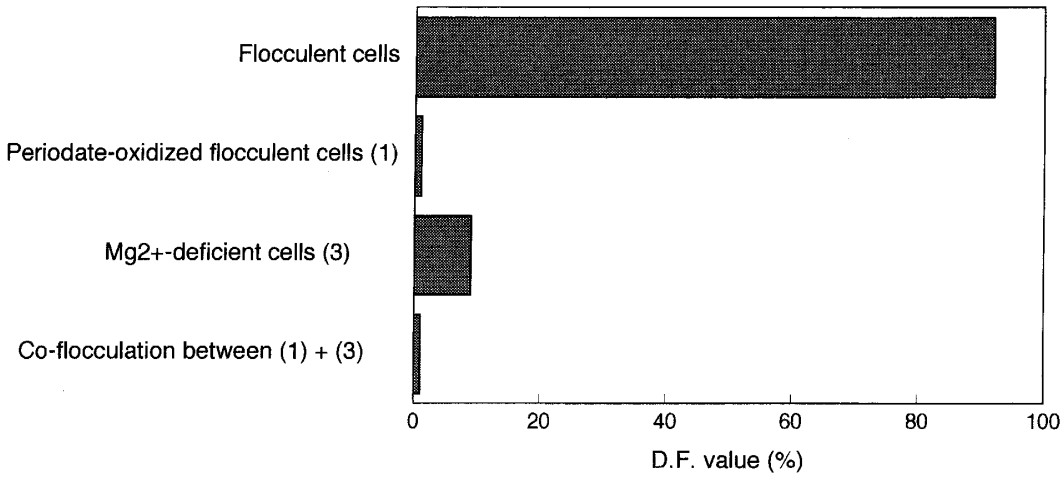


Figure 10. Effect of Oxidation with Periodate of Flocculent Cells on Co-flocculation with $Mg^{2+}$-deficient Cells.

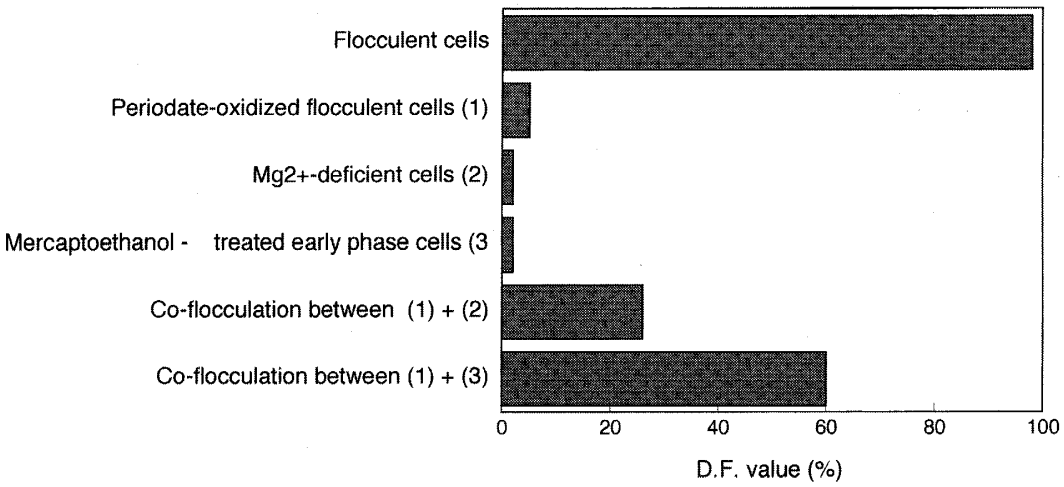


Figure 11. Effect of Mercaptoethanol-reduction of $Mg^{2+}$-deficient Cells on Co-flocculation with Flocculent Cells Oxidized with Periodate.

by periodate oxidation failed to co-flocculate with $Mg^{2+}$-deficient cells. Flocculent cells defocculated by periodate oxidation, however, co-flocculated with $Mg^{2+}$-deficient cells after treatment of $Mg^{2+}$-deficient cells with mercaptoethanol, as shown in Figure 11.

Therefore, it is evident that both protein and carbohydrate components on the cell surface play the essential roles in the flocculation of flocculent cells of *S. diastaticus* IFO 1958. On the other hand, $Mg^{2+}$-deficient cells are non-flocculent since they are not able to produce surface protein component essential for flocculation while they possess essential carbohydrate component.

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