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Flocculation of *Saccharomyces diastaticus* Grown with Starch

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Abstract

Cells of *Saccharomyces diastaticus* IFO 1958 did not flocculate 24 h after inoculation in the medium with starch as carbon source but flocculated 48 h after inoculation. Cycloheximide completely inhibited induction of floc-forming ability of cells grown for 24 h in the medium including starch. The effect of chemical modification of cell surface protein and carbohydrate components on the floc-forming ability of flocculent cells grown with starch for 48 h and 72 h was studied. Treatment with proteolytic enzymes destroyed the floc-forming ability. Photo-oxidation in the presence of methylene blue and reduction with mercaptoethanol eliminated the flocculent ability. Oxidation with sodium periodate also deprived the cells of floc-forming ability. High concentrations of protein-denaturants brought about reversible deflocculation of the flocculent cells. These findings suggest that cell surface protein and carbohydrate components play essential roles in flocculation of the cells grown with starch.

Key words : flocculation, *Saccharomyces diastaticus*, starch, chemical modification

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INTRODUCTION

The phenomenon of flocculation of yeast is important and interesting from both biochemical and industrial standpoints. It is described that flocculation of brewer's yeast is caused by interaction between cell surface protein and mannan[1),2)]. Hitherto flocculation of *Saccharomyces cerevisiae* has been mostly studied, whereas investigation on flocculation of *S. diastaticus* is scarce[3)]. *S. diastaticus* has ability to secret glucoamylase and is able to ferment starch to ethanol. In the preceding paper[4)], it was suggested that flocculation of cells of *S. diastaticus* grown with D-glucose as carbon source is similar to that of brewer's yeast cells and that Mg^{2+} plays an essential part in the flocculation of *S. diastaticus* too. In this paper, we will describe the flocculation of cells of *S. diastaticus* grown with starch.

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 before[5)], were washed three times with sterilized deionized water and inoculated at a cell concentration of 1 μg/ml into fresh medium of the same composition or fresh medium including 2 % soluble starch instead of D-glucose. 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 was estimated as described before[2)].

Addition of cycloheximide into growing culture

After 1 μg/ml of cycloheximide was added into culture grown with starch for 24 h, cells grown for 48 h and 72 h after inoculation were harvested and D.F.

values were determined.

Effect of protein-denaturants on flocculence

Cells were suspended in 8 M urea or 5 M guanidine HCl in the presence of 5 mM Ca^{2+} and D.F. values were estimated.

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

Proteolytic enzymes

10 mg of cells was incubated with trypsin or chymotrypsin as described previously[6].

Mercaptoethanol-reduction

10 mg of cells was treated with 0.1M mercaptoethanol in the presence of 8 M urea, as described before[7].

Photo-oxidation

10 mg of cells was photo-irradiated in the presence of methylene blue and 8 M urea at the room temperature, as described before[7].

Acylation with acetic anhydride

10 mg of cells was suspended in 10 ml of 0.4 M acetic anhydride and allowed to stand for 30 m at room temperarure with adjusting pH to 6.0 with NaOH, as described before[7].

Sodium periodate

10 mg of cells was treated with 20 mM $NaIO_4$ at 0℃ for 30 m in the dark, as described previouly[2].

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

RESULTS AND DISCUSSION

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

Table 1 shows a time course of flocculation of cells of *S. diastaticus* IFO 1958 grown with D-glucose and soluble starch. Cells cultivated with D-glucose strongly flocculated 24 h after inoculation while cells grown with soluble starch were still dispersed 24 h after inoculation and flocculated 48 h and 72 h after inoculation.

Effect of cycloheximide on induction of floc-forming ability of cells grown with starch for 24 h

Figure 1 shows effect of cycloheximide on induction of floc-forming ability of growing cells with starch for 24 h. Cycloheximide strongly inhibited the induction of floc-forming ability, suggesting that *de novo* protein synthesis at ribosomes is necessary for the induction of floc-forming ability of cells grown with starch for 24 h.

Inhibitory effect of protein-denaturants on flocculence

Effect of protein-denaturants on the flocculence of flocculent cells grown with starch for 48 h and 72 h was shown in Table 2. High concentrations of urea or guanidine HCl deflocculated the cells even in the presence of 5 mM Ca^{2+}.

Effect of treatment with proteolytic enzymes

Table 3 shows effect of proteolytic treatment of cells grown with starch for 48 h and 72 h on the floc-forming ability. It was demonstrated that the floc-forming ability is lost completely by treatment with trypsin or chymotrypsin.

Table 1. Time-course of growth and flocculation of cells of S. *diastaticus* IFO 1958 grown with D-glucose and starch as carbon source.

Cultivation time (h)	D-Glucose		Starch	
	Growth (mg/ml)	D.F. Value (%)	Growth (mg/ml)	D.F. Value (%)
24	2.5	60	0.9	5
48	2.5	69	5.6	63
72	2.8	72	6.9	66

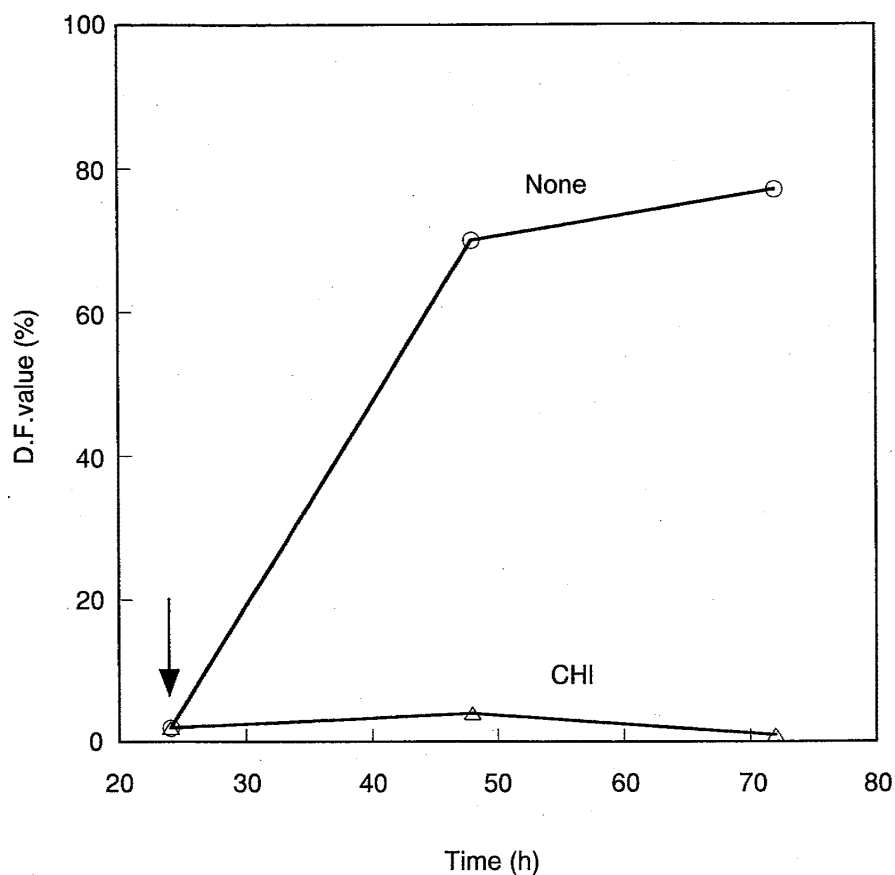

Figure 1. Effect of cycloheximide on induction of floc-forming ability of non-flocculent cells grown with starch.

Table 2. Effect of protein-denaturants on flocculence of flocculent cells grown with starch.

Protein-denaturant	D.F. value (%)	
	48 h	72 h
None	60	89
8M Urea	1	1
5M Guanidine HCl	1	1

Table 3. Effect of treatment with proteolytic enzymes on flocculation of flocculent cells grown with starch.

Proteolytic enzyme	D.F. value (%)	
	48 h	72 h
None	60	89
Trypsin	2	2
Chymotrypsin	10	2

Table 4. Effect of reduction with mercaptoethanol on flocculation of flocculent cells grown with starch.

Reagent	D.F. value (%)	
	48 h	72 h
None	56	75
Mercaptoethanol	2	3

Table 5. Effect of photo-oxidation on flocculation of flocculent cells grown with starch.

Treatment	D.F. value (%)	
	48 h	72 h
None	71	80
Photo-oxidation	6	13

Effect of reduction with mercaptoethanol

Table 4 indicates effect of reduction of cells grown with starch for 48 h and 72 h with mercaptoethanol on the flocculation. It is well known that mercaptoethnol reduces disulfide bonds of proteins. The treatment with mercaptoethanol in the presence of 8 M urea caused the complete loss of the flocculent ability of cells.

Effect of photo-oxidation in the presence of methylene blue

Table 5 shows effect of photo-irradiation in the presence of methylene blue and 8 M urea on floc-forming ability of cells grown with starch for 48 h and 72 h. Photo-irradiation in the presence of methylene blue brings about modification of imidazole groups of histidyl residues in proteins[8)]. Irradiation in the presence of the photo-sensitizer resulted in a significant destruction of the floc-forming ability.

Effect of acylation with acetic anhydride

Table 6 shows effect of acylation with acetic anhydride on floc-forming ability of cells grown with starch for 48 h and 72 h. Acetic anhydride react readily amino groups in proteins. Complete deflocculation was resulted from acylation with acetic anhydride even in the absence of urea.

Table 6. Effect of acylation with acetic anhydride on flocculent cells grown with starch.

Reagent	D.F. value (%)	
	48 h	72 h
None	73	85
Acetic anhydride	1	1

Table 7. Effect of oxidation with sodium periodate on flocculent cells grown with starch.

Reagent	D.F. value (%)	
	48 h	72 h
None	68	88
Sodium periodate	8	12

Effect of oxidation with sodium periodate

Table 7 shows effect of oxidation with sodium periodate on floc-forming ability of cells grown with starch for 48 h and 72 h. Treatment with periodate is well known to result in the C-C bond cleavage of vicinal dihydroxyl compounds including carbohydrates. The treatment with sodium periodate brought about a significant loss of the floc-forming ability.

Flocculent cells grown with starch as carbon source were deflocculated when treated with proteolytic enzymes and protein-modifying reagents. High concentrations of protein-denaturants, such as urea or guanidine HCl, deflocculated the flocculent cells even in the presence of 5 mM Ca^{2+}. Cycloheximide, which is known to repress the *de novo* protein synthesis at ribosomes, depressed the induction of floc-forming ability of non-flocculent cells grown with starch for 24 h. These findings suggest that cell surface protein plays an essential role in the flocculation of flocculent cells grown with starch for 48 h and 72 h. In addition, oxidation with periodate deprived the flocculent cells of floc-forming ability, suggesting that cell surface carbohydrate component (mannan fraction) also participates in the flocculation. The flocculation of cells grown with starch might be similar to that of cells grown with D-glucose.

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