JOURNAL OF BACTERIOLOGY
Vol. 88, No. 4, p. 929-936 October, 1964

INACTIVATION OF SEXUAL AGGLUTINATION IN *HANSENULA WINGEI* AND *SACCHAROMYCES KLUYVERI* BY DISULFIDE-CLEAVING AGENTS

NEIL W. TAYLOR

Pioneering Laboratory for Microbiological Chemistry, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois

Received for publication 15 May 1964

ABSTRACT

TAYLOR, NEIL W. (Northern Regional Research Laboratory, Peoria, Ill.). Inactivation of sexual agglutination in *Hansenula wingei* and *Saccharomyces kluyveri* by disulfide-cleaving agents. J. Bacteriol. **88**:929-936. 1964.—Mating types of both *Hansenula wingei* and *Saccharomyces kluyveri* can be activated to produce uniformly strong sexual agglutination by treatments with various solvents, such as 8 M LiBr. The strongly agglutinative mating-type preparations were irreversibly inactivated for sexual agglutination by various chemical treatments. Type 5 of *H. wingei* was inactivated by disulfide-cleaving reagents, but type 21 of *H. wingei* was not. Type 3 of *S. kluyveri* was more sensitive than type 26 of *S. kluyveri* to inactivation by disulfide-cleaving reagents. Comparison of sensitivities to these and other treatments, plus a moderately strong cross-agglutination between type 3 and type 21, indicated that the sexually agglutinative elements on type 3 are similar to type 5, and those of type 21 are similar to those of type 26. Inactivation-rate experiments showed a loss of agglutinative ability according to a sigmoid decrement with time for both types 5 and 21. The apparent extent of inactivation depended markedly on agglutination test conditions. Results of these experiments were interpreted to indicate tentatively, first, that the agglutinative elements of both types of a species are proteins and, second, that several agglutinating linkages are formed between any two cells in sexual agglutination.

Sexual agglutination of yeasts, wherein opposite mating types of a species agglutinate together, was discovered by Wickerham (1956) in *Hansenula wingei*. It is found in at least three additional genera of yeasts (Wickerham, 1958; Wickerham and Dworschack, 1960), and presumably occurs commonly in yeasts. Wickerham (1958) and Wickerham and Burton (1962) emphasized the close relation of sexual agglutination in species of phylogenetic lines to higher ploidy level as well as to recent evolutionary development.

Sexual agglutination is generally specific between opposite mating types of the same species. This specificity indicates that the mechanism of sexual agglutination is based on a selective interaction of the two types. The structures having this specificity are as yet unknown, and warrant further study.

Limited evidence is available concerning the nature and mechanism of sexual agglutination. Brock (1958*a*, *b*; 1959) showed that the ability of type 21 of *H. wingei* to agglutinate with the opposite mating type was destroyed by trypsin and some other proteolytic enzymes, but its opposite type 5 was not affected. Similarly, treatment with 80% phenol or hot dilute HCl inactivated type 21, but not type 5. On the other hand, type 5 could be more rapidly inactivated with dilute sodium periodate. From his evidence, Brock postulated that the elements responsible for agglutination on the surface of type 21 were protein. The higher sensitivity to periodate was somewhat tenuous evidence in favor of a carbohydrate on the surface of type 5. It was quite evident, though, that the two mating types were distinctly different in their responses to various agents, and thus substantially different structures were implied in their agglutinative elements.

The agglutinative elements of type 5 can be freed from the cell, at least in part, by treatment with snail enzymes (Taylor, 1964). The soluble agglutinating factor released from type 5 specifically agglutinates the opposite mating type 21.

Hunt and Carpenter (1963) suggested that sexual agglutination involves sterols, based on their observations that nystatin inhibited agglutination. Brookbank and Heisler (1963) found no immunological differences between opposite mating types of *H. wingei*.

To further examine sexual agglutination in yeasts, Brock's work was extended by use of other reagents and by tests on another species. In other experiments, the significance of quantitative agglutination measurements was explored. Some tentative conclusions have been drawn as to the nature of the elements involved in sexual agglutination.

MATERIALS AND METHODS

H. wingei NRRL Y-2340, mating types 5 and 21 (Wickerham, 1956), and *Saccharomyces kluyveri* (Phaff, Miller, and Shifrine, 1956) NRRL Y-4288, mating types 3 and 26 (Wickerham, 1960), were received from L. J. Wickerham of the Northern Laboratory. The organisms were grown for 2 days at 25 C in shaken flasks in YM medium (Haynes, Wickerham, and Hesseltine, 1955). (The medium contained 3.0 g of yeast extract, 3.0 g of malt extract, 5.0 g of peptone, and 3.0 g of glucose per liter of distilled water.) Reagents were the best obtainable from laboratory supply houses and most were analytical grade.

Turbidity of cell suspensions was measured in test tubes (13 by 100 mm) in a Bausch & Lomb Spectronic-20 colorimeter at 590 mμ. The test tubes were selected to give an optical density at 590 mμ of 0.51 $\pm$ 0.01 with an appropriate $KMnO_4$ solution and to read uniformly 0.00 $\pm$ 0.02 with water.

Agglutination was tested by mixing 1-ml volumes of suspensions of each mating type in the calibrated tubes; each cell suspension was made up to an optical density of 0.5 at 590 mμ in 0.1 ionic strength phosphate buffer of pH 6.6 (unless otherwise indicated). This concentration of cells, extracted as described below, corresponded to 0.2 mg (dry weight) of *H. wingei* per ml, or about 4×10^7 cells per ml. Mixtures of mating types of *H. wingei* were shaken for 5 min, and mixtures of *S. kluyveri* were shaken for 10 min, on a reciprocating shaker with a 6-cm stroke 260 cycles per min. After shaking, the mixtures stood 10 min, or longer where indicated, and then turbidities were read in a Spectronic-20 colorimeter for quantitative measurements; for qualitative measurements, extent of agglutination was estimated visually. In the experiments on rates of reaction, agglutination was tested in two different solvents as indicated. Of these, the 1% $MgSO_4 \cdot 7H_2O$, of about pH 5, gives results comparable to those in 0.1 ionic strength pH 6.6 buffer, except after acid inactivation as discussed below.

RESULTS AND DISCUSSION

Agglutination test. Sexual agglutination in mature cultures of *H. wingei* is usually a strong reaction. When approximately equal numbers of cells of both types are shaken together, marked agglutination occurs within a few minutes, even though reaction conditions are varied within large limits of pH, solvent, cell concentration, shaking vigor, and time. In other experiments, however, where weaker agglutination due either to solvent inhibition or to partial chemical inactivation was experienced, the extent of agglutination varied much more, both with agglutination conditions and with replication of identical experiments. With further experimentation, it became evident that the agglutination tests were not equilibrium values nor completed reactions. Very long reaction times (e.g., 24 hr or longer of gentle shaking) may be necessary for some weak agglutination reactions to reach completion. Other unknown factors may be involved. Owing to these complications, quantitative physical measurements should be interpreted with caution. As comparative tests, however, the agglutination tests are convenient; decisive in most cases; and, with the use of suitable control preparations, can give valid results as to changes in agglutinability of any of the mating types. The procedures adopted are given in Materials and Methods.

Activation of agglutinability. Agglutination between mating types 5 and 21 of *H. wingei* varied for different preparations, and could be enhanced in the weaker preparations by treating the cells in various ways. Brock (1959) found that boiling in water intensified sexual agglutination, and such treatment was used here to prepare preliminary stocks. However, in other experiments, boiling for extended times sometimes inactivated the preparations, and other activating treatments were sought. Other procedures that activated agglutination were extraction with solvents at room temperature, such as 0.1 M NaOH for 10 min; or 2-hr treatments with 8 M LiBr (pH 5.6), 8 M guanidine-HCl (pH 5.6), or 10% phenol (pH 7.8). LiBr of 6 M or less was less effective, as were also 8 M urea (pH 3) or 8 M and 1 M HCl. The pH is probably not important in the range of 3 to 8. Mixing for 3 min in a high-speed

blender (VirTis Co., Inc., Gardiner, N.Y.) had no effect. Because the acid, base, or boiling-water treatments might partly inactivate the agglutination factors on the cells, treatment with 8 M LiBr or 8 M guanidine-HCl is probably safer. Treatment with 8 M LiBr (pH 5.6) for 2 hr at room temperature was adopted as convenient, and all stocks used were so treated. The treatment extracted about 27% of the dry weight of the cells for both types 5 and 21, and rendered them considerably smaller. Boiling produced a similar effect. However, the ratio of optical density at 590 mμ to cell count was essentially unchanged for either treatment. No cofactors were necessary for agglutinating extracted cells, as suggested by Brock, except a small amount of salt or buffer.

Much of the variation of agglutination intensity between mating types of both *H. wingei* and *S. kluyveri* depended on the age of the cultures. Untreated 16-hr cultures were inactive, and 2- or 3-day-old cultures increased in activity. However, after treatment with 8 M LiBr, all cultures of all four mating types were intensely agglutinating with the opposite type, regardless of age. The younger cultures contained the agglutinating elements, but they were evidently inhibited.

As to the mechanism for activation, the effects of 8 M LiBr and 8 M guanidine-HCl, both of which solvents tend to disperse proteins and polysaccharides, suggest that some material which tends to inhibit agglutination is being extracted from the cell wall. A highly charged, greatly extended polymer on the cell surface, or a more specific inhibiting substance, might be involved. In *S. carlsbergensis*, self-flocculation as the cultures age has been associated with a decreasing content of mannan (Masschelein et al., 1963). One possibility is that sexual agglutination is also inhibited by such a substance, which similarly disappears as growth terminates.

Inactivation of sexual agglutinability. Ability of either mating type of a species to agglutinate with its opposite type can be destroyed by various treatments. The action of different reagents on the four mating types is compared in Table 1. For reasons discussed below, the data are simply given as complete inactivation (++), partial inactivation (+), or no effect (−). When agents other than acid or base were used, no effect was shown on otherwise identical controls at the same pH and with urea where applicable. All work reported in this section was performed with one preparation of each mating type; other preparations gave similar results, however.

TABLE 1. *Inactivation of sexual agglutination*

No.	Treatment*			Extent of inactivation†			
	Reagent‡	pH	Time	*Hansenula wingei* type		*Saccharomyces kluyveri* type	
				5	21	3	26
			hr				
1	5 M HCl		1	−	+	−	+
2	5 M HCl		21	++	++	++	++
3	3% Acetic acid, 75 C		1	−	+	−	+
4	0.1 M NaOH		1	−	+	++	++
5	1.0 M NaOH		1	++	++	++	++
6	0.05 M Ag^+, 4 M urea	5.9	1	+	−	++	++
7	0.25 M RSH	7.9	2	++	−	++	++
8	0.25 M RSH + 8 M urea	7.9	2	++	−	++	++
9	0.25 M RSH + 8 M urea	7.9	21	++	−	++	++
10	0.1 M Cysteine	8.0	1	+	−	++	−
11	0.1 M Cysteine	8.0	16	+	−	++	−
12	0.1 M Cysteine	5.5	4	−	−	++	−
13	0.1 M Na_2SO_3	7.0	1	−	−	++	−
14	0.1 M Na_2SO_3	7.0	16	−	−	++	−
15	0.1 M Na_2SO_3 + 4 M urea	7.8	1	++	−		
16	0.1 M NaCN	9.4	1	−	−	+	−
17	0.1 M NaCN	9.4	21	−	−	++	−
18	0.003 M $NaIO_4$, 37 C	5.6	1	+	−	+	−
19	0.003 M $NaIO_4$, 37 C	5.6	4	++	+	++	+
20	10% Phenol	2.5	1	−	+	−	+
21	10% Phenol	6.5	1	−	−	−	+
22	10% Phenol	6.5	21	−	−	−	+
23	0.1% DNFB	8.0	2	−	−	−	−
24	0.1% DNFB	8.0	16	−	++	−	++

* Temperature was 25 C unless otherwise specified.

† Data are given as complete inactivation (++), partial inactivation (+), or no effect (−).

‡ Abbreviations: RSH, mercaptoethanol; DNFB, 2,4-dinitrofluorobenzene.

No inactivation was observed in the following solvents for any of the four mating types in the pH range 2.0 to 7.5: water, 14 days at 1 C or 6

days at 25 C; 8 M urea, 1 day at 25 C; 8 M guanidine-HCl, 1 day at 25 C or 5 days at 1 C; and 8 M LiBr, 1 day at 25 C or 5 days at 1 C. No inactivation was observed for any of the four types on treatment at 25 C with 0.1 M *N*-ethylmaleimide (pH 8 for 16 hr), 0.1 M acrylonitrile (pH 8 for 16 hr), 0.01 M $K_4Fe(CN_6)$ (pH 7.1 for 1 hr), or 1 M $NaNO_2$ (pH 4.1 for 30 min).

Comparison of the data in Table 1 indicates that the opposite types are quite different in sensitivity to chemical treatment, as shown by Brock (1959) for *H. wingei*. The results of acid, 1 M NaOH, periodate, and the 2-hr 2,4-dinitrofluorobenzene (DNFB) treatments on the *H. wingei* types qualitatively agree with Brock's findings. However, type 21 was somewhat more sensitive than type 5 to 0.1 M NaOH.

When results (Table 1) are compared between species, the data imply that type 5 is similar to 3, and 21 to 26, except that, superimposed on these similarities, both mating types of *S. kluyveri* are considerably more sensitive to some reagents, particularly NaOH and the disulfide-cleaving reagents (Table 1, treatment no. 6 to 17). The similarity of response of type 5 to 3, and of 21 to 26 (in treatments 1, 3, 10, 11, 18, 20, and 24), suggests the hypothesis that the agglutinating elements on the surface of type 5 correspond to those on type 3, and those on type 21 to type 26. Alternatively, the material connecting the agglutinating elements to the cell surfaces might be similar in each pair. The former of these alternatives is the more likely, because the correspondence pattern is confirmed by 16-hr DNFB treatment, one which is less likely to hydrolyze connecting bonds than to cover exposed groups necessary for agglutinability in types 21 and 26.

This correspondence hypothesis was tested further by mixing the four combinations of pairs of mating types from opposite species. Types 3 and 21 agglutinated fairly well, but none of the other combinations showed any reaction. If this cross-reaction is specific sexual agglutination, then evidently type 3 can substitute for 5, or type 21 can substitute for 26 as a complementary agglutinating partner; and, therefore, either type 3 or 21 is similar in a highly specific way—perhaps in having identical agglutinating elements—to its corresponding type of the other species.

The usual difficulties appear when interpretation of the various inactivation treatments is attempted. The reagents are of varying specificity, and perhaps none is absolutely specific for one chemical grouping. The most specific are treatment with mercaptoethanol (RSH) or cysteine, which should cleave disulfide bonds. Effects of these and other agents that cleave disulfide bonds, such as Na_2SO_3, NaCN, and Ag^+ (Cecil and McPhee, 1957), indicate that type 5 and the similar type 3 contain disulfide bonds needed for sexual agglutination to occur. Type 26 was also affected, but more gentle treatments with disulfide-cleaving agents (Table 1) indicate type 3 to be more sensitive. NaCN, a weaker disulfide-cleaving agent, was effective only on type 3.

After inactivation of type 5 with mercaptoethanol, reactivation by recombination of disulfide bonds was attempted by White's (1960) procedure of bubbling air through washed cells. No reactivation of agglutination occurred, but reactivation was not precluded because only a few experiments were performed.

As for inactivation by DNFB, amino groups should be covered in the 2-hr treatment (Hirs, Halmann, and Kycia, 1961). The negative results obtained with all four types in 2 hr (Table 1) indicate that underivatized amino groups are not necessary for sexual agglutination in either species, and confirm Brock's similar observation on *H. wingei*. In a subsequent 16-hr treatment, in which inactivation did occur on types 21 and 26, some other chemical group may be involved, such as other amino acids. The absence of any effect on treatment with *N*-ethylmaleimide or acrylonitrile indicates intact sulfhydryl groups also are not necessary in sexual agglutination. These conclusions are tentative, because either amino groups or sulfhydryl groups might be masked in the active agglutinating elements.

As for acid inactivation under mild conditions, a complicating phenomenon was encountered. After treatment of type 21 with 8 M urea (pH 2.8) for 2 hr at 25 C, agglutination with type 5 in 1% $MgSO_4 \cdot 7H_2O$ was poor. However, agglutination of a portion of the same treated cells in pH 6.6 buffer was good. This difference was a matter of pH. Agglutination was as poor in 0.1 ionic strength, pH 4.91, acetate buffer as in 1% $MgSO_4 \cdot 7H_2O$, which had a pH of 5.0. *S. kluyveri* type 26 displayed a similar dependence on pH after acid-urea treatment. Thus, the activity of treated types 21 and 26 depended on the pH of subsequent suspensions in a pH region where the

untreated types were unaffected. This behavior implies that acid urea produced a transition in structure of types 21 and 26 which was reversed only above pH 5. Inactivation observed in treatments 1, 2, and 3 of Table 1 was not of this reversible type.

Inhibition of agglutination. Agglutination is inhibited in 8 M urea (Brock, 1959). Lower concentrations of such strong dispersing agents may partially inhibit agglutination, and their effect depends on pH. Figure 1 shows typical results for equal concentrations of type 5 and 21 cells equivalent to an optical density (OD) of 0.5 at 590 mμ in water; solvent and cells were mixed, shaken for 5 min, and rested for 30 min. Guanidine-HCl is the most effective inhibitor on a molar basis. Inhibition was reversible by washing the solvent from the cells with 0.1% NaCl solution. There is a minimal inhibition for guanidine-HCl at about pH 4.5. Replicate experiments under slightly different conditions confirm that the minimum is close to this value. The pH variability shows that sexual agglutination is affected by charged titratable groups on the cell surface. Their effect could be either cell-to-cell repulsion due to general surface properties or a more specific action on the agglutinating elements.

Agglutination between types in 2.0 M guanidine-HCl at pH 4.5 is evidently quite weak. A similar solvent system, but containing 2.5 M guanidine-HCl, was used in testing rate of inactivation of agglutinability to observe early effects of inactivating agents.

Rates of inactivation. Inactivation of sexual agglutinability was explored further by measuring the rates of inactivation by RSH and by NaOH. If the reagent is in sufficient excess to be essentially constant through the reaction interval, then one might expect chemical bonds to be broken according to a first-order reaction. The results obtained experimentally could then be used to evaluate the significance of quantitative agglutination measurements.

When the inactivation rate of type 5 cells by RSH was measured, agglutination with type 21 cells decreased; i.e., turbidity increased, in a sigmoid function with time (Fig. 2, curve A). Samples of type 5 cells treated for various reaction times were obtained by withdrawing samples of the reaction mixture, diluting and cooling to stop the reaction, and washing the cells after centrifugation. Agglutination was tested by mixing equal volumes of treated type 5 and untreated type 21, both made up in 1% $MgSO_4 \cdot 7H_2O$ and of OD (590) equal to 0.5. The time required to reach the midpoint of the inactivation curve (half-time)

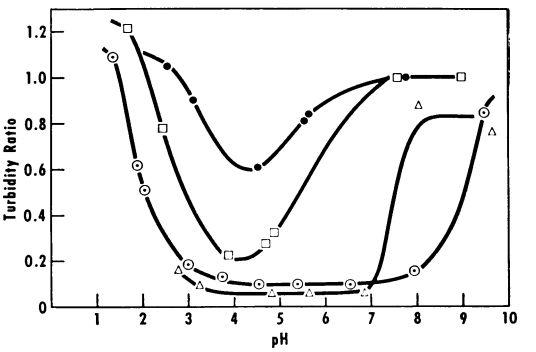

FIG. 1. *Turbidity (optical density at 590 mμ) of equal volumes of Hansenula wingei type 5 and type 21 cells, in different solvents, as a function of pH. Turbidity is given as the ratio to the turbidity of the same concentration of cells, but of one mating type only, in the same solvent and of pH 6.6. Buffers are all 0.1 ionic strength. A low turbidity ratio indicates the cells agglutinated and sedimented to the bottom after shaking and settling. Solvents are water (⊙), 3 M urea (△), 3 M LiBr (□), or 3 M guanidine-HCl (●).*

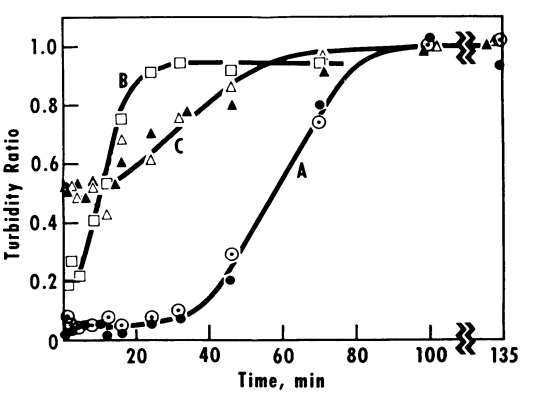

FIG. 2. *Time course of inactivation of agglutinability of type 5 by 0.2% mercaptoethanol at 25 C, as measured by various agglutination tests. Turbidity (optical density at 590 mμ) of mixtures of treated type 5 for various treatment times with untreated type 21. Turbidity is given as the ratio to turbidity of the same concentration of cells but of one mating type only, in the same solvent. Agglutination was assayed on two different occasions (indicated by opened and closed symbols) by three different methods as described in the text. A (⊙, ●): in 1% $MgSO_4 \cdot 7H_2O$. B (□): in 2.5 M guanidine-HCl (pH 4.5). C (△, ▲): 1:10 cell ratio, in 1% $MgSO_4 \cdot 7H_2O$.*

was 59 min. During the first 30 min or so, there was a lag period in which no apparent inactivation occurred. However, if the same series of treated samples was assayed by performing the agglutination tests in 2.5 M guanidine-HCl at pH 4.5 (curve B), then the half-time was only 12 min. The agglutination response in 2.5 M guanidine-HCl was much more sensitive to RSH. Again, there was a lag period. Finally, if agglutination was tested with 1 volume of treated type 5 cells to 9 volumes of untreated type 21 cells (curve C) each in 1% $MgSO_4$ and of OD (590) equal to 0.5, then the half-time was 32 min, a time which is also less than that for curve A.

As indicated in the section on the agglutination test, the quantitative results on partially active samples was sensitive to agglutination reaction conditions. A few tests of agglutination activity of the type 5 cells of Fig. 1 with longer agglutination times indicated that the sigmoid curves of Fig. 1 may become steeper and displaced to longer times, but the points at which agglutinating activity becomes completely absent appeared not to be changed appreciably.

The sigmoid response curve of type 5 treated with RSH is decidedly not apparent first-order with respect to loss of agglutinating ability. Particularly, the lag period (Fig. 2, curve A) demonstrates that a certain amount of reaction is necessary before inactivation can be perceived in the agglutination test. This response suggests that there is an excess of biologically active agglutinative elements on the surface of type 5, a number of which can be inactivated without hindering agglutination with type 21. A formally similar response was found in ultraviolet-light inactivation of intracellular bacteriophage by Luria and Latarjet (1947), for which they developed such a multiplicity hypothesis.

The increased sensitivity of type 5 cells to RSH when assayed in guanidine-HCl (Fig. 1, curve B) tends to confirm the multiplicity hypothesis. The conditions chosen here were such that even normal agglutination was weak, so that any deterioration in agglutinability would be noticeable. Thus, more nearly every agglutinative element on type 5 cells appears necessary under these more adverse conditions, whereas less of the normally available elements are required for agglutination in 1% $MgSO_4 \cdot 7H_2O$.

Similar reasoning applies to assaying agglutinability at unfavorable cell ratios (Fig. 1, curve C). Increased sensitivity of type 5 cells, over curve A, is shown when greater demands are made on the available surface of type 5 cells to agglutinate type 21.

In a test involving only one reaction time, the sigmoid inactivation response of Fig. 2 shows that considerable inactivation of the agglutinating sites could have occurred without being perceived. Such a test gives essentially a yes-or-no response, with some possibility of partial inactivation being found. For this reason, the inactivation data in Table 1 were reported in simplified form. However, in this approach it is assumed that all agglutination sites on each mating type are essensentially equivalent. As discussed below, this appears not always to be the case.

Rates of inactivation by other agents, as well as replicate experiments with RSH, are shown in Table 2 as half reaction times. Also calculated were lag times, the time at which the linear extension of the horizontal lag curve joins the linear extension of the rising curve (Fig. 1). The ratios of lag time to half time (lag ratio, Table 2) are indicated. Duplication in half times and lag ratios for RSH inactivation of type 5 is not precise. However, the relative values of half times by the three assays are consistent among treatments. Inactivation of both types of *H. wingei* with NaOH gave sigmoid response curves. Treatment of type 21 cells with 0.1 M NaOH, followed by assays by the three techniques, gave results similar to those of type 5, suggesting that a multiplicity of elements occurs on the surface of this type also.

Nature of agglutinative elements. The elements on type 21 active in sexual agglutination appear to contain protein, because type 21 is inactivated by proteinases (Brock, 1959). The other types, 5, 3, and 26, are inactivated by RSH and other disulfide-cleaving agents, and thus seem to have disulfide bonds involved. From this information and the general analogous behavior of types 3 and 5 and of types 21 and 26, it is possible that the agglutinative elements of both types in both species are proteins, or contain essential protein constitutents. This interpretation is tentative, but suggests caution in adopting Brock's original hypothesis that type 5 has a carbohydrate. On the other hand, his hypothesis that type 21 contains a protein appears valid; but the protein contains no disulfide bonds necessary to agglutinate.

TABLE 2. *Half reaction times and lag periods for inactivation of Hansenula wingei mating types*

Treatment	Type	Assay					
		In 1% $MgSO_4 \cdot 7H_2O$		Cell ratio of 1:10 in 1% $MgSO_4 \cdot 7H_2O$		In 2.5 M guanidine-HCl	
		Half time	Lag ratio	Half time	Lag ratio	Half time	Lag ratio
		min		*min*		*min*	
RSH*, 0.2%	5-A†	59	0.6	32	0.3	12	0.3
Repeat assay of above		59	0.7	33	0.3		
RSH, 0.2%	5-A	135	0.7	79	0.5	27	0.2
RSH, 0.2%	5-A	48	0.4	38	0.6	13	0.5
RSH, 0.2%	5-A	51	0.4				
RSH, 0.2%	5-B	56	0.3				
NaOH, 0.3 M	5-A	51	0.8				
NaOH, 0.1 M	21-A	98	0.4				
NaOH, 0.1 M	21-B	74	0.4	42	0.6	18	0.6
NaOH, 0.3 M	21-B	22	0.4				

* RSH, mercaptoethanol.

† A and B refer to separate stocks of prepared cells for either mating type.

Evidence is developing that agglutinative elements on the cell surfaces of type 5 may be heterogeneous, in that all the elements do not react alike. When type 5 was treated with snail enzymes to remove the soluble agglutinating factor, the agglutinative activity of the cells diminished to a constant low value which was unaffected by an additional treatment with fresh enzyme (Taylor, 1964). When type 5 was treated with cysteine at pH 8 without urea (Table 1), a similar effect was observed, in that a further 16-hr treatment produced no more inactivation after a 1-hr treatment. Philip Snider (*personal communication*) has observed in type 5 a resistance to further inactivation with periodate after treatment under appropriate conditions. Brock (1959) showed a similar effect in type 21. Evidently, a fraction of the agglutinative elements on type 5, and perhaps on 21, is more resistant to attack. Whether partial resistance of type 5 in all three cases is due to the same fraction of resistant agglutinative elements remains to be tested. Any such heterogeneity may be a complicating factor in quantitative agglutination tests.

A tentative, but reasonable, model of sexual agglutination is that the two mating types of a species have complementary agglutinative elements on their surfaces, in excess over the minimum necessary for agglutination. In an average contact between cells of opposite type, several pairs of these complementary elements combine and thus hold the two cells together.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge that this problem was suggested by Lynferd J. Wickerham. Dennis Patterson assisted in some of the experimental work involved.

LITERATURE CITED

BROCK, T. D. 1958*a*. Mating reaction in the yeast *Hansenula wingei*. Preliminary observations and quantitation. J. Bacteriol. **75**:697–701.

BROCK, T. D. 1958*b*. Protein as a specific cell surface component in the mating reaction of *Hansenula wingei*. J. Bacteriol. **76**:334–335.

BROCK, T. D. 1959. Mating reaction in *Hansenula wingei*. Relation of cell surface properties to agglutination. J. Bacteriol. **78**:59–68.

BROOKBANK, J. W., AND M. R. HEISLER. 1963. Immunology of the yeast *Hansenula wingei*. J. Bacteriol. **85**:509–515.

CECIL, R., AND J. R. MCPHEE. 1957. Further studies on the reaction of disulphides with silver nitrate. Biochem. J. **66**:538–543.

HAYNES, W. C., L. J. WICKERHAM, AND C. W. HESSELTINE. 1955. Maintenance of cultures of industrially important microorganisms. Appl. Microbiol. **3**:361–368.

HIRS, C. H. W., M. HALMANN, AND J. H. KYCIA. 1961. The reactivity of certain functional

groups in ribonuclease A towards substitution by 1-fluoro-2,4-dinitrobenzene. Inactivation of the enzyme by substitution at the lysine residue in position 41. *In* T. W. Goodwin and O. Lindley [ed.], Biological structure and functions. Academic Press, Inc., New York.

HUNT, D. E., AND P. L. CARPENTER. 1963. Sterols as components in the mating reaction of *Hansenula wingei*. J. Bacteriol. **86**:845–847.

LURIA, S. E., AND R. LATARJET. 1947. Ultraviolet irradiation of bacteriophage during intracellular growth. J. Bacteriol. **53**:149–163.

MASSCHELEIN, C. A., C. JEUNEHOMME-RAMOS, C. CASTIAU, AND A. DEVREUX. 1963. Mechanism of phenotypic variations in the flocculence character of yeast. J. Inst. Brewing **69**:332–338.

PHAFF, H. J., M. W. MILLER, AND M. SHIFRINE. 1956. The taxonomy of yeasts isolated from *Drosophila* in the Yosemite region of California. Antonie van Leeuwenhoek J. Microbiol. Serol. **22**:145–161.

TAYLOR, N. W. 1964. Specific, soluble factor involved in sexual agglutination of the yeast *Hansenula wingei*. J. Bacteriol. **87**:863–866.

WHITE, F. H., JR. 1960. Regeneration of enzymatic activity by air-oxidation of reduced ribonuclease with observations on thiolation during reduction with thioglycolate. J. Biol. Chem. **235**:383–389.

WICKERHAM, L. J. 1956. Influence of agglutination on zygote formation in *Hansenula wingei*, a new species of yeast. Compt. Rend. Trav. Lab. Carlsberg **26**:423–443.

WICKERHAM, L. J. 1958. Sexual agglutination of heterothallic yeasts in diverse taxonomic areas. Science **128**:1504–1505.

WICKERHAM, L. J., AND K. A. BURTON. 1962. Phylogeny and biochemistry of the genus *Hansenula*. Bacteriol. Rev. **26**:382–397.

WICKERHAM, L. J., AND R. G. DWORSCHACK. 1960. Extracellular invertase production by sexually agglutinative mating types of *Saccharomyces kluyveri*. Science **131**:985–986.