Antibodies against the α-Factor Pheromone of *Saccharomyces cerevisiae*

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The mating pheromone of baker's yeast, the α-factor, is a dodeca-/tridecapeptide, which is not antigenic by itself. It was coupled to succinylated thyroglobulin by the carbodiimide procedure to facilitate selective coupling of the α-factor mainly by its N-terminal region. Antibodies against this conjugate were raised in rabbits. After selective precipitation of the rabbit antiserum with succinylated carrier prior to the radial double diffusion test (Ouchterlony) specific antibodies against the coupled α-factor could be detected.

Introduction

In the course of mating (conjugation) of the heterothallic yeast *Saccharomyces cerevisiae* an elongation ("shmooing") of the spherical haploid a and α cells occurs. This morphological change is under pheromonal control of peptides mutually produced by the haploids of opposite mating type, α-cells secrete α-factor, a mixture of dodeca- and tridecapeptides affecting a-cells. The latter produce a-factor, a mixture of undecapeptides that act upon α-cells [1, 2]. The zygote generated by the fusion of the haploid cells buds off diploid progeny.

The pheromones are currently detected and measured by means of several bioassays. In order to overcome the inherent variability of such tests we set out to develop a sensitive enzyme-linked immunosorbent assay (ELISA) for the precise quantitation of the α-factor. A crucial drawback lies in the fact that the pheromone is a small molecule not necessarily antigenic by itself. For the first time we have succeeded in raising specific antisera against the α-factor – a result prompting this report.

Dedicated to Prof. Dr. Martin Bopp on the occasion of his 60th birthday.

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Materials and Methods

Organisms and growth conditions

The standard laboratory yeast strains, X 2180-1A (a) and X 2180-1B (α) were kindly provided by W. Duntze. The conditions for their cultivation have been described [3].

Chemicals

Chemicals were of the highest grade commercially available. Biologically active α-factor was isolated from yeast α-cell culture fluid in batches of 50 liters by the method of Duntze [3]. Isolated α-factor was finally subjected to HPLC and shown to be highly purified. Thyroglobulin (porcine) was purchased from Sigma, Freund's adjuvant from Difco.

Bioassay and Ouchterlony test

The liquid bioassay was performed as described by Khan *et al.* [4] and the radial double diffusion test (Ouchterlony) [5] as follows.

The test was performed on microscopy slides covered with 1% (w/v) agar in veronal-acetate buffer (17 mM diethylbarbiturate, 17 mM sodium acetate, pH 7.4). After filling the wells with the appropriate solutions the slides were left for 24 h at room temperature in a humid chamber, washed for 48 h with 0.9% (w/v) NaCl solution, stained 10 min with 0.6% (w/v) Amido black 10 B in methanol : acetic acid : water (5 : 1 : 4, v/v/v) and washed 3 times for 10 min with methanol : acetic acid : water (5 : 1 : 4, v/v/v).

Immunization

0.1 ml of the conjugate (10 mg/ml) was homogenized in an equal volume of complete Freund's adjuvant and then administered intradermally in the back of each of three rabbits. After three weeks the animals were boosted with incomplete Freund's adjuvant and the same conjugate concentration as with the first immunization. The animals were bled by ear vein puncture.

Results and Discussion

In order to raise antibodies against a small peptide like the α-factor the pheromone (2.5 mg/ml)

was coupled to succinylated thyroglobulin (10 mg/ml) by the carbodiimide procedure as described by Mroz and Leeman [6]. The carrier protein was succinylated by the method of Klapper and Klotz [7] to facilitate selective coupling of the α-factor mainly via its N-terminal end. It was assumed that disadvantageous interference with the pheromone's biological activity could be avoided in this way [8]. Based on spectroscopic determinations, 50% of the added α-factor was recovered in the succinylated conjugate.

Ten days after the second immunization antisera titer were checked. Antibodies were raised in all three animals (Fig. 1). It had to be shown, however, that the entire antibody population contained antibodies not only against the carrier protein but specific for the coupled hapten. In order to prove this antiserum was mixed with succinylated thyroglobulin (10 mg/ml), kept on ice (16 h) and the precipitate was centrifuged down.

As shown by Fig. 2a, the antiserum contained in fact antibodies against the protein carrier of the thyroglobulin-α-factor conjugate (Fig. 2a, well 2, 6). Uncoupled α-factor formed no immune precipitation (Fig. 2a, well 4). As expected the supernatant (purified antiserum) contained antibodies specific for the coupled α-factor: a faint but clearly visible precipitation line is formed only with the succinylated thyroglobulin-α-factor conjugate (Fig. 2b,

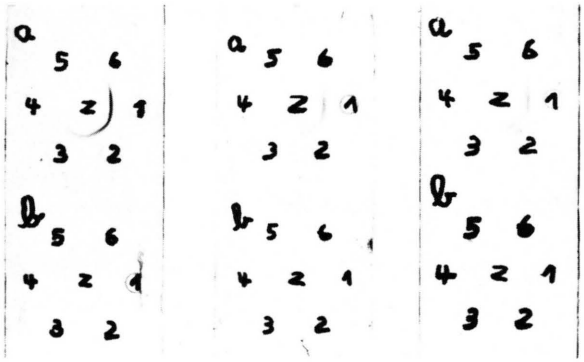

Fig. 1. Radial double diffusion Ouchterlony test with antiserum against succinylated thyroglobulin-α-factor conjugate. (a) The center well (z) contained 10 µl of the conjugate (2 mg/ml), while the outer wells contained 10 µl of differently diluted antiserum: undiluted (1), diluted with 0.9% (w/v) sodium chloride solution 1:2 (2), 1:4 (3), 1:8 (4), 1:16 (5), 1:32 (6). (b) The same numbering and dilution program as in (a) but with antiserum drawn from rabbits before immunization.

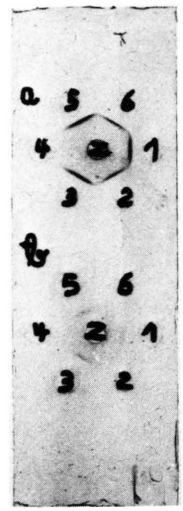

Fig. 2. Radial double diffusion Ouchterlony test with complete and purified antiserum against succinylated thyroglobulin-α-factor and different antigens. (a) The center well (z) contained 10 µl of antiserum 1:2 diluted with preimmune serum of rabbit number one (Fig. 1, left side slide), while the outer wells contained 10 µl of conjugate (1 mg/ml) (1, 3, 5), 10 µl of α-factor (4 mg/ml) (4), 10 µl of succinylated thyroglobulin (1 mg/ml) (2, 6). (b) The center well (z) contained 10 µl of purified antiserum recovered after mixing the total antiserum with succinylated thyroglobulin (see text). Numbering and contents of outer wells as in (a).

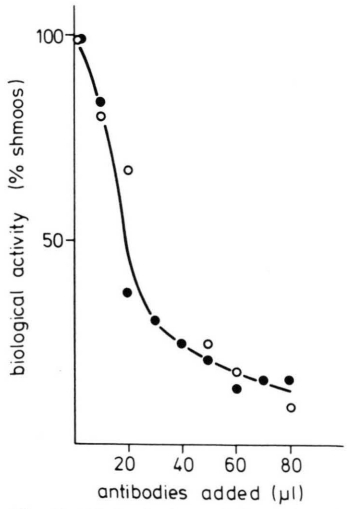

Fig. 3. Biological activity of α-factor after addition of antibodies against the pheromone. The graph represents the data from two out of four independent experiments (experiment 1: ○, experiment 2: ●). For bioassaying the α-factor (6×10^{-7} M) different amounts of the antibodies (original concentration 0.57 mg protein/ml) were mixed with the pheromone test solution followed by the addition of yeast cells (X 2180-1A). After incubation (4 h) the number of "shmoos" was determined (see Materials and Methods).

well 1, 3, 5) but not with the carrier protein alone (Fig. 2 b, well 2, 6).

In order to further prove the specificity of the raised antibodies they were added to a α-factor solution which was then bioassayed for α-factor activity (Fig. 3). The added antibodies very efficiently reduced the biological activity of the α-factor from ca. 100% (control level) to approximately 10%.

Our results confirm other experiments showing that synthetic peptides containing only seven and eleven amino acids were sufficient for raising antibodies [9].

Further experiments in our laboratory with purified antibodies against the above used α-factor conjugate have established an ELISA for α-factor not only with authentic peptide but also for crude preparations of yeast culture fluid. Details will be published elsewhere.

Acknowledgements

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