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Abstract

Response of slime strains to concanavalin A

Terenzi, H. F., M. M. Flawia and H. N. Torres. A distinctive response to concanavalin A-mediated agglutination shown by cells from two different "slime" strains.

We demonstrated that N. crassa strains having a cr-1 ("crisp") morphological mutation are deficient in adenylate cyclase activity (Terenzi et al. 1975 Biochem. Biophys. Rer. Commun. 58: 990). The enzymatic defect seems to be directly involved in the expression of the "crisp" phenotype, since the addition of cyclic 3',5'-AMP or its dibutyryl derivative to the culture medium restored in the mutants some of the morphological characteristics of the wild type (Torres et al. 1975 In D rummond et al. (Eds.) Advances in cyclic nucleotide research. Vol. V, p. 67. Raven Press, New York). We first noticed the cyclaseless condition of cr-1 mutants by comporing the enzymatic activities of cell extracts prepared from two different "slime" strains which were obtained from the Fungal Genetics Stock Center. These strains possess different genotypes: FGSC 326, A; fz;sg;os-1;arg-1;cr-1;aur, is the original "slime" isolated by Emerson after UV mutagenesis. The other strain: FGSC 1118, A; fz;sg;os-1 is a recombinant obtained after several crosses to wild types (Emerson 1963 Genetica 34: 162). We observed that the former strain, that is FGSC 326, was devoid of adenylate cyclase activity.

Since Neurospora adenylate cyclase is a membrane-bound enzyme (Flawia and Torres 1972 J. Biol. Chem. 247: 6873) we conridered of interest to compare some membrane properties of the two "slime" strains in order to gain some insight on the molecular basis of the 'crisp" phenotype. Among several experimental approaches we assayed plant lectins; there have been widely used as membrane prober in many cellular systems (Lis and Sharon 1973 Ann. Rev. Biochem. 42: 541). We report here evidence demonstrating that cells originated from FGSC 326 and FGSC 1118 "slime" cultures exhibited a markedly different response to concanavalin A-mediated agglutination.

Concanavalin (Con A) from Canavalia ensiformis was purchased from Sigma. Cells to be assayed were obtained from cultures mode on slants of Vogel solid complete medium (Woodward and Woodward 1968 Neurospora Newsl. 13: 18; starch was not added to the medium). After 2 to 3 days of incubation at 30° C, 3 ml of sodium phosphate buffer, 0.1 M, pH 6.0, containing 0.1 M NaCl, 2mM $MnCl_2$ and 2mM $CaCl_2$ (PBS buffer) were added to the slants. Cell suspensions were carried out by scraping very gently the surface of the slant with a 1 ml pipette, and then by sucking up and ejecting the liquid two or three timer. This procedure permitted us to resuspend homogeneously practically the totality of the cells, without disturbing at all the agar surface. The cell suspensions were transferred to 12 x 100 mm test tuber, and allowed to stand for a few minutes at room temperature. After that time, part of the cells in suspension were agglutinated spontaneously. It was regularly observed that about 25 to 30 of the cells obtained from FGSC 1118 cultures decanted spontaneously; this phenomenon was much less evident in suspensions from FGSC 326 cultures. Once the initial clump was formed, it could not be disgregated by gentle swirling or by dilution in PBS buffer. On the other hand the cells which remained in suspension did not agglutinate spontaneously, even after several hours of standing at room temperature. There cells were used for all the experiments. Occasionally, cells obtained from cultures mode in complete liquid medium were also assayed, and they behaved essentially like those obtained from cultures mode in solid medium, in terms of Con A agglutination. In the former case, however, the cells must be washed repeatedly by pelletting and resuspending in PBS buffer. This procedure causes some cell damage and also makes it more difficult to obtain homogeneous suspensions. In view of that, we decided to employ in the experiments cells obtained from cultures made on solid medium and to avoid the washing step.

Con A-induced agglutination was measured as the decrease in absorbance at 540nm in a Coleman Junior spectrophotometer. The assays were performed at room temperature. "Slime" cells were incubated at room temperature in Coleman tubes (12 x 75mm)which contained variable amounts of Con A in PBS buffer. The amount of cells was adjusted to hove an initial absorbance of 0.4-0.5; the final volume was 2 ml. α-Methyl glucoside and a-methyl mannoside were added in some experiments. The tuber were allowed to stand until no changes in absorbance were detected. Agglutination was usually completed after one hour. In control tubes, made in the absence of Con A, the change in absorbance due to spontaneous settling or lysis of the cells represented less than 5% of the initial O.D.

Figure 1 shows the results of a typical experiment. Cells from FGSC 1118 were agglutinated by Con A depending on the concentration of the lectin. A fifty percent decrease in absorbance was attained with 50 µg per ml. At a concentration of about 200 µg

Per ml complete agglutination was observed (as confirmed by microscopic examination). Agglutination was inhibited by 5 mM a-methyl mannoside. By contrast, under the same conditions a-methyl glucoside had no effect (data not shown). In contrast to these results cells from FGSC 326 cultures were not agglutinated by Con A, even at a very high concentration. After a brief treatment with 0.1 Percent trypsin, a moderate, though erratic, tendency to be agglutinated was observed in these cells (data not shown).

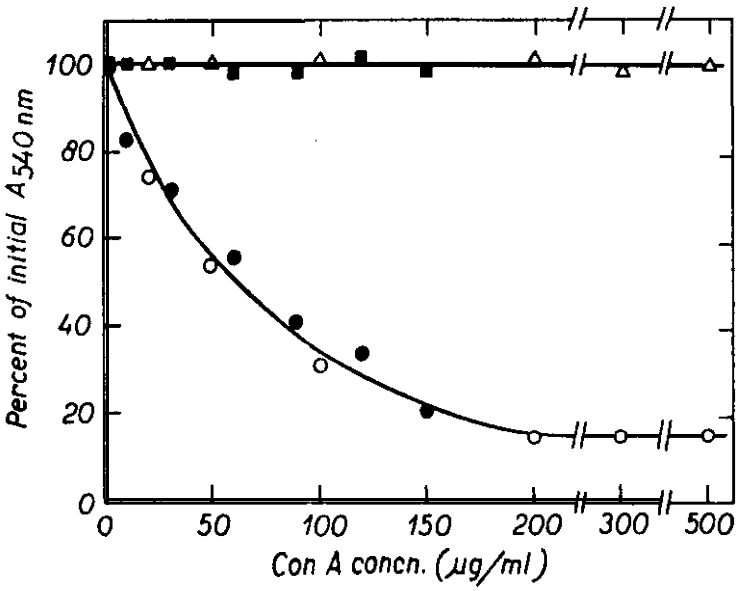

Figure 1. Con A-mediated agglutination in cell suspensions from cultures of FGSC #1118 and FGSC #326 "slime" strain. Cells were suspended in sodium phosphate buffer, 0.1 M, pH6.0, containing 0.1 M NaCl, 2mM $CaCl_2$ and 2mM $MnCl_2$, and Con A as indicated. The amount of cells was adjusted to have an initial absorbance at 540nm of 0.4-0.5, and the final volume was 2ml. The tubes were allowed to stand at room temperature until no changes in absorbance were detected. The experimental points represent the percent of the initial absorbance remaining after the agglutination was completed. Symbols: ● ,0: FGSC #1118, data from two different experiments; ● : FGSC #1118 with 5mM a-methyl monnoside added to the incubation mixture; Δ : FGSC #326.

We decided to investigate whether the binding sites for Con A were or were not Present at the surface of cells from the "slime" FGSC 326. Con A was labeled with ^{125}I(Na ^{125}I, NES-033 A, New England Nuclear) by the Chloramine T method (Allan and Crumpton 1973 Expti. Cell Res. 78: 271) The labeled lectin was absorbed onto a Sephadex G-75 column equilibrated with 1 .O M NaCl, washed with 2 volumes of the same saline solution, and eluted with 0.1 M glucose in 1.0M NaCl. Fractions were collected, and those representing the peok of radioactivity were pooled and dialized extensively against PBS buffer. Aliquots of the cell suspensions were incubated for 15 minutes at room temperature with the radioactive lectin, in PBS buffer. Final volume was 0.15ml

Table I. Binding of ^{125}I Con A to cells from cultures of FGSC 1118 and FGSC 326 "slime" strains.

Assay conditions[a]	FGSC 1118[b]		FGSC 326[b]	
	cpm x 10^3 (minus blank)	percent of remaining activity	cpm x 10^3 (minus blank)	percent of remaining activity
2.5 μg ^{125}I Con A[c]	102	100	126	100
2.5 μg ^{125}I Con A plus 5mM a-methyl glucoside	112	109	112	91
2.5 μg ^{125}I Con A plus 5mM a-methyl mannoside	26	25	30	23
2.5 μg ^{125}I Con A plus 200 μg unlabeled Con A	15	14	22	17

"Cells were incubated at room temperature in sodium phosphate buffer, 0.1 M, pH6.0, containing 0.1 M NaCl, 2mM $CaCl_2$ and 2mM $MnCl_2$. ^{125}I Con A, unlabeled Con A, a-methyl glucoside, and a-methyl mannoside were added as indicated. Final volume was 0.15ml. After incubation 3ml of PBS buffer were added and the cells were washed three times by centrifugation. Blanks without cells were also run. The activity of these always represented less than ten percent of that of the samples.

[b]The number of cells added for the assays was approximately the same in both cases.

[c]Specific activity was about 10^5 cpm per μg Con A.

After incubation, the samples were diluted with 3 ml of PBS buffer and the cells were pelleted and washed three times with the same solution. The liquid was finally decanted and the radioactivity in the cell pellet was meowed in a Gamma counter. The results shown in Table I demonstrate that cells from both "slime" strains were able to bind radioactive Con A. The binding was competed with by an excess of unlabeled Con A or by α-methyl mannoside, but not by a-methyl glucoside. These data demonstrate that binding sites for Con A are indeed present at the cell surface of both "slime" strains. However, we cannot conclude whether the two strains differ in terms of the number of binding sites present per unit of surface, or whether these sites hove the same affinity to bind Con A. Kinetic experiments, which ore beyond the scope of this note, should provide the answer.

The results reported here strongly suggest that the original "slime" obtained by UV treatment and the recombinant strain possess structural differences at the cell surface level. It is tempting to speculate on whether such differences ore or are not related to the cr-1 mutation. Crossing tests, which ore under way, should clarify this matter.

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