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Surface Membrane Hydrophobicity of *Paramecium primaurelia* Mating Types: Analysis under Immaturity and Maturity Conditions

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ABSTRACT—*Paramecium primaurelia* stock 90 clones were examined in their mating immaturity and maturity stages, under starving conditions triggering mating reactivity in mature cells. Living samples were labelled by cycloheptaamilose-dansyl chloride (CDC) complex, which has been proved to be an effective fluorescent marker of outer membrane proteins in living ciliates, as well as a sensitive probe towards microenvironment hydrophobicity. The analysis of CDC-labelled surface membrane region, usually engaged in conjugation, of immature and unpaired mature cells of mating types I and II, reveals differences in emission spectra, which can be referred to different hydrophobicity conditions. These findings lead us to assume that variations in surface membrane chemico-physical properties, differently affecting CDC fluorescence spectra, would be mating type-dependent and related to the functional states of cell immaturity or maturity, thus supporting the suggested role of hydrophobic interactions in *Paramecium* pair formation.

INTRODUCTION

The development of *Paramecium primaurelia* vegetative progeny yielded from conjugation, consists of the sequential stages of immaturity and maturity, referred to as cells' inability and capacity to conjugate in response to starvation, respectively. Conversely, the two products of the first fission following autogamy, are already able to conjugate under starving conditions [1, 13]. The initial event of conjugation is mating reaction involving in *Paramecium* cell-cell interactions at the tip surface of ventrally located cilia [7, 12] and triggering nuclear activation [6] as well as the sequential series of structural events, leading to establishment of contact sites between antero-ventral surface membranes of paired conjugants and formation of fusion areas [8].

The findings that hydrophobic interactions between an artificial substratum and ventral ciliary surface of *P. caudatum* mating reactive cells induce nuclear activation [10] and that cell attachment to substratum increases throughout mating reaction [11], suggested that hydrophobic interactions between the surface membrane regions engaged in conjugation, would play a role in pair formation.

This paper focuses on chemico-physical properties of *P. primaurelia* surface membrane region, usually involved in conjugation, labelled *in vivo* with cycloheptaamilose-dansyl chloride (CDC) complex. CDC-labelling occurring at the outer membrane of living ciliates involves surface proteins [16, 18], and is related to specific binding of the dansyl group with amino acids [2, 9]. Moreover, CDC emission spectrum is significantly affected by variation in hydrophobicity of the microenvironment, because of the sensitivity of dansyl chloride fluorochrome to the polarity of the medium [2]. We have analyzed mating types I and II mature unpaired cells of exautogamous clones as well as immature and mature unpaired cells of mating types I and II of exconjugant clones, under starving conditions triggering mating reactivity in mature cells. Beside the characterization of conjugation sites in *P. primaurelia*, with regard to their hydrophobicity, the results of this work can be exploited in more general studies on membrane-membrane interactions.

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MATERIALS AND METHODS

Culturing conditions and experimental designs

Paramecium primaurelia stock 90 was grown in monoxenic lettuce medium bacterized with *Enterobacter aerogenes* [14].

Two experimental designs were performed. The first experimental design (experiments A-A* and B) [4] refers to the two mature progeny derived vegetatively from the division products of an autogamous cell and named sister karyonides of the exautogamous clone. They were separately grown at 25°C and, in response to starvation, their mating type, I or II, was expressed when they reached early stationary growth phase.

The second experimental design (experiment C) refers to the vegetative progeny derived from an intraclonal cross of sister exautogamous karyonides, examined before and after transition to matu-

rity, in order to compare fluorescence intensities within a material as genetically homogeneous as possible. The four immature karyonides derived from the first fission of both exconjugants were allowed to divide once, and their two products referred to as sister subkaryonides and characterized by the same mating type expression, I or II [15], were differently cultured. Four subkaryonides were maintained at 18°C and their vegetative progeny were allowed to reach early stationary growth phase after a few fissions from conjugation, i.e. within the immaturity period. The other four subkaryonides were kept at 25°C as long as some fissions had occurred; thereafter, they were allowed to multiply in order to enter maturity before reaching early stationary growth phase. The mating type, I or II, of mature cells was revealed by their capacity to give mating reaction with stock 90 mating type II or stock P mating type I tester cells, respectively. Likewise, immaturity was directly proved by the inability of cells to give mating reaction with any testers.

Cytochemical procedures and measuring techniques

Immature and unpaired reactive cells of mating types I and II were transferred into a 0.005 M Tris-HCl buffer (pH 7.6), stained with a 5% cycloheptaamilose-dansyl chloride (CDC) (Polisciences, Washington, USA) solution in 0.1 M phosphate buffer (pH 7.4) for 7 min in absence of light, washed in Tris-buffer, transferred onto the slide and allowed to dry. Measuring of each experiment samples was performed separately and within 12 hr from the CDC-labelling of living paramecia.

The spectral analysis of the CDC-labelled outer membrane proteins was performed, cell by cell, on a small area of the antero-ventral surface region framed by a narrow rectangular-shaped diaphragm and focused by a 40× objective. In experiment A*, fluorescence of mating type I and mating type II reactive cells, was recorded on unreactive surface membrane regions, different from the reactive antero-ventral one.

Fluorescence emission spectra were measured by means of a Leitz Microspettrograph (Wetzlar, Germany) under epi-illumination conditions; equipped with an optical multichannel analyzer (EG & Princeton Applied Research, NJ), mounting an intensified silicon photodiode array detector (Model 1420/512). An HBO 100 W Hg lamp (Osram, Germany), combined with KG 1 and BG 38 antithermic filters was used as an excitation source. Excitation band was selected by means of 366 nm (±5) interference filters and TK 405 dichroic mirrors. The fluorescence emission was analyzed over the 400–750 nm range.

RESULTS AND DISCUSSION

Fluorescence emission spectra recorded on CDC-labelled antero-ventral surface membrane region, usually involved in conjugation, exhibit a clear difference in the position of their peak which has shifted to a longer wavelength in mating type I than in mating type II exautogamous cell lines, when analyzed under mating reactivity conditions (Fig. 1, experiment A). This result is consistent with the finding that the mean values of the ratios of fluorescence intensity emissions, measured at two selected wavelengths and recorded on surface reactive region, are significantly higher ($P<0.05$) in mating type I than in mating

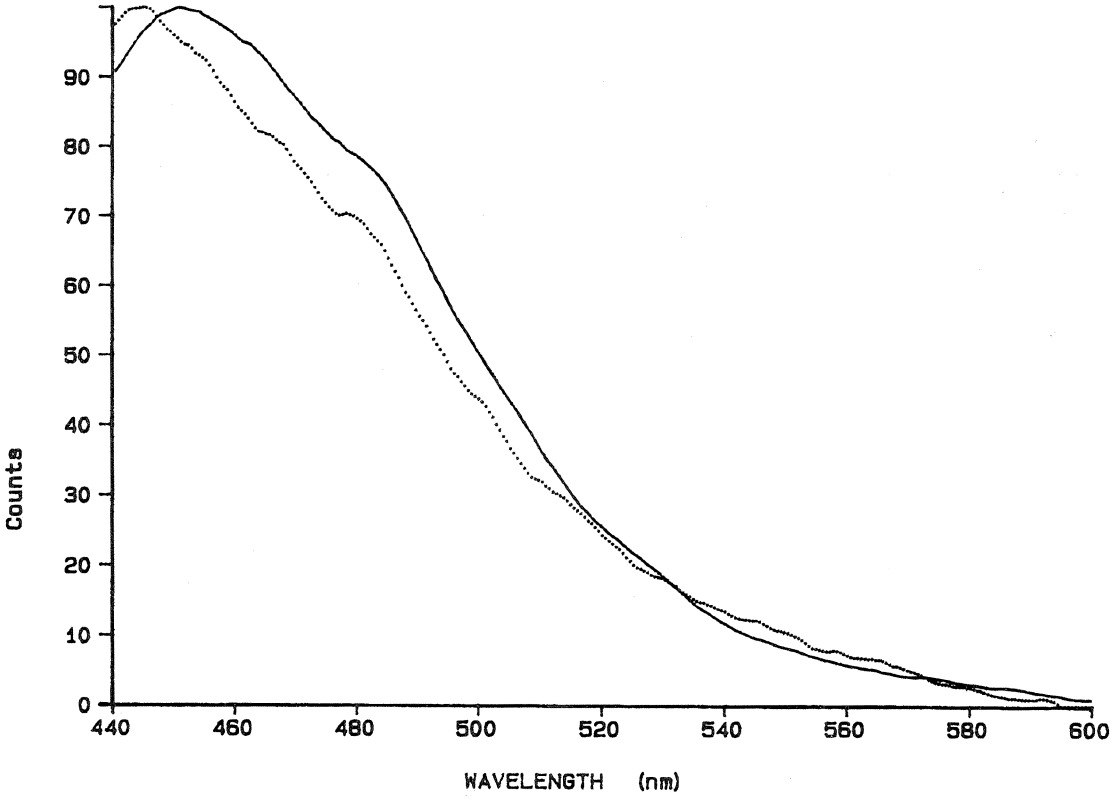

FIG. 1. Fluorescence emission spectra recorded on antero-ventral surface membrane region usually engaged in conjugation, in CDC-labelled mating type I (——) and mating type II (·····) cells of the exautogamous clone, analyzed under mating reactivity conditions (experiment A).

type II cells (Table 1, experiments A and B). Conversely, when fluorescence was recorded on unreactive surface membrane areas, no difference ($P>0.05$) appears between mating type I and mating type II reactive cells (Table 1, experiment A*). These data also prove that the change of condition of membrane zone to be measured, that is from reactive to unreactive one, affects CDC emission spectra, so that the difference between mating types disappears. Moreover, within both mating type I and mating type II populations, significant differences ($P<0.05$) in the ratio of fluorescence intensities are found between the reactive and unreactive regions, showing lower and higher values, respectively (Table 1, experiments A-A*). This observation points out the advantage of the membrane *in situ* analysis, as this method makes it possible to examine selectively the condition of the cell surface region engaged in conjugation.

TABLE 1. Spectral shape, expressed as mean value of the ratios of fluorescence intensities (FI) measured at two selected wavelengths, recorded on surface membrane of CDC-labelled mating type I and mating type II mature unpaired cells of exautogamous clones, analyzed under mating reactivity conditions (experiments A-A* and B)

Surface region	FI 465 nm/ FI 445 nm	FI 465 nm/ FI 445 nm	
	Mating type I	Mating type II	
Reactive (exp. A)	0.90±0.04	0.84±0.03	$P<0.05$
Unreactive (exp. A*)	1.12±0.04	1.09±0.02	$P>0.05$
	$P<0.05$	$P<0.05$	
Reactive (exp. B)	0.85±0.01	0.78±0.03	$P<0.05$

Mean±standard deviation. Student's *t*-test was used to compare the means. The number of examined cells per sample was comprised between 18 and 21. In experiments A and B, fluorescence was recorded on the antero-ventral surface membrane region; in experiment A*, fluorescence was recorded on another surface region different from the antero-ventral one.

Fig. 2 shows the fluorescence emission spectra recorded on CDC-labelled antero-ventral surface membrane region of mating type I and mating type II exconjugant cell lines analyzed in their immaturity and maturity stages, under starving conditions (experiment C). The four emission curves differ from each other in their patterns, depending both on the functional states of immaturity or maturity and the mating types, I or II. Actually, either in mating types I and II, spectral patterns have shifted to shorter wavelengths in mature than in immature cells. Moreover, shifts towards longer wavelengths are shown by the fluorescence emission patterns of mating type I immature and mature cells, when

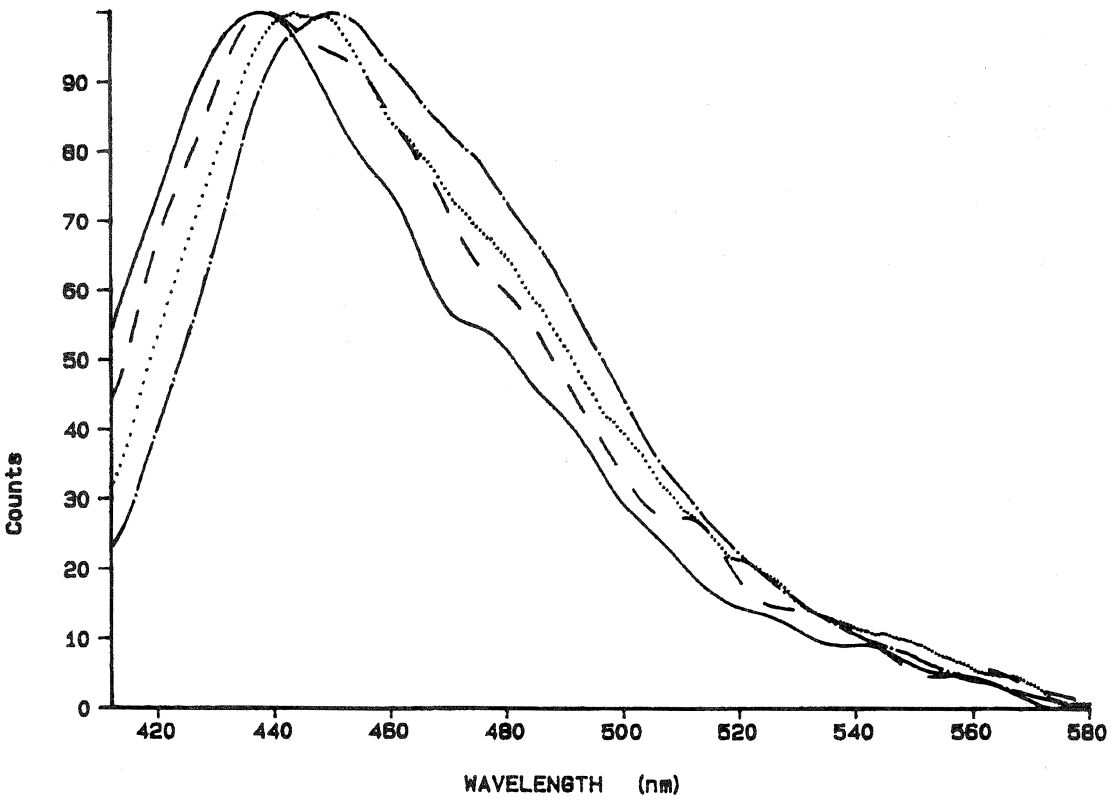

FIG. 2. Fluorescence emission spectra recorded on antero-ventral surface membrane region usually engaged in conjugation, in CDC-labelled mating types I and II cells of the exconjugant clones, analyzed in their immaturity (—·— and ·····, respectively), and maturity stages (– – – and ——, respectively), under starving conditions (experiment C). The number of measured cells per sample was comprised between 12 and 15.

compared with those of mating type II immature and mature cells, accordingly.

Spectral analysis of CDC-labelled membrane reveals the effect of membrane protein structure, occurring prior to labelling, on this fluorescent probe [17]. Therefore, conformational changes in membrane protein(s) leading to hydrophobicity variations would account for shifts of CDC fluorescence emission spectrum. Our results of spectral analysis of the CDC molecules bound to outer membrane proteins, reveal that the functional states of mating incompetence or competence are associated in *P. primaurelia* to variations in chemico-physical properties of the surface membrane region usually engaged in conjugation. Indeed, CDC emission spectra recorded on immature and mature cells indicate that development of mating competence would involve variations towards a more hydrophobic state, as a consequence of structural changes of membrane protein(s). Moreover, it appears that mating type I and mating type II *P. primaurelia* cells differ from each other in their hydrophobicity, which is enhanced in mating type II cells both in immaturity and maturity. Based upon this evidence, mating type-dependent differences in chemico-physical properties of surface membrane region engaged in conjugation, would be also related to the functional states of cell immaturity or maturity. This finding is consistent with previous results proving that mating type I and mating type II cell lines of *P. primaurelia*, characterized by a genetical homogeneity as possible, are two discrete physiological types [3, 5]. It can be suggested that enhanced hydrophobicity in mature cells *vs* immature ones, would depend on mating type-specific structural changes of surface membrane protein(s), which are possibly due to loss or masking of hydrophilic groups.

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