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INHIBITION OF CONJUGATION IN *TETRAHYMENA PYRIFORMIS* BY CERULENIN

POSSIBLE REQUIREMENT FOR DE NOVO LIPID SYNTHESIS

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Summary

Conjugation in *Tetrahymena pyriformis* is induced by the mixing of two starved complementary mating types. Addition of the antibiotic cerulenin, a specific inhibitor of de novo lipid synthesis, upon mixing of the mating types inhibited the conjugation process. The inhibition of conjugation was found to be reversible upon washing the cells.

Cerulenin inhibited [^{14}C]acetate incorporation into the lipid fraction of the cells, while it did not affect the incorporation of [^{3}H]leucine into proteins. Analysis of the fatty acid composition of the whole cells revealed that during conjugation the ratio of saturated to unsaturated fatty acids is markedly changed. While the ratio of saturated:unsaturated fatty acids is 0.30 in unconjugated cells, it reached a value of 0.45 in conjugated cells.

Introduction

Processes of cell recognition, cell agglutination and membrane fusion have attracted much interest in recent years. Among membrane components, proteins, and particularly glycoproteins, were suggested to mediate cell recognition and cell agglutination. Glycoproteins may be involved in such phenomena since they extend from the membrane, may attain a high degree of specificity and their synthesis may be rigorously controlled. On the other hand, very little attention has been paid to the active role of the lipids which constitute the matrix of the membrane in which the other components are embedded. It has been shown that specific phospholipids and lysophospholipids are able to induce membrane fusion when added to cells in culture [1]. Based on these observations, it was suggested that membrane phospholipids may play an active role in natural fusion processes such as mating and fertilization.

The use of specific inhibitors was of much importance in elucidating the requirement for de novo synthesis of proteins in phenomena of cell recognition and agglutination [2]. The lack of a specific inhibitor for lipid biosynthesis prevented such studies with lipids. Recently an antibiotic which interferes primarily with de novo synthesis of lipids was isolated from the culture filtrate of *Cephalosporium caerulens* [3,4]. Cerulenin, an antibiotic of the structure 2,3-epoxy-4-oxo-7,10-dodecadienoylamide [5—7], is a specific inhibitor of the condensing enzyme in the fatty-acid synthetase complex [8], β-ketoacyl-acyl carrier protein synthetase in the non-associated fatty-acid synthetase [9], and 3-hydroxy-3-methyl-coenzyme A synthase [10] in sterol synthesis. It was used in many systems [7] both for studies of fatty-acid biosynthesis and of the biosynthesis, structure, and functions of membranes in diverse microorganisms or cells in which fatty acid synthesis occurs.

During the last few years we have studied in our laboratory the conjugation process of *Tetrahymena pyriformis* in order to elucidate the involvement of proteins and glycoproteins in cell recognition and membrane fusion [11—13]. This sytem is also suitable for studying the requirements for de novo synthesis of lipids for the above membrane events. The various steps in the conjugation process occur in the following order: After mixing of starved complementary mating types, there is a lag period after which the cells specifically meet and adhere (cell recognition and agglutination). Eventually their membranes fuse in a restricted part of the cells, and cytoplasmic bridges between the cells are established [14]. Thus, it would be of interest to study the role of lipids and, particularly, fatty acids in this naturally occurring, yet controlled, system.

In the present work we have used cerulenin to study the requirement for de novo fatty acids and lipid synthesis for the various stages of the conjugation in *T. pyriformis*. In addition, the effect of cerulenin on the incorporation of fatty and amino acids into the cells of *T. pyriformis* was monitored.

Materials and Methods

Materials

Fatty acids (Sigma, 99% pure) were in the form of K^+ salt and were dissolved in slightly alkaline water/ethanol solution (1 : 3, v/v).

Cerulenin (Makor Chemicals Ltd., Israel) was dissolved in distilled water at 60°C to a concentration of 1 mg/ml.

DL-[2-^{14}C]Mevalonic acid (23 mCi/mmol) was obtained from The Radiochemical Centre, Amersham. [1-^{14}C]Oleic acid (54 mCi/mmol) was obtained from The New England Nuclear Corp. [1-^{14}C]Acetate (59 mCi/mmol) and [^{3}H]leucine (38 Ci/mmol) were obtained from The Nuclear Research Center, Negev, Israel.

T. pyriformis mating type I (strain WH-6) and mating type III (strain WH-52) were obtained from the American Type Culture Collection and were grown under sterile conditions at 26°C, as previously described [13].

Medium

The medium used for washing of cells, starvation, and conjugation experiments was 20 mM tricine/NaOH, pH 7.4.

Induction and determination of conjugation

Conjugation was induced in starved cells essentially as previously described [13]. After washing of the growth medium each of the mating types was suspended in 20 mM tricine/NaOH, pH 7.4 (approx. 10^6 cells/ml) and incubated at 30°C for 24 h (starvation period). For conjugation, 0.5 ml of each of the starved mating types was introduced into 20-ml glass flasks and incubated at 30°C without shaking. If not otherwise stated, conjugation was determined after 4 h of incubation. Percent of conjugation was estimated by counting cells under the phase microscope, as previously described [13]. All the experiments in the present work were performed in duplicate and each sample was counted twice. Each time 75—100 cells were counted. The results given are an average of two separate counts.

Incorporation of [^{3}H]leucine

Conjugation was performed in a total volume of 2 ml. [^{3}H]Leucine, 10 μCi (10 mCi/nmol) was added to the conjugation medium right after mixing of the mating types and samples of 0.1 ml were withdrawn at different times and loaded on chromatography papers (2.5 cm in diameter). The loaded papers were immersed in a solution of 5% cold trichloroacetic acid, washed three times with cold 5% trichloroacetic acid (approx. 10 ml for each sample), and incubated again for 20 min in 5% trichloroacetic acid warmed to 90°C. After three additional washes with cold 5% trichloroacetic acid, the acid was removed by immersing the chromatography papers once in acetone/ether (1 : 1) and once in pure ether. After drying the paper, disks were immersed in 3 ml toluene scintillation liquid and were counted in a scintillation counter (Packard).

The amount of protein in the cell suspensions was determined according to Lowry et al. [15] and bovine serum albumin served as a standard.

Incorporation of radioactive precursors into lipids

[1-^{14}C]Acetate (15 μCi, 1 mM) or [2-^{14}C]mevalonate (3.7 μCi, 1 mM) or [1-^{14}C]oleic acid (2.5 μCi, 7 mM) were added to 10-ml portions of cell suspensions. At different times, samples of cells (1 ml) were transferred to cooled tubes containing 10 ml of cold buffer solution. After centrifugation at 2000 $\times g$ for 5 min at 4°C, the cells were washed with 10 ml of fresh buffer. The cell pellet obtained after centrifugation was extracted successively with 4-ml portions of ethanol/ether (3 : 1, v/v) and chloroform/methanol (2 : 1, v/v). The combined extracts were evaporated to dryness, and the residue dissolved in 3 ml of acetone/ethanol (1 : 1, v/v) (total lipid fraction). Samples were taken for radioactivity measurement (0.1 ml). The remainder of the total lipid solution was evaporated to dryness, dissolved in 1 ml water and 0.3 ml 50% KOH, and saponified at 100°C for 30 min. This solution was extracted three times with 5-ml portions of petroleum ether (40°C to 60°C b.p.). The petroleum ether solution was evaporated to dryness, and the residue was dissolved in 10 ml of toluene scintillation solution and counted for radioactivity (nonsaponifiable fraction). Following acidification of the above aqueous solution to pH 2 with 6 M HCl, the fatty acids were extracted with three 5-ml portions of petroleum ether (fatty acid fraction). Samples were taken for radioactivity measurement.

Fatty acid analysis

The fatty acid methyl esters were prepared from the fatty acid fraction, as described by Moss et al. (ref. 16, Method B). A Varian 1200 gas chromatograph equipped with flame ionization detector was used for analysis of samples of methyl esters of fatty acids. Samples were analyzed on stainless steel columns packed either with 20% diethylene-glycol-succinate (DEGS) on Chromosorb W (60/80 mesh), or with 10% Apiezon L on Chromosorb W/AN/DMCS (70/80 mesh) (Applied Science Co., State College, Pa.). The methyl esters' peaks were tentatively identified by comparison of their retention values with highly purified methyl ester standards (Applied Science Co.). Peak areas of methyl esters were determined by weighing, and the percentage of each fatty acid was calculated from the ratio of the area of its peak to the total area of all peaks.

Results

Inhibition of conjugation by cerulenin

Fig. 1 shows the effect of increasing concentrations of cerulenin on the conjugation process when added upon mixing of the mating types. The inhibitory effect of cerulenin follows a linear shape and, at a concentration of 7 μg/ml, the inhibition is total.

The events which follow mixing of the mating types and lead to permanent conjugating pairs may consist of several distinct steps [14]. Therefore, it is of great interest to check the effect of the inhibitor when added at various times after the process has been initiated (mixing of the mating types). Fig. 2 shows that addition of cerulenin at various times after conjugation has already started blocks any further increase in the number of conjugating pairs and even causes

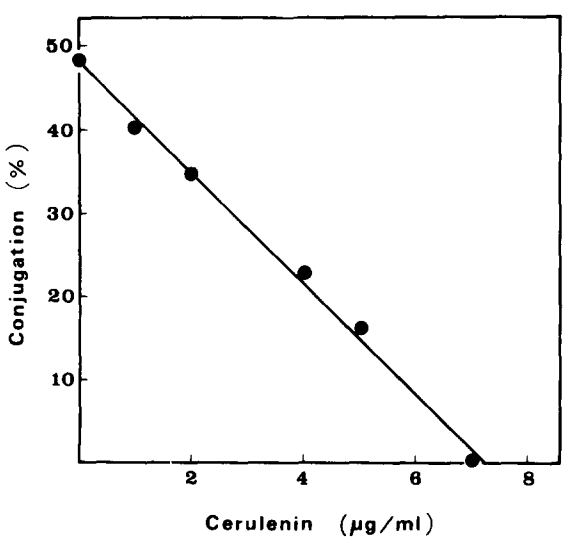

Fig. 1. The effect of increasing concentrations of cerulenin on the conjugation process. Cerulenin was added upon mixing of the mating types and conjugation was determined 4 h later, as described under Materials and Methods. In this experiment the degree of conjugation in a control system without cerulenin was 48%.

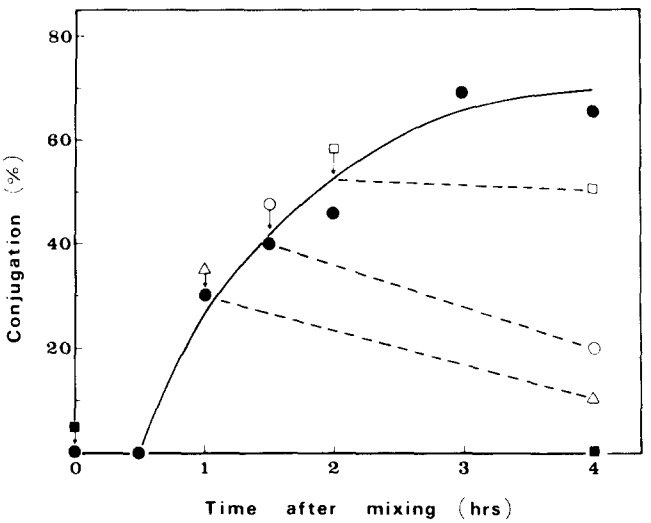

Fig. 2. Inhibition of conjugation by cerulenin added at various times during the conjugation. Conjugation was performed in parallel in several glass flasks in a total volume of 1 ml. At the time indicated by the arrows, 7 μg/ml of cerulenin was added to each flask. Samples of 0.05 ml were withdrawn for determination of conjugation before and after addition of cerulenin. ●, Control without inhibitor. The inhibitor was added at: ■, zero time; △, 1 h; ○, 1.5 h; □, 2 h after mixing of the mating types.

partial dissociation of pairs already formed. For example, cerulenin caused a decrease of about 50% in the conjugation when added 1 h after mixing (from 30% to 15%) (Fig. 2).

The concentrations which were used routinely (8—20 μg/ml) were about 10 times smaller than the concentration which caused cell death (200 μg/ml), and under these conditions viability of the cells was not affected. This was assessed by (a) microscopic observation of the shape and motility of the cells; (b) reversibility of the inhibitory effect upon removal of the antibiotic by washing with

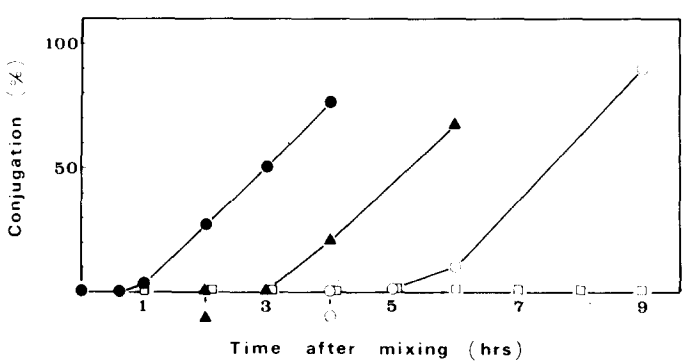

Fig. 3. Restoration of conjugation by removal of cerulenin from the conjugation medium by washing with fresh medium. Conjugation was performed in parallel in several glass flasks in a final volume of 1 ml. At zero time the two mating types were mixed and cerulenin (8 μg/ml) was added. At the times indicated by arrows the content of the vials was washed three times with 20 mM tricine-NaOH, pH 7.4, resuspended to its original volume (1 ml), and reincubated at 30°C. Samples of 0.05 ml were withdrawn for conjugation determination. ●, Control without cerulenin. Samples containing cerulenin were washed twice with 20 mM tricine-NaOH, pH 7.4: ▲, 2 h; ○, 4 h after mixing of the mating types, □, Unwashed cells.

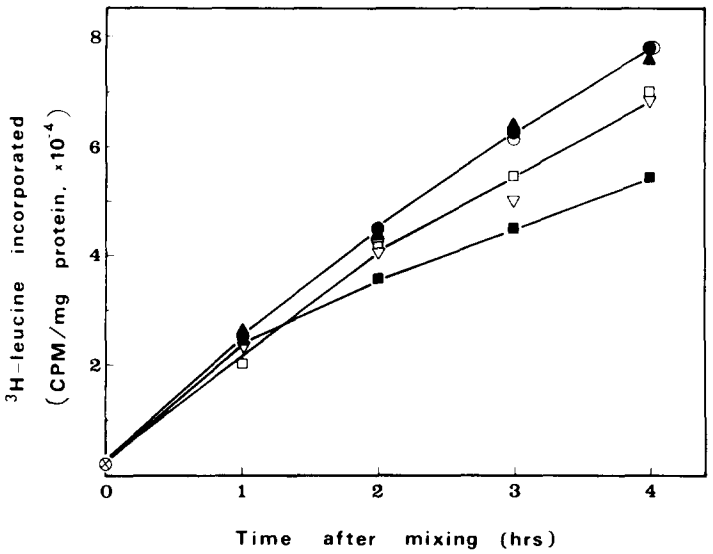

Fig. 4. The effect of cerulenin on the incorporation of [^{3}H]leucine into the mating types of *T. pyriformis*. 1-ml samples of starved cells (10^6 cells/ml) of each mating type were mixed in 20-ml vials and various amounts of cerulenin were added to the different systems upon mixing. [^{3}H]Leucine, 10 μCi (10 mCi/mmol) was added to the conjugation medium 10 min after mixing, and sampling started from that moment. Determination of the incorporation is described under Materials and Methods. ●, Control. Control plus cerulenin: ○, 8 μg/ml; ▲, 10 μg/ml; □, 15 μg/ml; △, 20 μg/ml; ■, 30 μg/ml.

fresh medium (Fig. 3); and (c) [^{3}H]leucine incorporation (Fig. 4).

The inhibition of the conjugation caused by cerulenin could be overcome by washing the inhibitor from the medium. Fig. 3 shows that even after 4 h of incubation of the two mating types with cerulenin, the cells did not lose the ability to conjugate (conjugation after washing even exceeded the degree of the unwashed control). As can be seen in Fig. 3, after the removal of the inhibitor, the cells resumed conjugation and followed the same kinetics as cells which were just mixed. Also, the lag time of 1 h, before the appearance of the first pairs, was still necessary, although the two mating types were mixed already for 4 h.

Effect of cerulenin on protein synthesis and de novo lipid synthesis

Fig. 4 describes the effect of various concentrations of cerulenin on the incorporation of [^{3}H]leucine into the proteins of *T. pyriformis*. Concentrations of 8—10 μg/ml of cerulenin, which completely blocked conjugation, did not affect the incorporation of [^{3}H]leucine. Higher concentrations of 15 and 20 μg/ml inhibited the incorporation of [^{3}H]leucine by 10%. The highest concentration used (30 μg/ml) showed a biphasic shape with a turn point at 1 h. That may reflect an indirect effect of cerulenin on the protein synthesis.

Under conditions where cerulenin blocked conjugation but did not affect protein synthesis, it caused marked inhibition of [^{14}C]acetate incorporation into total lipid fraction. As can be seen in Fig. 5, the degree of inhibition of [^{14}C]acetate incorporation depends upon the concentration of cerulenin used and is correlated to the inhibition of conjugation. Fig. 6 shows the effect of

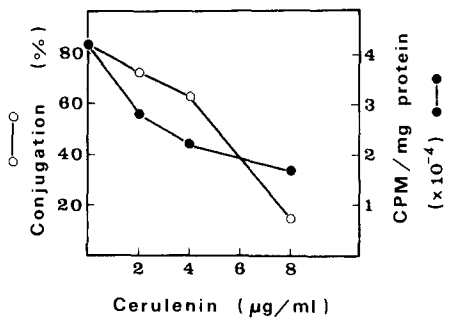

Fig. 5. The effect of increasing concentrations of cerulenin on [^{14}C]acetate incorporation and on the conjugation process. Conjugation was performed in parallel in several glass flasks in a final volume of 5 ml. At zero time the suspensions of the two mating types were mixed, and cerulenin in various concentrations was added. [^{14}C]Acetate (3 μCi, 0.5 mM) was added 10 min after mixing of the mating types. Samples of 1 ml were withdrawn at 4 h after mixing of the mating types for determination of [^{14}C]acetate incorporation into the total lipid fraction, and samples of 0.05 ml were used for determination of percent conjugation (see Materials and Methods). ○, Conjugation; ●, [^{14}C]acetate incorporation.

cerulenin on the incorporation of [^{14}C]acetate into the various lipid fractions.

Fractionation of the lipids into two groups: (a) fatty acids, and (b) non-saponifiable material (sterols and neutral lipids) showed that the incorporation of [^{14}C]acetate to both fractions was inhibited almost to the same extent (80% to non-saponifiable fraction and 90% to fatty acid fraction) (Table I and Fig. 6). This finding is consistent with the postulated mechanism of action of the inhibitor and resembles its effect in other organisms [7].

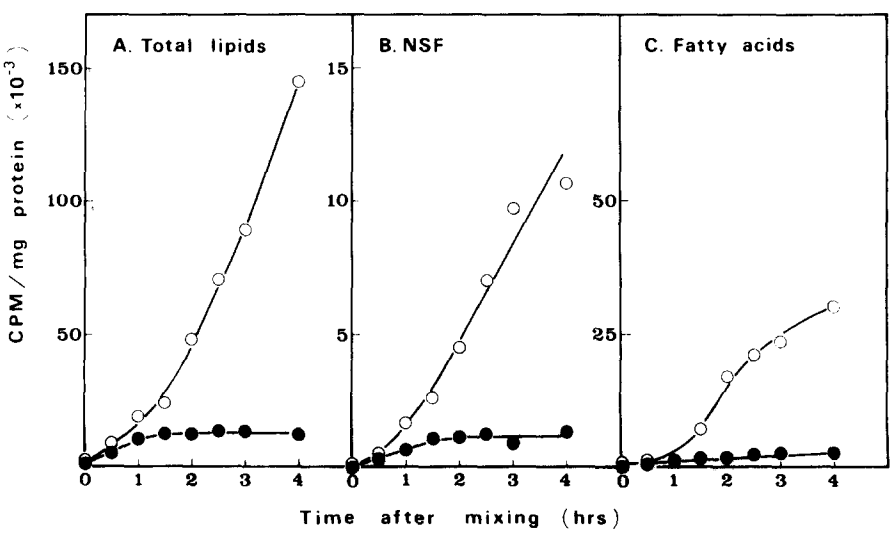

Fig. 6. The effect of cerulenin on the incorporation of [^{14}C]acetate into various lipid fractions of mating types of *T. pyriformis*. 5 ml of starved cells (10^6 cells/ml) of each mating type were mixed in a 2-l Erlenmeyer flask and allowed to conjugate, in the absence and in the presence of 8 μg/ml cerulenin, which was added upon mixing. [^{14}C]Acetate was added 10 min after mixing, and sampling started from that time. Determination of the incorporation to the various fractions is described under Materials and Methods. A, Total lipids; B, non-saponifiable fraction (NSF); C, fatty-acid fraction. ○, ●, Absence and presence of cerulenin, respectively.

TABLE I

EFFECT OF CERULENIN ON THE INCORPORATION OF [1-^{14}C]ACETATE, [1-^{14}C]OLEIC ACID AND [2-^{14}C]MEVALONIC ACID INTO LIPIDS OF MATING TYPES OF *TETRAHYMENA PYRIFORMIS*

5 ml of starved cells (10^6 cells/ml) of each mating type were mixed in a 2 l Erlenmeyer flask in the presence or in the absence of cerulenin (8 µg/ml). The radioactive compound was added 10 min after mixing. Determination of conjugation and of incorporation into the various fractions was performed 4 h after mixing. For experimental conditions see Materials and Methods.

Radioactive compound	Cerulenin (µg/ml)	Conjugation (%)	Total lipids (cpm/mg protein)	Fatty-acids (cpm/mg protein)	Non-saponifiable (cpm/mg protein)
[1-^{14}C]Acetic acid	0	60	145 000	32 500	11 100
[1-^{14}C]Acetic acid	8	0	15 000	2 500	1 150
[1-^{14}C]Oleic acid	0	60	41 470	12 020	—
[1-^{14}C]Oleic acid	8	0	45 700	13 800	—
[2-^{14}C]Mevalonic acid	0	60	2 190	—	180
[2-^{14}C]Mevalonic acid	8	0	2 430	—	179

Since cerulenin inhibits only the first stages of fatty-acid and sterol synthesis, the incorporation of metabolites beyond the inhibition point should not be affected by cerulenin. Indeed, as Table I shows, the incorporation of oleic acid into the fatty-acid fraction and mevalonic acid into the non-saponifiable fraction were not influenced by cerulenin, whereas acetic-acid incorporation into both fractions was strongly inhibited.

Cells inhibited by cerulenin were supplemented with various lipids, and their ability to restore conjugation was checked. The following lipids were tested: fatty acids (such as oleic, myristic, palmitic, cis-vaccenic, linoleic, and linolenic), sterols (such as squalene and cholesterol) and mevalonic acid. Mixtures of 2 of 3 of the pure lipids, lipid extracts of either of the mating types and of cells undergoing conjugation, were also checked. In none of the cases could conjugation be restored in the presence of cerulenin (not shown).

Changes in lipids during conjugation

The fact that cerulenin inhibits conjugation may suggest that de novo synthesis of lipids is required for conjugation. Mixing of the mating types may induce a net increase in the lipid synthesis or synthesis of new specific lipid species. Alternatively, a new distribution of the lipids may occur.

Fig. 7 shows the incorporation of [^{14}C]acetate into the lipid fraction of the cells during conjugation as compared to the rate of incorporation into the unmixed mating types. This figure shows that the two mating types have different rates of incorporation and that the rate of total lipid synthesis in the conjugants is slightly higher (approx. 10—15%) than the calculated average of the rate of the two unmixed mating types. This finding repeats itself when we measured the incorporation of [^{14}C]acetate to the non-saponifiable fraction. However, no increase was found in the incorporation of [^{14}C]acetate into the fatty-acid fraction of conjugated cells. Therefore, we examined the composition of fatty acids and the ratio of saturated to unsaturated fatty acids of both mating types of *T. pyriformis* before mixing, and of conjugants which were produced 4 h after mixing. As can be seen in Table II, the ratio of saturated to unsatur-

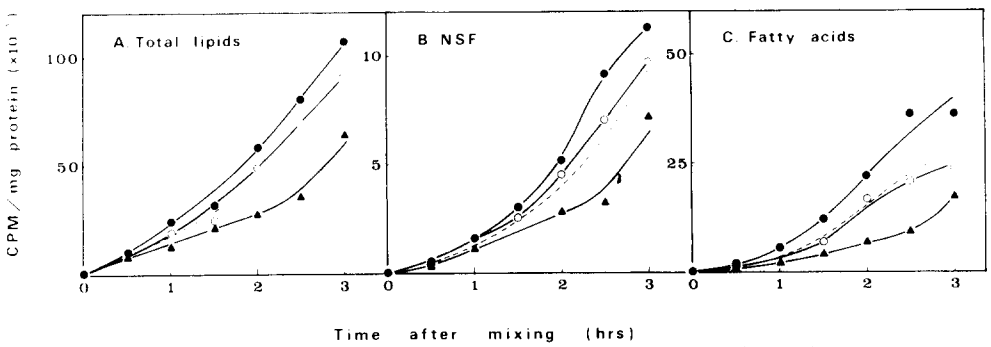

Fig. 7. Incorporation of [^{14}C]acetate into the mating types of *T. pyriformis* during conjugation. Conjugation was induced by mixing 5 ml of starved cells (10^6 cells/ml) of each mating type in a 2-l Erlenmeyer flask. In parallel, 10 ml of each mating type were incubated separately under the same conditions. [^{14}C]-Acetate, 15 μCi, was added 10 min after mixing and sampling started from that time. Determination of the incorporation into total lipid fraction is described under Materials and Methods. NSF, non-saponifiable fraction. ▲, Mating type I; ●, Mating type III; ○, conjugation; - - - - - -, calculated average of mating type I and mating type III.

rated fatty acids of *T. pyriformis* cells was 0.25 for mating type I and 0.35 for mating type III, while conjugants had a ratio of 0.45. This means that the ratio increased from 0.30 at the moment of mixing (the calculated average value of the two mating types) to 0.45 when conjugation reached its maximum. In the case where cerulenin was present in the mixture of the two mating types and conjugation was prevented, the ratio of saturated to unsaturated fatty acids

TABLE II

THE COMPOSITION OF FATTY ACIDS AND THE RATIO OF SATURATED TO UNSATURATED FATTY ACIDS OF THE MATING TYPES AND OF CONJUGANTS OF *TETRAHYMENA PYRIFORMIS*

20 ml (10^6 cells/ml) of each of the starved mating types were mixed and allowed to conjugate in the absence and in the presence of 8 µg/ml cerulenin. In parallel, 40 ml of each of the mating types were incubated separately under the same conditions. After 4 h conjugation was determined and all the samples were prepared for a fatty acid analysis. Conjugation reached 60% in control and 0% in the presence of 8 µg/ml cerulenin. For other experimental conditions see Materials and Methods.

Fatty acid *	Mating type I	Mating type III	Calculated average of mating types I + III	Conjugation	Mixture of mating types I + III + Cerulenin
14 : 0	2.0	4.6	3.3	4.6	3.6
15 : 0	1.3	1.0	1.15	1.6	3.0
16 : 0	8.0	9.7	8.85	10.4	7.7
16 : 1	7.7	7.0	7.35	9.0	6.6
17 : 0	0.5	0.8	0.65	1.2	0.9
18 : 0	8.2	9.9	9.05	13.1	7.1
18 : 1	16.8	16.0	16.4	10.9	7.8
18 : 2	18.0	22.1	25.05	24.0	34.3
18 : 3	27.5	28.9	28.2	25.2	29.0
Saturated fatty acids / Unsaturated fatty acids	0.25	0.35	0.30	0.45	0.29

* Number preceding colon indicates the number of carbon atoms, and number following colon indicates the number of double bonds in the fatty acids.

was the same as for uninduced cells (0.29). The increase in the ratio of saturated to unsaturated fatty acids during conjugation was observed in another experiment, although the absolute values were a little different probably due to minor changes in the experimental conditions (temperature, volume, etc.).

Discussion

The events of the conjugation process of *T. pyriformis*, from mixing of the two starved mating types up to the formation of the cytoplasmic bridges between the cells, can be divided into two stages: (a) "costimulation", during which the two complementary mating types randomly collide, and (b) the pairing. The formation of the pairs depends on specific recognition between the complementary mating types and proceeds through stages of cell agglutination and, subsequently, fusion of the two adjacent membranes [14].

In the present work we have shown that cerulenin inhibited the conjugation process of *T. pyriformis* when added to the conjugation medium upon mixing of the mating types. The antibiotic cerulenin inhibited conjugation even when it was added after the cells had proceeded through the first stages of recognition and agglutination, and even partially dissociated preformed pairs. This suggests that there is requirement for a continuous de novo synthesis of lipids throughout the whole process.

It has been previously shown that the stages of recognition and agglutination require de novo synthesis of glycoproteins [11]. The requirement for lipid synthesis may arise either from the need for a lipid in one of the steps of the biosynthesis of these glycoproteins [17] or that the macromolecule responsible for recognition and agglutination is in fact a lipoglycoprotein. The synthesis of receptor molecules may have an auto-catalytic character and this may explain the results of the present work showing a demand for a continuous synthesis of lipids, which may be coupled to the corresponding proteins.

Two kinds of mechanisms were suggested for membrane fusion: one of them states that the proteins are the major components which are responsible for the interaction between the membranes [18] and the other sees the intermixing of the lipids as the main event [1]. The latter mechanism is based on experiments showing that the changes in the composition of the lipid phase facilitates the process of membrane fusion. Therefore, the results of the present work showing the involvement of lipids in the stage of membrane fusion is not surprising.

Further support for the involvement of the lipid fraction of the membrane in initiation of the fusion process comes from ultrastructural studies. Recently it has been shown that during secretion of histamine from mast cells, the intracellular zymogen granules are fused with the plasma membrane in regions free of intramembranous particles, generally thought to represent protein-free lipid areas [19]. In addition, membranes of human erythrocyte ghosts have been shown to fuse, especially in regions which appear smooth, depleted of intramembranous particles, after freeze-fracturing [20]. Similar structures, namely regions poor in intramembranous particles, were also observed by Satir [21] during fusion of mucocytes in *T. pyriformis* or by Goodenough during conjugation of *Chlamydomonas* [22].

Recently we have found that the membrane area between the two mating

types of *T. pyriformis*, which is formed during conjugation, is completely devoid of intramembranous particles and that its size increases as the process progresses [23]. This protein-free, lipid-rich area can be formed either by displacement of intramembranous particles due to the relative movement of pre-existing lipid molecules, or by insertion of new lipid molecules which are directed to this restricted, specialized area. The lipids in this particular area may have some specific properties which will help the process of intermixing of the lipid layers of the two cells. These changes may involve either alteration in charge distribution, or some physical parameters as chain length or unsaturation.

Our results indicate only a small change in the overall rate of lipid synthesis during the conjugation, while we do find a considerable difference in the distribution of fatty acids. We have found that the ratio of saturated to unsaturated fatty acids increases during the conjugation process from 0.30 to 0.45 (Table I). As we have extracted lipids of the whole cell and the fusion itself is limited to a small area, our data may represent a lower estimate. Nozawa et al. [24] have shown that alterations in fatty acid composition of the membrane of *T. pyriformis* is accompanied by a change in its fluidity. The higher the ratio of saturated to unsaturated fatty acids becomes, the lower the fluidity goes. In light of this concept, we may conclude that the fluidity of the *T. pyriformis* membranes decreases during the conjugation process. This finding may look intriguing because one would have expected the membranes that fuse to be more fluid. A possible explanation might be that a membrane area of large dimensions, which lacks proteins, must have higher rigidity in order not to collapse.

Interestingly, Ohno et al. [25] found that in the sporulation process of *Saccharomyces cerevisiae* the ratio of saturated to unsaturated fatty acids increases from 0.11 (for presporulated yeast) to 0.164 (for sporulated yeast).

Our failure to restore conjugation in the presence of cerulenin by supplying exogenous lipids may reflect a highly controlled mechanism in which the synthesis and insertion of proteins and lipids is tightly coupled.

The fact that conjugation in *T. pyriformis* is a natural and dynamic process, helped us to point out that beside their passive participation in the phases of recognition, agglutination and fusion, lipid biosynthesis is specifically induced for these purposes.

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