EFFECTS OF MEMBRANE FLUIDITY CHANGES ON LECTIN BINDING IN *TETRAHYMENA PYRIFORMIS*

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Abstract—The supposed interrelationship between membrane fluidity and membrane saccharide composition was studied in model experiments on *Tetrahymena pyriformis* cells. Membrane fluidity changes were induced by different environmental (culturing) temperatures, and by the membrane perturbing agent ergosterol. The modifications of the membrane saccharide organization position and their interrelationships with membrane fluidity were assessed from changes in the binding capacity for seven lectins of different ligand specificity.

The membrane fluidizing effect of long-term or short-term cold exposure was associated with an increase in the membrane glucose, mannose (plus derivatives) and N-acetyl-galactosamine organization, whereas heat exposure enhanced membrane rigidity by an opposite effect on the same saccharide components. The effect of ergosterol, although it depended greatly on the interaction of other factors acting on membrane fluidity, was similar to that of warm culturing conditions or heat shock, yet ergosterol-induced membrane rigidity was due primarily to a N-acetyl-glucosamine organization increase.

Key words: Tetrahymena, ergosterol, temperature-changes, lectin-binding, membrane-perturbation

EFFETS DES MODIFICATIONS DE LA FLUIDITÉ DE LA MEMBRANE SUR LA LIAISON LECTINE DE *TETRAHYMENA PYRIFORMIS*

Résumé—Les interrelations supposées entre la fluidité de la membrane de *Tetrahymena*, et la composition de la membrane en sacharides ont été étudiées au cours d'expériences sur les cellules de *Tetrahymena pyriformis*. Les changements de fluidité de la membrane on été induits par différentes températures environantes et par un agent perturbateur de la membrane, l'ergostérol. Nons avons enregistré des modifications des sacharides par la mesure de la liaison de ligand spécifique de sept différentes lectines, et nous en avons conclu la corrélations possibles entre la fluidité de la membrane et les sacharides examinés.

La membrane fluide, résultat d'un refroidissement à court ou long terme, a été suivi par l'accroissement quantitatif des glucose et mannose de la membrane et de leurs dérivés et de N-acétyl-galactosamine. La membrane rigide, effet de la température opposée, était causée par les composants sacharides ci-dessus qui avaient une distribution opposée. L'effet de l'ergostérol, bien que dépendant de l'interaction d'autres facteurs agissant sur la fluidité de la membrane, était similaire à ceux mentionnés lors des températures élevées ou basses qui déterminent la rigidité ou la fluidité de la membrane, et est suivi en premier lieu par la multiplication des molécules de N-acétyl-glucosamine.

Mots-clefs: Tetrahymena, ergostérol, changement de température, liaison de la lectine, perturbation de la membrane

INTRODUCTION

The cell membrane, with its receptor and marker structures, plays a key role in interactions between the cell and its environment. The continuous environmental changes greatly contribute to the remarkable alterability of the cell membrane. However, certain characteristic components of the cell membrane are conservative structures, which seem to account for cellular integrity at both the unicellular and the multicellular level. Cellular integrity is probably the issue of the interrelationship between internal regulation and external influences.

Unicellular organisms in aquatic indigenous surroundings are particularly sensitive to environmental changes. *Tetrahymena pyriformis*, used in the present study as a model cell, is also noted for its extraordinary responsiveness to environmental influences (Kovács, 1984; Nozawa and Thompson, 1979).

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Environmental temperature changes can alter the structure of the cell membrane (Fukushima *et al.*, 1976; Wunderlich *et al.*, 1973). High and low ambient temperatures equally affect the lipid and phospholipid composition of the membrane, but while a warm environment increases its fluidity, a cold environment renders it more rigid. These changes are transient, for, after a certain time, the cell adapts to the changed environmental conditions by modifying the structure of its membrane. The final result of membrane modification, which seems to involve the flow of microsomal lipids towards the surface (Ramesha and Thompson, 1982), is that the warm environment increases ultimately the rigidity, while the cold environment affects the fluidity of the membrane relative to its original state (Kameyama *et al.*, 1980; Morris *et al.*, 1984; Umeki *et al.*, 1983).

Certain materials fluidize the cell membrane by other than temperature-dependent effects. Ergosterol, for example, develops action partly by incorporation into the membrane, partly by modifying the membrane lipid composition (Ferguson *et al.*, 1971; Nozawa *et al.*, 1975; Nozawa, 1980).

Ergosterol modifies not only the membrane lipids, but indirectly, also other membrane components, among them the superficial saccharide chains, by altering their steric orientation and number. The membrane fluidity-dependent membrane saccharide changes can be followed up by lectin binding studies. In the present experiments we used seven lectins of different ligand specificity to obtain information about temperature-induced and ergosterol-induced changes in membrane fluidity.

MATERIALS AND METHODS

Tetrahymena pyriformis GL cells, cultured in 1% Bacto Tryptone medium (Difco, Michigan, U.S.A.) for 48 hr, were used. Part of the culture was set up in a plain medium to serve as a control, part in the presence of 8 µg/ml ergosterol (Sigma, Missouri, U.S.A.). The experimental and control cultures were divided into two equal groups for culturing at 15°C and 28°C. After culturing for 24 hr at these temperatures, part of the culture originally maintained at 15°C was exposed to 28°C for 1 hr, and vice versa, that originally maintained at 28°C was exposed to 15°C for the same period of time (heat-shocked and cold-shocked cultures). Thus eight schemes of treatment were applied in eight groups. After treatment, the cells were fixed in 4% formaline, washed in phosphate buffer (PBS; 0·05 M phosphate buffer, pH 7·2 in 0·9% NaCl), and incubated for 1 hr in the presence of the following lectins: Concanavalin A, *Datura stramonium*, *Lens culinaris*, *Pisum sativum*, *Phaseolus vulgaris*, *Lycopersicon esculentum*, *Helix pomatia*. All lectins were added to the incubation medium at 0·4 mg/ml concentration, and all

were labeled with fluoresceine–isothiocyanate (FITC.BDH Chemical Ltd.). After incubation, the cells were washed in PBS, spread on slides, and examined for lectin binding by cytofluorimetry, using a Zeiss Fluoval cytofluorimeter, which was connected with a HP-41C calculator for evaluation and registration of the results. Twenty cells were assayed for fluorescence in each group, and each experiment was performed in three replicates.

RESULTS AND DISCUSSION

We examined the influence of environmental (culturing) temperature and of the exogenously added ergosterol on membrane fluidity in a unicellular model system. The membrane rigidity-enhancing action of high ambient temperature, and the membrane-fluidizing action of low ambient temperature, developed by modification of the phospholipid composition and by enhancing unsaturated fatty acid accumulation, respectively, also applied to this system. Ergosterol influenced the membrane by a dual effect, i.e. indirectly by modifying its phospholipid composition, and directly by replacing its tetrahymanol component. Both effects increased the rigidity of the membrane structure.

The influence of ambient (culturing) temperature on the membrane structure was assessed by comparison of the lectin-binding capacity of ergosterol-treated and control cells between the two culturing temperatures (15 and 28°C). The impact of short-term heat shock and cold shock was assessed by comparison of the lectin binding relations before and after the shock treatment. Information on the effect of ergosterol was obtained by comparisons between the ergosterol-treated groups and the adequate control groups under all schemes of treatment.

(1) The cells grown in plain medium bound more Concanavalin A (250%), *Pisum* (130%), *Phaseolus* (125%) and *Helix* (115%) lectin, but less *Lycopersicon* lectin (84%) at 28°C than at 15°C (Fig. 1a).

(2) In the presence of ergosterol, Concanavalin A, *Helix*, *Phaseolus* and *Datura* lectin binding was greater (186%, 132%, 115% and 110%, respectively) at 28°C, whereas the binding of the other lectins did not appreciably differ between the two culturing temperatures (Fig. 1b).

(3) At 28°C, ergosterol increased only the binding of the *Pisum* (115%) and *Phaseolus* (110%) lectins over the control. The binding relations of the other lectins did not appreciably differ from the control (Fig. 2a).

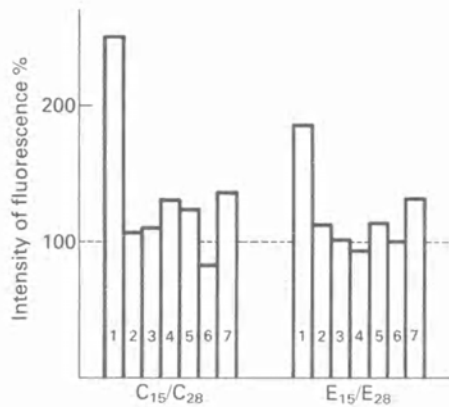(4) At 15°C, ergosterol had a still smaller

Fig. 1. Lectin binding of control and ergosterol-treated *Tetrahymena* cells, cultured on two different temperatures (15°C, 28°C). Legend to figures: C = control culture, E = ergosterol-treated culture. The indices show the culturing temperature and the trends of temperature change. 100% is the value assessed for the group used as denominator. Symbols used: 1 = Concanavalin A, 2 = *Datura* lectin, 3 = *Lens* lectin, 4 = *Pisum* lectin, 5 = *Phaseolus* lectin, 6 = *Lycopersicon* lectin, 7 = *Helix* lectin.
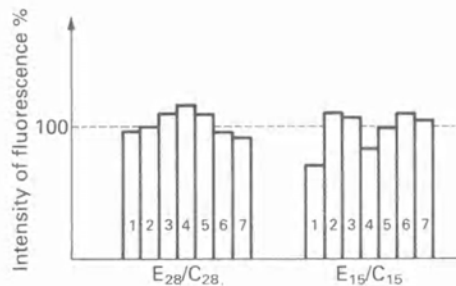
Fig. 2. Lectin binding of ergosterol-treated and control cultured *Tetrahymena* cells, cultured on two different temperatures (15°C, 28°C). Values refer to the actual control. Explanation of figures: see Fig. 1.
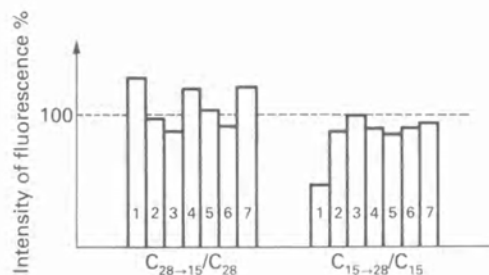
Fig. 3. Lectin binding of control *Tetrahymena* cells. Effect of cooling and heating, values refer to the starting temperature. Explanation of figures: see Fig. 1.
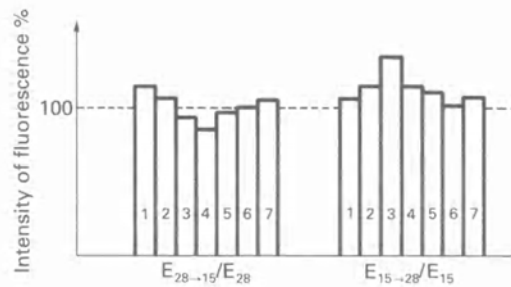
Fig. 4. Lectin binding of ergosterol-treated *Tetrahymena* cells. Effect of cooling and heating, values refer to the starting temperature. Explanation of figures: see Fig. 1.
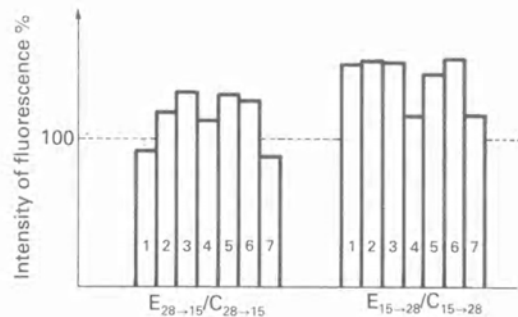
Fig. 5. Lectin binding of *Tetrahymena* cells. Effect of cooling and heating on ergosterol-treated cells, values refer to the tempered control cells. Explanation of figures: see Fig. 1.

influence. The cells so treated bound more *Datura* (110%) and *Lycopersicon* lectin (110%), but less Concanavalin A (70%) and *Pisum* lectin (84%) relative to the control (Fig. 2b).

Exposure of the cells to heat shock and cold shock has disclosed some important aspects of short-term changes in membrane fluidity and composition.

(5) Abrupt reduction of the culturing temperature from 28°C to 15°C caused a relative increase in Concanavalin A (128%), *Pisum* (120%) and *Helix* lectin (121%) binding, and a relative decrease in *Lens* lectin binding (88%) (Fig. 3a).

(6) Abrupt elevation of the culturing temperature from 15°C to 28°C depressed the binding of all lectins except *Lens* lectin (99%) to an appreciable degree, such as that of Concanavalin A (47%), *Datura* (88%) and *Phaseolus* lectin (86%) (Fig. 3b).

(7) Response to cold shock was partially modified by ergosterol, inasmuch as only two lectins, Concanavalin A (114%) and *Pisum* (85%), showed a measurable change in their binding relations; the binding values of these lectins represented the limits

of change under the influence of cold shock plus ergosterol (Fig. 4a).

(8) Heat shock plus ergosterol accounted for a relative increase in *Datura* (114%), *Lens* (135%) and *Pisum* lectin (114%) binding (Fig. 4b).

The influence of combined heat or cold shock plus ergosterol treatment on membrane fluidity, and, consequently, on membrane saccharide composition, was assessed by comparison of lectin binding response to abrupt temperature changes in the presence and absence of ergosterol.

(9) In the cold-shocked cultures, *Datura* (118%), *Lens* (131%) and *Phaseolus* (111%) lectin binding was increased, whereas that of *Helix* lectin was decreased (88%) in the presence of ergosterol (Fig. 5a).

(10) In the heat-shocked cultures the binding of all lectins increased over the control in the presence of ergosterol (Concanavalin A 151%, *Datura* 154%, *Lens* 153%, *Pisum*, 116%, *Phaseolus* 144%, *Lycopersicon* 156%, *Helix* 117%) (Fig. 5b).

It follows from the foregoing observations that long-term or short-term temperature changes, and ergosterol treatment as well, can measurably modify the membrane saccharide organization. Lectin binding obviously depends not only on the membrane glycolipids, but also on the neighbouring structures. Investigations into the agglutination of glycolipid–phospholipid vesicles containing the synthetic glycolipid octadiol–maltobionaminide (OMBA) or phosphatidyl ethanolamine, phosphatidylcholine, phosphatidylinositol, etc. by Concanavalin A have indicated a considerable influence of the phospholipids surrounding the membrane glycolipid on lectin binding to the latter. The polar part of these molecules controls the extent of wedging of the carbohydrate chain into the phospholipid layer. If a critical chain length is achieved, no lectin binding can take place, despite the presence of the ligand in the membrane (Sundler, 1982). In view of this, one cause of the changed binding relations may as well be the altered array or quantitative relations of the phospholipids and fatty acids surrounding the glycolipid, as a quantitative increase or decrease of the directly interacting glycolipid itself, or its steric alterations. In the following we shall mention all these by name of changes in organization of membrane.

In the case of the *Tetrahymena*, elevation of the culturing temperature modifies primarily the membrane composition by increasing the amount of phosphatidyl ethanolamine, myristic and palmitic acids, and decreasing that of the 2-amino-phospholipids and γ-linolenic acid (Nozawa, 1980; Umeki *et al.*, 1983). Reduction of the culturing temperature increases the amount of linolenic acid, but decreases that of palmitic acid (Nozawa, 1980; Ramesha and Thompson, 1982). These changes are sequelae to active membrane transformation, for, as it is known, high environmental temperatures increase first the fluidity, and only later the rigidity of the cell membrane, while cold environmental temperatures cause ultimately a fluidization thereof by enhancing the accumulation of unsaturated fatty acids. Thus the membrane modifications associated with short-term and long-term changes in the environmental (culturing) temperature can throw light on the underlying mechanisms.

Increase in the Concanavalin-A binding of the cultures durably maintained at 15°C relative to those maintained at 28°C, indicated that membrane fluidization was due to accumulation, or capable organization for binding, of glucose, mannose or their derivatives. The binding capacity for *Pisum* lectin, which binds to the D-isomers of the latter sugars, also showed a relative increase at the lower temperature, and membrane fluidization associated increase in N-acetyl-D-galactosamine was indicated by a relative increase in *Phaseolus* and *Helix* lectin binding. However, the lower temperature accounted for a relative decrease in the N-acetyl-glucosamine content, to judge from the reduced binding of *Lycopersicon* lectin at 15°C.

Abrupt short-term temperature changes (heat-shocked and cold-shocked control cells) had similar effects to the long-term exposures, although they allowed only 1 hr for membrane transformation. Reduction of the culturing temperature from 28 to 15°C accounted for a (concentration) increased organization of membrane saccharides (glucose, mannose and their D-isomeres) and of N-acetyl-D-galactosamine, to judge from the greater binding of Concanavalin-A, *Pisum* and *Helix* lectins, and a decreased organization of N-acetyl-glucosamine, as indicated by a relative decrease in *Lens* lectin binding. Thus short-term cold exposure was also sufficient for membrane fluidization; the fact that complete membrane transformation would have required more than the 1 hr available is indicated by the minor differences in *Helix* (N-acetyl-D-galactosamine) and *Phaseolus* (N-acetyl-galactosamine) lectin binding.

Abrupt elevation of the culturing temperature from 15 to 28°C had the opposite effect of cold shock, in that it increased the rigidity rather than the fluidity of the cell membrane. The binding relations

of Concanavalin A indicated a quantitative decrease in membrane saccharides in their number or organization, (glucose and mannose) and those of *Phaseolus lectin* indicated concentration or orientation changes in the N-acetyl-galactosamine component. It is interesting to note that while *Lens* lectin binding indicated N-acetyl-glucosamine organization increase, *Datura* lectin, which binds to N-acetyl-D-glucosamine, indicated an organization decrease, thereof.

It follows that temperature changes can in themselves elicit fluidity-dependent membrane modifications, which can be reliably characterized by alterations in the lectin binding capacity of the *Tetrahymena*.

Long-term and short-term cold exposure equally accounted for glucose, mannose (plus derivatives), N-acetyl-galactosamine and N-acetyl-D-glucosamine increase (binding sites), and for a considerable decrease of N-acetyl-D-glucosamine and its oligomeres. Long-term and short-term heat exposure had the opposite effect, in that both accounted for a decrease in the glucose, mannose (plus derivatives) and N-acetyl-glucosamine and N-acetyl-D-glucosamine components differed between the groups: while cold shock decreased, and heat shock, in contrast, increased, the amount of N-acetyl-D-glucosamine, to judge from changes in *Lens* lectin binding, alteration of the N-acetyl-glucosamine component seemed to be the result of adaptation to lasting cold exposure.

Ergosterol, too, alters membrane fluidity or, more precisely, causes membrane rigidity, by modifying the membrane lipid configuration. This is achieved by a dual effect. Firstly, ergosterol replaces the membrane lipid tetrahymanol (Ferguson *et al.*, 1971), secondly, by analogy of the heat effect, it increases the myristic acid, palmitic acid and phosphatidylethanolamine concentration, but simultaneously decreases the oleic acid, γ-linolenic acid and phosphatidylcholine concentrations (Nozawa, 1980). We studied the effect of these changes on the membrane of the *Tetrahymena* at two culturing temperatures (15 and 28°C) and in conditions of cold shock and heat shock.

The lectin binding of the ergosterol-treated cells did not appreciably differ from the control at either culturing temperature: an increase was noted in membrane glucose and galactose organization (and derivatives) as well as in the N-acetyl-D-galactosamine and N-acetyl-galactosamine organization as judged from the greater binding of Concanavalin A, *Phaseolus* and *Helix* lectins.

However, increase in *Datura* lectin binding indicated an accumulation of N-acetyl-glucosamine oligomeres in the presence of ergosterol.

Cold-shocked ergosterol-treated cultures showed an increase in membrane glucose, mannose and their derivatives over the control, as judged from the greater binding of Concanavalin A, and a decrease in the D-isomeres of these sugars, as judged from a relative decrease in *Pisum* lectin binding. Heat shock, in contrast, elicited a concentration increase of the latter D-isomeres and of lectin-binding N-acetyl-D-glucosamine molecules, as indicated by changes in the binding relations of *Pisum*, *Datura* and *Lens* lectin. Thus the effects of cold shock and heat shock were similar in the presence and absence of ergosterol, except that cold shock increased the amount of the D-isomeres in itself, whereas heat shock only in the presence of ergosterol, and short-term heat exposure had no influence on the N-acetyl-galactosamine organization of the membrane, if ergosterol was present.

Comparison of lectin binding capacity between cultures treated and not treated with ergosterol in the same temperature conditions has shown that while ergosterol caused a relative increase of N-acetyl-glucosamine (*Datura* and *Lycopersicon* binding), and a relative decrease of glucose, mannose, their derivatives (Concanavalin A-binding) and D-isomeres (*Pisum*-binding) at 15°C, it increased the organization of D-isomeres and N-acetyl galactosamine at 28°C (*Pisum* and *Phaseolus* lectin binding).

During cold shock, ergosterol increased (the concentration of) N-acetyl-glucosamine over the control, and modified the molecular-level composition of N-acetyl-galactosamine (D-isomeres, beta conformation), as indicated by increased *Phaseolus* and decreased *Helix* lectin binding. During heat shock, ergosterol increased the amount of all membrane saccharides, although to different degrees. The greatest relative increase was observed in the oligomeric and D-isomeric N-acetyl-glucosamine component (as judged from the considerably greater binding of *Datura*, *Lycopersicon* and *Lens* lectins), and the least increase in D-isomeric glucose and mannose (as judged from *Pisum* lectin binding).

It follows that ergosterol, an agent causing membrane perturbation and rigidity, has a characteristic influence on membrane composition, which may be modified to a certain extent by other external factors acting on membrane fluidity. Thus, depending on the membrane fluidity or rigidity enhancing action of the culturing temperature,

ergosterol increased the organization of N-acetyl-glucosamine and its oligomeres in the fluid membrane (at 15°C), and that of the D-isomeres of glucose and mannose as well as of N-acetylglucosamine in the rigid membrane (at 28°C). In this light, the main effect of ergosterol seems to be the induction of a concentration increase of lectin-binding N-acetyl-glucosamine molecules in the cell membrane.

Summarizing, investigations into changes in the lectin binding capacity of *Tetrahymena* membrane saccharides under the influence of two external factors, such as environmental (culturing) temperature and ergosterol, have shown that the membrane fluidizing effect of lasting or short-term cold exposure increased the organization of glucose, mannose (and derivatives) and N-acetyl-galactosamine, but decreased that of N-acetyl-glucosamine, whereas long-term and short-term heat exposures had the opposite effects on the membrane saccharide composition. The effect of ergosterol, although it depended greatly on the interaction of other factors acting on membrane fluidity, was similar to that of warm culturing conditions or heat shock, yet ergosterol-induced membrane rigidity was associated primarily with a N-acetyl-glucosamine organization increase.

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