LIPID DYNAMICS DURING THE SPONTANEOUS REACTIVATION OF A MEMBRANOUS SIALYLTRANSFERASE COMPLEX IN *ESCHERICHIA COLI* K-235

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Membranes from *Escherichia coli* K-235 contain a sialyltransferase complex (STC) that catalyzes the synthesis of sialyl polymers (SP). Sialylmonophosphorylundecaprenol (NeuNAc-P-C_{55}) is an intermediate in the formation of SP and the solid-fluid state of the membrane controls insertion of exogenous P-C_{55} into a functional enzyme complex (1). In membranes containing an increased unsaturated fatty acyl content, obtained by growing cells at 15°C, SP synthesis is uncoupled from synthesis of NeuNAc-P-C_{55}. Reconstruction experiments showed the derangement in SP synthesis to result from the inability of 15°C membranes to assemble a functional endogenous acceptor rather than from a defect in synthesis of the ST (2). In vitro reactivation of SP synthesis occurs in these "inactive" membranes after incubation at 33°C for 2–4 h. The present study was undertaken to examine the biochemical and physical interactions which may occur in these 15°C membranes during the 2–4 h lag preceding reactivation.

MATERIALS AND METHODS

Preparation of the STC

Membranous STC associated with inner membrane (IM) and outer membrane (OM) was prepared from cells grown at 15°C and 37°C as described previously (1, 2). A membrane fraction highly enriched in OM (OME) was obtained from 15°C cells by sedimentation of the cell-free homogenate at 30,000 *g* for 30 min. Centrifugation of the 30,000 *g* supernatant at 200,000 *g* for 1 h at 4°C resulted in a membrane fraction enriched in IM (IME). Sucrose gradient centrifugation was used to localize ST activity in membrane fractions. Resolution of the various membrane systems employed in the in vitro reactivation system was confirmed by an analysis of their characteristic protein profiles on SDS-PAGE (2).

In Vitro Synthesis of ^{14}C-labeled Sialyl Polymers

The in vitro synthesis of radiolabeled SP was carried out as described (2).

NMR Measurements

^{31}P-NMR spectra were recorded on a 200-MHz spectrometer (Nicolet Technology Corporation, Mountain View, CA) operating at a frequency of 80.988 MHz (12-mm probe) with broad-band proton decoupling. Experiments carried out were pulse-free precession, bilevel decoupling (3), or T_1 inversion and recovery with alternating phases (4). Spectra of membranes were recorded in 50 mM Tris, pH 8.0 containing 25 mM Mg^{2+} and 20% D_2O or 99% D_2O containing 0.9% NaCl. Phospholipids were dissolved in NMR grade $DCCl_3$.

RESULTS

Biochemical Correlates of Reactivation

In vitro reactivation of SP synthesis in 15°C membranes occurred in OME but not IME fractions, presumably because the latter lacked endogenous acceptor. This conclusion was verified by the observation that both membrane fractions contained the ST enzyme since they both synthesized SP when provided an exogenous acceptor. In contrast, endogenous SP synthesis occurred in both the OME and IME fractions isolated from cells grown at 37°C. Under these conditions, both fractions contained ST and endogenous acceptor because of the incomplete resolution of these membrane systems when isolated from cells grown at 37°C.

Unexpectedly, reactivation of SP synthesis in 15°C membranes also occurred in a very low density (ρ = 1.11–1.12 g/cm^3) fraction. This fraction contained IM, OM and cytoplasmic-specific proteins plus significant amounts of phosphatidylethanolamine (PE). This fraction may be enriched in Bayer junctions, regions of attachment between IM and OM. Reactivation also required a soluble factor (SF) of <500 daltons, present in both the periplasm and cytoplasm. Ca^{2+} ion and K^+ could partially replace the requirement for SF.

Trypsin was used as a probe to assess the topological orientation of STC. In 37°C membranes, SP synthesis utilizing either an endogenous or exogenous acceptor was trypsin labile. In contrast, only the ST responding to exogenous acceptor in 15°C membranes was sensitive to trypsin. Thus, reactivation was unaffected by trypsin or pronase and could not be rendered sensitive by detergents or sonication. These data indicate a possible difference in the transbilayer orientation of STC between 37°C and 15°C membranes, resulting perhaps from the increased *cis* unsaturation of the membrane lipids in cells grown at 15°C.

^{31}P-NMR Investigation of In Vitro Reactivation

The ^{31}P-NMR spectra for unfractionated membranes isolated from cells grown at 15°C and 37°C differed in spectral linewidth, chemical shift, and spin lattice relaxation times (T_1). Identical ^{31}P-NMR spectra of PL extracted with $CHCl_3$:MeOH from 15° and 37°C membranes were obtained when run in $DCCl_3$. This established that the PL headgroups were identical and indicated that the spectral differences in the isolated membranes must arise from differences in motional properties of the PL *in situ*.

T_1 values for the envelope of lines indicated a greater motional restriction of PL headgroups in 37°C membranes (230–300 ms) relative to 15°C membranes (580–700 ms). There was a temporal dependence of the T_1 in 15°C membranes, decreasing from an initial 580 ms to 300 ms within 3 h at 33°C. There was no linewidth dependence upon frequency at 40.45 MHz, and only a 21 Hz linewidth variation as a function of temperature between 15°–42°C. Similarly, no temporal variation of linewidth was observed. We conclude from these data that the observed ^{31}P-NMR spectral differences do not arise from chemical shift anisotropy effects and that there is no apparent vesicle fusion during or proceding in vitro reactivation.

^{31}P-NMR spectra on 15°C IME and OME fractions provided information on the relative contribution of each membrane to the spectral envelope. The broader resonances of the envelope were contributed by the IM, and the narrower resonances, dominated by PE, by the OM.

A time-dependent change in the chemical shift of the phosphorous atoms in 15°C but not 37°C membranes occurred concomitantly with reactivation of SP synthesis. Diminution of a component at −0.9 ppm and a new, broad resonance appearing at ~ 4 ppm was observed. The time-dependent motional changes within the lipid matrix have been confirmed using fluorescence emission shifts of the membrane probes *cis*- and *trans*-paranaric acid.

DISCUSSION

These studies have described an in vitro reactivation of SP synthesis in membranes from 15°C grown cells. They do not provide, however, a complete description of the events occurring during the 2–4 h lag before reactivation. Reactivation is prevented by energy uncouplers (azide, 2,4-dinitrophenol); no proteolytic processing of membrane proteins has been observed. Reactivation is inhibited by cerulenin, an antibiotic that inhibits β-ketoacyl synthetase, thereby affecting PL and LPS synthesis. While the significance of this result is under study, it adds further credence to the possibility that the 15°C derangement in SP synthesis reflects a modulation of ST activity induced by the altered thermotropic properties of the lipids. The ^{31}P-NMR data, showing motional changes within the lipid matrix that correlate temporally with in vitro reactivation, provide additional evidence in support of this hypothesis.

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