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Effect of ganglioside GM3 on the activity and conformation of reconstituted Ca^{2+}-ATPase

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Abstract Trace amounts of gangliosides were found in rabbit skeletal muscle sarcoplasmic reticulum and their main part was shown, by high performance thin layer chromatography, to be GM3. Addition of GM3 to the soybean phospholipids used for reconstitution of proteoliposomes markedly increased ATP hydrolysis as well as Ca^{2+} uptake activity of sarcoplasmic reticulum Ca^{2+}-ATPase incorporated into the proteoliposomes. Conformation changes of Ca^{2+}-ATPase induced by GM3 were also observed by intrinsic fluorescence and circular dichroism measurements.

Key words: Ganglioside GM3; Proteoliposomes; Ca^{2+}-ATPase; Sarcoplasmic reticulum

1. Introduction

Apart from phospholipids that constitute the majority of building blocks of biological membranes, gangliosides are characteristic components of the plasma membranes of most vertebrate cells. Even though the effects of gangliosides on plasma membrane proteins, e.g. Na^+,K^+-ATPase [1], Ca^{2+}-ATPase [2], Ca^{2+} channels [3], phospholipase C [4], protein kinase C [5] and epidermal growth factor receptor [6,7], have been well documented in recent years, the distribution and role of gangliosides in the intracellular membranes are still poorly understood.

The sarcoplasmic reticulum (SR) of skeletal muscle cell is a highly specialized subcellular membrane system for the storage and rapid release of calcium ions. The membrane-bound Ca^{2+}-ATPase is among the most important of the calcium transport systems across intracellular membranes. It catalyzes active uptake of Ca^{2+} into SR vesicles coupling to ATP hydrolysis. Michelangeli et al. [8] and Tu and Yang [9] have reported the activity of the SR Ca^{2+}-ATPase to be sensitive to the structure and composition of the phospholipid molecules surrounding it in phospholipid bilayers. However, the distribution of gangliosides and their interactions with membrane transport proteins in the SR membranes remain uncertain. Owing to the vital importance of Ca^{2+} as a second messenger, and the pivotal position of SR Ca^{2+}-ATPase in maintaining intracellular calcium homeostasis, here SR Ca^{2+}-ATPase is chosen as the target membrane protein to study its interaction with gangliosides.

In the present paper, the following three questions are answered. (1) Does SR have gangliosides as the topological basis of its interaction with SR proteins? If so, what is the main ganglioside species? (2) Could the main ganglioside species affect SR Ca^{2+}-ATPase reconstituted into proteoliposomes? (3) Could the main ganglioside species induce a conformational change in the enzyme?

2. Materials and methods

2.1. Materials

HPTLC plates were from E. Merck, Darmstadt; ganglioside standards (GM3, GM2, GM1, GD1a, GD1b and GT1b) were from Fidia; octyl glucoside, reactive red-120 agarose, soybean phospholipids and ADP were from Sigma. GM3 from canine erythrocytes was prepared by the method described by Tsui et al. [10], its purity was 90%.

2.2. Isolation, purification and HPTLC analysis of SR gangliosides

This was performed according to the method of Ladisch and Gillard [11], as modified by Zhang et al. [12].

2.3. Preparation of SR Ca^{2+}-ATPase

Rabbit SR was prepared according to Meissner et al. [13], and SR Ca^{2+}-ATPase according to Coll and Murphy [14], with a slight modification. The enzyme was homogeneous according to SDS-PAGE.

2.4. Preparation of proteoliposomes containing SR Ca^{2+}-ATPase

Preparation of proteoliposomes was based on the methods described by Gould et al. [15] and Tu and Yang [9]. The lipid/protein ratio was 100:1 (μmol/μmol). For the preparation of GM3-containing proteoliposomes, the GM3/soybean phospholipid ratio (μmol/μmol) was 0.25/40, 0.5/40, 1/40, 1.5/40 or 2/40. Electron microscopic examination of the negatively stained proteoliposomes revealed well-sealed intact vesicles, indicating successful preparation.

2.5. ATP hydrolysis and Ca^{2+} uptake by Ca^{2+}-ATPase

The ATP hydrolysis activity was monitored at 30°C by continuous spectrophotometry following the oxidation of NADH as described by Carafoli et al. [15]. Ca^{2+} uptake activity was measured at 30°C by following the decrease in the absorbance of arsenazo III used as Ca^{2+} indicator (675–685 nm), in a Hitachi model 557 spectrophotometer as described by Gould et al. [16].

2.6. Fluorescence measurement

The intrinsic fluorescence of Ca^{2+}-ATPase was measured using a Hitachi F4010 spectrophotometer equipped with a temperature control. Measurement was made in the ratio mode with excitation and emission band widths of 5 nm. Enzyme concentration was 200 μg/ml. Samples were excited at 285 nm, and the emission light monitored at 300–400 nm [17].

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Abbreviations: CD, circular dichroism; Cer, ceramide; Gal, galactose; GM3, NeuNAcα 2→3Galβ 1→4Glcβ 1→β 1′Cer; HPTLC, high performance thin layer chromatography; NADH, reduced form of nicotinamide adenine dinucleotide; NeuNAc, *N*-acetylneuraminic acid; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SR, sarcoplasmic reticulum; GT1b, NeuNAcα 2→3Galβ 1→3GalNAcβ 1→4 (NeuNAcα 2→8NeuNAcα 2→3)Galβ 1→4Glcβ 1→1′Cer; GD1a, NeuNAcα 2→3 Galβ 1→3 GalNAcβ 1→4 (NeuNAcα 2→3) Galβ 1→4Glcβ 1→1′Cer; GD1b, Galβ 1→3 GalNAcβ 1→4 (NeuNAcα 2→8NeuNAcα 2→3) Galβ 1→4Glcβ 1→1′Cer; Glc, glucose; GM1 (=GM1a), Galβ 1→3 GalNAcβ 1→4 (NeuNAcα 2→3) Galβ 1→4Glcβ 1→1′Cer

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2.7. Circular dichroism

Hitachi JASCO-J-500 CD spectrometer was used to monitor the CD spectra of Ca^{2+}-ATPase. The spectra were matched with four standard secondary structures by computer to estimate their percentage contents.

2.8. Other methods

Lipid-bound sialic acid was determined according to Aminoff [18]. Protein was determined according to Lowry et al. [19].

3. Results and discussion

3.1. Rabbit SR does contain gangliosides and the main species is GM3

Purified rabbit SR (purity 96%) was subjected to total ganglioside extraction and purification. The total ganglioside content, in terms of lipid-bound sialic acid, was 0.36 ± 0.02 nmol/mg protein, which was much less than that of the plasma membrane (4.91 ± 0.25 nmol/mg protein). The HPTLC pattern of SR gangliosides revealed that the main ganglioside species was GM3 (Fig. 1). Densitometric scanning of the chromatogram after developing the plate by a resorcinol-HCl spray reagent gave the percentage contents of GM3 (86%) and GM1 (8%).

Gangliosides have generally been known to be localized primarily in the plasma membrane. Subcellular contents and distribution of gangliosides have been reported in hepatocytes by Matyas and Morre [20], but their distribution in the skeletal muscle SR membranes has not been documented so far. The above results revealed that rabbit skeletal muscle sarcoplasmic reticulum does contain trace amounts of gangliosides and the main species is GM3. Thus it is reasonable to choose GM3 as the exogenous ganglioside used in the present study.

3.2. GM3 markedly increases the activity of Ca^{2+}-ATPase in reconstituted proteoliposomes

Ca^{2+}-ATPase of rabbit SR was purified and reconstituted into soybean phospholipid proteoliposomes with and without GM3. Then, ATP hydrolysis activities of Ca^{2+}-ATPase in these two kinds of proteoliposomes were determined and compared. From Table 1, it can be seen that GM3 stimulated ATP hydrolysis in a concentration-dependent manner. The highest enzyme activity was observed in proteoliposomes with a GM3/soybean phospholipid ratio of 1/40 (μmol/μmol).

Furthermore, Ca^{2+} uptake activities of Ca^{2+}-ATPase in proteoliposomes with or without GM3 were determined. As shown in Table 1, GM3 increased drastically the Ca^{2+} transport rate of Ca^{2+}-ATPase. At a GM3/soybean phospholipid ratio higher than 1/40, neither ATP hydrolysis nor Ca^{2+} uptake activity of Ca^{2+}-ATPase was further increased significantly.

Although glycosphingolipids are small molecules compared to proteins and account mostly for only a few percent of the total cell lipids, they comprise 30–60% of the lipid molecules on the outer half of the plasma membrane, forming a sort of sugar lawn on the cell surface, and play important roles in cell differentiation, morphogenesis, and oncogenic transformation [21]. Formation of clusters or aggregation of trace amounts of glycolipids on the cell membrane surface are considered to be important in regulating the structure and function of many membrane proteins. It is possible that some glycosphingolipids may also be highly concentrated in intracellular membranes and modulate functions of subcellular organelles, as well as in plasma membranes. The present study clearly showed that addition of GM3 to the reconstitution system could markedly increase the ATP hydrolysis as well as Ca^{2+} uptake activity of SR Ca^{2+}-ATPase. The results suggest that GM3 present in SR membrane is not just acting as a 'passer-by', but is involved in the modulation of SR Ca^{2+}-ATPase activity.

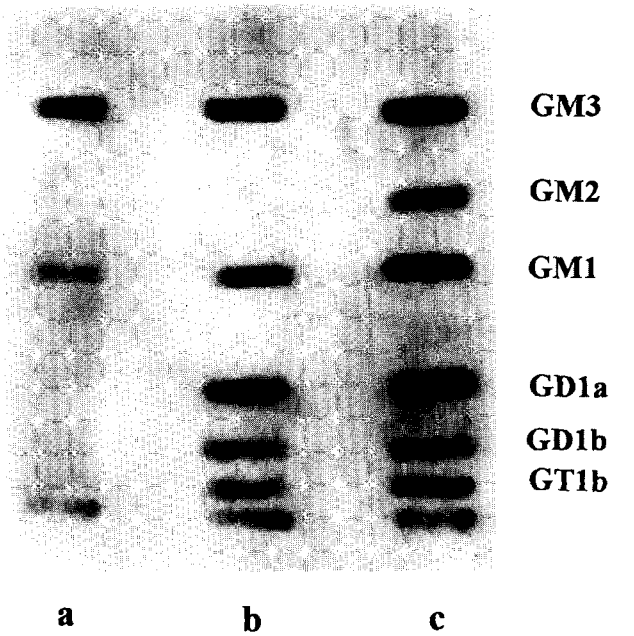

Fig. 1. HPTLC pattern of SR gangliosides from rabbit skeletal muscle. The plate was developed in a mixture containing chloroform/methanol/0.25% $CaCl_2$ (60:40:9, v/v/v), and visualized by a resorcinol-HCl spray, then the plate was covered with a glass plate and heated in an oven at 110°C for 15 min. a: SR; b: plasma membrane; c: ganglioside standards.

3.3. GM3 induces a conformational change of SR Ca^{2+}-ATPase reconstituted into proteoliposomes

In order to compare the conformation of Ca^{2+}-ATPase in reconstituted proteoliposomes with and without GM3, fluorescence and CD spectroscopy have been used.

The fluorescence emission spectra of Ca^{2+}-ATPase in pro-

Table 1
Effect of GM3 on the activities of Ca^{2+}-ATPase proteoliposomes

Ratio of GM3 and soybean phospholipid (μmol/μmol)	ATP hydrolysis (μmol/min·mg)	Ca^{2+}-uptake (nmol/mg)
0	2.20 ± 0.01	30 ± 1
1/80	3.12 ± 0.03	45 ± 2
1/40	7.41 ± 0.02	86 ± 2
1/27	7.34 ± 0.05	87 ± 2

Results are expresssed as means ± S.E. for four experiments.

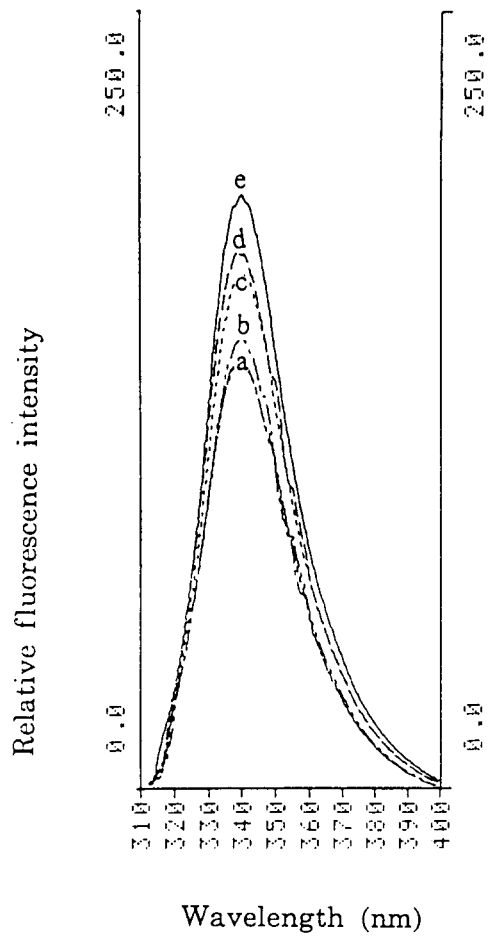

Fig. 2. Effect of GM3/soybean phospholipid ratio (μmol/μmol) on the intrinsic fluorescence spectra of reconstituted Ca^{2+}-ATPase. a: no GM3; b: 1/200; c: 1/150; d: 1/100; e: 1/40. Total lipid/protein ratio was 100:1.

teoliposomes with and without GM3 are shown in Fig. 2. Fluorescence was measured with excitation at 285 nm, and difference in emission intensity was recorded at 300–400 nm. As can be seen from Fig. 2, the intrinsic fluorescence intensity of Ca^{2+}-ATPase increased when GM3 was added to the system and was GM3 concentration-dependent, but the emission maximum at 340 nm did not shift significantly. The results implied that the microenvironment of Trp residues of Ca^{2+}-ATPase has been altered due to conformational changes caused by GM3. A molecule of SR Ca^{2+}-ATPase contains 13 tryptophan residues which produce the intrinsic fluorescence. Various experiments have confirmed that the fluorescence changes of Trp residues are associated with conformational changes of SR Ca^{2+}-ATPase during Ca^{2+} transport [22] and transmembrane Ca^{2+} gradient formation [23].

CD spectra of Ca^{2+}-ATPase in proteoliposomes in the wavelength range of 200–250 nm with and without GM3 were also measured and the results showed that GM3 increases the content of α-helix (+16%), and decreases that of β-turn (−19%) and random coils (−30%).

It is well known that GM3 is an amphipathic molecule asymmetrically located in the membrane with its hydrophobic portion, the ceramide, inserted into the lipid core and with hydrophilic oligosaccharide chains protruding into the surrounding medium [24]. GM3 on the whole increased the compactness of the enzyme molecules (unpublished results), and the more compact molecules might exhibit a higher activity. It is possible that GM3-mediated changes in conformation and activity of SR Ca^{2+}-ATPase are modulated either by direct interaction between glycolipids and enzyme molecules or by changes in lipid physical state, or by both factors. Related further studies are in progress.

To sum up, rabbit SR does contain trace amounts of gangliosides, and the main species is GM3. GM3 can significantly activate Ca^{2+}-ATPase by inducing an optimal conformation for higher activity to maintain the intracellular Ca^{2+} homeostasis.

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