PRESENCE OF GLYCOSPHINGOLIPIDS IN THE SARCOPLASMIC RETICULUM FRACTION OF RABBIT SKELETAL MUSCLE

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1. Introduction

With the realisation that gangliosides are probably present in most if not all mammalian tissues (cf. [1]), increasing attention has been paid to their subcellular location in extraneural tissues. The available evidence indicates that while they are notably enriched in plasma membrane fractions [2–9], they are also present in other intracellular membranes, such as smooth and rough endoplasmic reticulum [7,10], Golgi apparatus [7] and the membranes of primary [11] and secondary [12] lysosomes. They may be absent from mitochondria [2]. Several recent reports have described the presence of gangliosides and neutral glycosphingolipids (GSLs) in skeletal muscle [13–16]. Several sugar residues have been detected subsequent to hydrolysis of lipid extracts of the sarcoplasmic reticulum (SR) fraction of rabbit skeletal muscle [17], indicating the presence of glycolipids in this membrane system. Sarzala et al. [18] have also provided preliminary evidence of the presence of GSLs in this SR fraction. In order to extend the available information on the possible distribution of gangliosides and neutral GSLs in intracellular membranes we have investigated their presence in the SR fraction of rabbit skeletal muscle, an intracellular membrane fraction of relatively simple composition that can be prepared in high purity [19].

2. Materials and methods

Albino rabbits weighing 3–3.5 kg were killed by cervical dislocation and decapitation. White muscle from the dorsum and legs totalling about 400 g was removed from each rabbit. The procedure of MacLennan [19] was used to prepare the SR fraction (R_1 washed fraction). This preparation is free of mitochondrial ATPase and of $Na^+ + K^+$ ATPase activities. The Ca^{2+}-dependent ATPase (R_4 fraction) of the SR was prepared by the method of MacLennan et al. [20]. Gangliosides were extracted from whole muscle preparations and from SR fractions by the method of Suzuki [21], as previously utilized by our laboratory [22]. After a preliminary mild alkaline methanolysis to remove alkali-labile lipid contaminants, the lipid-bound sialic acid (LBSA) content of the ganglioside fractions was estimated by the procedure of Svennerholm [23] as modified by Miettinen and Takki-Luukkainen [24]. The lower phase of the Folch extract was dried, its weight determined and designated as the weight of the total lipid fraction. The lower phase lipids were also subjected to mild alkaline methanolysis and their neutral GSL and sulfatide components purified by a modification [25] of the procedure of Vance and Sweeley [26]. For facilitation of description, sulfatide is subsequently classed with the neutral GSLs, as operationally it was obtained with these compounds. Analysis of the patterns of gangliosides and neutral GSLs in the various preparations was performed utilizing separations by thin layer chromatography (TLC) [22]. The sialic acid content of individual gangliosides separated by TLC was quantitated by the method of MacMillan and Wherrett [27]. For the purpose of partial structural studies and/or quantitation, the GM_3 ganglioside and monoglycosylceramide species were isolated by preparative TLC and their sugar composition determined by analyses by

Table 1
A. Ganglioside content of rabbit skeletal muscle and the SR fraction

Sample	μg LBSA per mg Protein	μg LBSA per mg Lipid
Muscle [7]	0.040 ± 0.002	0.36 ± 0.05
SR [7]	0.613 ± 0.043	0.84 ± 0.07
Ca^{2+} ATPase [1]	0.320	0.53

B. Percentage distribution of sialic acid in individual gangliosides

Sample	GM_3	GM_1	GD_{1a} and GD_{1b}	GT
Muscle [2]	46.5	14.5	31.0	8.0
Sr [2]	47.5	18.0	27.0	7.5

LBSA, protein and lipid were determined as described in the text. The percentage distribution of sialic acid in the individual gangliosides was measured by the method of MacMillan and Wherrett [27]; the results are expressed as a percentage of the total sialic acid recovered. The composition of the individual gangliosides of rabbit skeletal muscle has previously been reported by Lassaga et al. [14]. Analysis by GLC of the individual GM_3 species of total muscle and of the SR fraction revealed them to have identical sugar compositions consisting of approximately equimolar amounts of glucose, galactose and *N*-acetylneuraminic acid. Their fatty acid compositions were also similar, both showing a predominance of 16:0 and 18:0 species (cf. [14]).

gas–liquid chromatography (GLC) of the trimethylsilyl derivatives [26]. Gangliosides, galactosyl-ceramide, sulfatide and neutral GSL standards were prepared from specimens of human brain and kidney obtained at autopsy. The ganglioside nomenclature used is that of Svennerholm [28]. Protein determinations were performed by the method of Lowry et al. [29].

3. Results

Quantitative data on the LBSA contents of the whole muscle and SR preparations are presented in table 1. In general, the SR preparations were enriched approximately 15-fold over whole muscle in their content of LBSA when the data were expressed as μg sialic acid per mg protein. However, when the data were expressed relative to total lipid, the SR was enriched only approximately 2.3-fold in its ganglioside content as compared with whole muscle. The percentage distribution of sialic acid in the individual gangliosides of whole muscle and SR fractions is also presented in this table; it is evident that GM_3 was the principal ganglioside of both fractions and that the distribution of the individual gangliosides in whole muscle and in the SR fraction was very similar. Further confirmation of this observation is presented in fig. 1A, a photograph of a separation by TLC of the gangliosides of whole muscle and of the SR fraction along with a standard extract of human brain gangliosides. The principal gangliosides of both the whole muscle and SR fraction corresponded in migration to GM_3, GM_1, GD_{1a}, GD_{1b}, and GT, with GM_3 the most prominent. The ganglioside content of one preparation of the Ca^{2+}-dependent ATPase of the SR was also determined (table 1). The enrichment of LBSA in this preparation was similar to that shown by the total SR fraction. Analysis by TLC showed that the pattern of gangliosides in this preparation was similar to that exhibited by the total SR fraction. As shown in fig. 1B, the major neutral GSL in both the muscle and SR preparations corresponded in chromatographic migration to a standard of monogalactosylceramide. Zones corresponding in migration to diglycosylceramide, sulfatide and triglycosylceramide were also evident in both preparations. Analyses by GLC showed that the monoglycosylceramide species of both muscle and SR contained mainly galactose (65%), but an appreciable amount of glucose (35%) was also detected. The enrichment of monoglycosylceramide in the SR fraction was approximately 20-fold that of the whole muscle value when expressed relative to protein and 2.5-fold when expressed relative to total lipid.

4. Discussion

Previous studies have demonstrated the presence of GSLs in rat [13], rabbit [14,16] and human [15] skeletal muscle, with considerable structural analyses on individual GSLs having been reported in the two latter investigations. The pattern of gangliosides observed by us in rabbit muscle (fig. 1A and table 1) agrees with that previously reported for this tissue [14] in demonstrating that GM_3 is the principal ganglioside and also

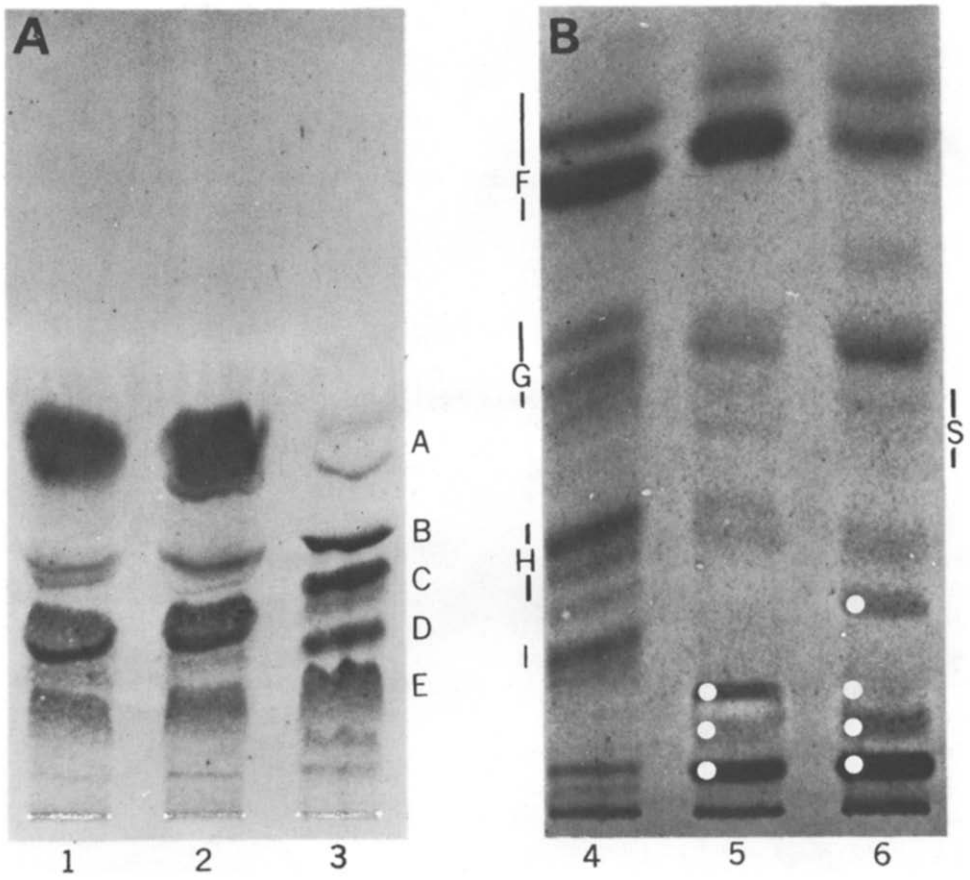

Fig. 1A. Thin layer chromatogram of the gangliosides from rabbit skeletal muscle and a derived SR fraction. Gangliosides of: 1, skeletal muscle; 2, SR fraction; 3, human brain. Aliquots of gangliosides corresponding in amount to approximately 20 μg of LBSA were spotted in each channel. The plate was developed in chloroform: methanol: ammonia: water (60 : 35 : 1 : 7, v/v/v/v) and sprayed with resorcinol reagent. The ganglioside nomenclature is that of Svennerholm [28]. A, GM_3; B, GM_1; C, GD_{1a}; D, GD_{1b}; E, GT.
Fig. 1B. Thin layer chromatogram of the neutral GSLs and sulfatide of rabbit skeletal muscle and a derived SR fraction. Neutral GSLs of: 4, standard mixture of human brain and kidney; 5, SR fraction; 6, skeletal muscle. Aliquots of GSLs corresponding in amount to approximately 1 mg were spotted in each channel. The plate was developed in chloroform: methanol: water (65:25:4, v/v/v) and sprayed with aniline–diphenylamine reagent. The compounds indicated by the white dots are contaminants and are neither neutral GSLs nor sulfatides. The GSLs characteristically resolved as double bands. F, monoglycosylceramide; G. diglycosylceramide; H, triglycosylceramide; I, tetraglycosylceramide; S, sulfatide.

that significant amounts of the more complex gangliosides are present. The SR fraction showed an almost identical pattern of gangliosides to that exhibited by whole muscle (fig. 1A), but was moderately enriched in its ganglioside content (approximately 2.3-fold) as compared with whole muscle (table 1) when the data were expressed relative to total lipid content. The pattern of neutral GSLs of the SR fraction was also very similar to that obtained from whole muscle (fig. 1B), with their enrichment in the SR (as revealed by quantitative determination of the monoglycosylceramide content) being of the same order as that of the gangliosides. The relatively striking enrichments of ganglioside and neutral GSL content in the SR when expressed relative to protein probably reflects the high contribution of contractile proteins in muscle to the total protein content of muscle. It is apparent that expression of data relative to both protein and lipid content is essential

if one is to obtain a realistic picture of the distribution. Albarracin et al. [16] have recently demonstrated the occurrence of neutral GSLs and sulfatides in rabbit skeletal muscle, but did not report analyses by TLC. The finding that the principal members of the neutral GSL class of both SR and whole muscle were compounds corresponding in migration to standards of mono-, di- and triglycosylceramides and classical sulfatide is thus of interest. This pattern is similar to that described by Svennerholm et al. [15] for human skeletal muscle, although monoglycosylceramide was the principal neutral GSL observed by us, whereas it was only a minor component of human muscle. Svennerholm et al. [15] attributed the presence of galactosylceramide and sulfatide in human muscle to contamination by peripheral nerve. We consider it unlikely that the purified SR fraction would be contaminated to the same extent as whole muscle by peripheral nerve. However, even if the galactosylceramide and sulfatide were contaminants derived from nerve, GM_3, monoglucosylceramide and the di- and triglycosylceramide species detected in SR and whole muscle would appear to be bona fide constituents of muscle, since these compounds are not significant components of rabbit peripheral nerve [30]. Moreover, since the SR fraction of rabbit muscle is a relatively pure subcellular fraction (see Materials and methods), it appears reasonable to conclude that at least these latter GSLs are integral components of this membrane system. The purified Ca^{2+}-dependent ATPase, whose content of other major lipid classes has previously been reported to be identical to that of the total SR [20,31], also revealed a ganglioside pattern identical with that of the total SR; it will be of interest to determine whether these GSLs play any role in the activity of this lipid-dependent enzyme.

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