Institute of Experimental Morphology and Anthropology

Acta morphologica et anthropologica, 9
Sofia • 2004

Gangliosides: Chemical Characterization, Isolation, Biological Functions, Role in Autoimmune Demyelinating Diseases

V. Kolyovska

Institute of Experimental Morphology and Anthropology, Bulgarian Academy of Sciences, Sofia

Gangliosides, sialic acid-containing glycosphingolipids, are normal constituents of vertebrate cell membranes and are particularly abundant in the nervous system. They are found mainly in plasma membranes of neurons, glial cells and myelin, as well as in non-cell associated forms in blood plasma and other body fluids. Several procedures have been described for the isolation and purification of gangliosides. They have various important biological functions as membrane receptors, in regulation of cell grouth, differentiation, adhesion, oncogenic transformation, in synaptic transmition and in trans-membrane transfer of information. Gangliosides could serve as tumour markers. Numerous studies have demonstrated their immunological properties and the role of gangliosides in the pathogenesis of autoimmune demyelinating nervous system diseases, especially in chronic relapsing experimental allergic encephalomyelitis and multiple sclerosis.

Key words: gangliosides, autoimmune demyelinating disease, chronic relapsing experimental allergic encephalomyelitis, multiple sclerosis.

The term "Gangliosides" was coined 1942 by Klenk to designate lipids of the nervous system that contained sialic acid, to signify their predominant location in ganglion cells and in the grey brain matter as well as their glicosidic nature. Gangliosides are glycosphingolipids that consist of a sialic acid-containing carbohydrate residue linked to ceramide, i.e., a N-fatty acyl derivative of a sphingoid long carbon chain base. Throughout cellular evolution of bacteria, fungi, plants and animals, this two hydrocarbontailed ceramide has served for the attachment of carbohydrate to biomembranes, in particular to that of the outer cell surface [21, 43]. Within vertebrates they occur in virtually all tissues and tissue fluids – in each species and each tissue their molecular patterns differ and undergo changes during development and under various physiological influences. The gangliosides may consist of one to six molecules of N-acetyl neuramine (sialic) acid. The ceramide is formed by the aminoalcohol sphingosine and a fatty acid chain. The hydrocarbon chain of the sphingosine contains from fourteen to twenty-two carbon atoms. The carbohydrate chain can contain from two to nine different components: galactose, N-acetyl galactose, glucose, fucose, etc. Gangliosides display a heteropolar nature: a negative charge from the carboxyl group of the sialic acid and a more weakly pronounced charge from the amino-group [28].

The composition and structure of the molecule determine their unique physical and chemical properties. They get dissolved both in water and organic solvents which allows then to thrive both in the lipid phase of the membrane and the water medium of the cytoplasm. Gangliosides have the capacity of transition from the cytoplasm into the membrane and vice versa. Gangliosides may not be evently distributed on the cell surface, but rather exist in clusters surrounding membrane-bound proteins as a functional aggregate with molecular weight from 18 000 to 800 000 [1, 27, 28]. They exist not only in monomeric form but in water medium they form aggregations consisting of 200 to 400 monomers. The lipophilic part of their molecule (ceramide) is anchored to the cell membrane while the oligosaccharide chain protrudes from the membrane surface as a sort of antenna. Since they are negatively charged they bind to proteins in areas with a positive electric charge. The bonds between the gangliosides and the membrane proteins are of a different type – from a polar to a hydrophobic one [39, 40]. Gangliosides are extracted from the tissues by means of typical lipid solvents such as chloroform, methanol, etc. after which they can be separated from most of the other lipids as a result of their water solvency. The latter amphiphilic nature of gangliosides is due to their carbohydrate components and mainly to the highly polar units of sialic acid which are responsible for the acidity of their structure. Gangliosides display as well surface activity properties.

According to the structure and composition of the oligosaccharide chain several series of gangliosides are classified: gala-, globo-, neolacto-, gangliotriosis, gangliotetraose, hematosides, etc. [1, 43]. The phenomenon of homology is typical of the gangliosides. With the increase of the number of the attached residues from the sialic acid the polarity of gangliosides grows. Their great diversity (about 90 types) is due to the oligosaccharide structure and the binding site of sialic acid. Saturated and unsaturated fatty acids have been found as well as hydroxyderivatives which are otherwise characteristic of the sphyngomyelins and cerebrosides [42, 43]. Gangliosides also contain neutral saccharides and glycosamines.

Classification: Various schemes for classification of the gangliosides have been proposed the one of Svennerholm [39] accompanied by the corresponding symbols being the most popular: GM1, GD1a, GT1b, etc. The main Latin letter in the index denotes the number of sialic residues (mono-, di-, etc.) in the molecule, the Arab figure – (5-n) where n is the number of monoses and the small letter means that it refers to an isomer according to the binding site of sialic acid.

Localization: Gangliosides are situated on the outer surface of the plasma membrane in the tissues of all vertebrates [27]. Their concentration is highest in the nervous system where they account for 10 per cent of the total lipids content [42]. The brain of the higher vertebrates contains four main types of gangliosides: GM1, GD1a, GD1b and GT1b, which account for 80-90% of the total ganglioside content [1]. Ganglioside content is highest in the neurons [1]. The main gangliosides in the neuron are GM1 and GD1a [1]. They are namely found in the membranes of the neuronal perikaryon, axon, dendrite, nerve endings, synapses. Per neuronal membrane there are from (5 to10)^{15} numbers of ganglioside molecules. A part of the gangliosides is found in the cytoplasm where they are synthesized in the Golgi apparatus and transported along the axon. In the myelin sheath which contains 70-85% lipids the gangliosides represent 0.3-0.7% of the total brain lipids (dry weight) [5]. The most common gangliosides in the human myelin are the GM1[1]. It has been established that the periods of active ganglioside synthesis in the neuronal cytoplasm coincide with these of myelination.

The major ganglioside of the peripheral nervous system (PNS) is LM1, a monosialogangliosides, differing from the brain gangliosides by the presence of N-acetylgalactosamine [41]. It is predominant also in the human PNS myelin. In the my-

elin of the motor peripheral nerve the GM1 content is much higher – around 15 % of the total ganglioside content compared to the myelin of the sensor peripheral nerves [30].

Gangliosides are found not only in the cells and tissues but also in the tissue fluids. The main ganglioside type in the serum is GM3. Fifty four per cent of the total gangliosides in the normal human serum are monosialo-, 30 % – disialo-, 10 % – trisialo- and 6 % – tetrasialo – gangliosides [23]. The ganglioside content of the serum is exceptionally constant and does not show major variations due to age and sex in healthy individuals[37]. In pathological states, however, the serum ganglioside spectrum undergoes significant changes. The ganglioside level in the blood serum is too low, the described concentrations in literature being very diverse [11]. Out of 11 studied human sera S e n n et al. [37] have obtained 10.5 ±3.2 nmolgangl/ml serum. The sialic acid obtained is the marker. The total concentration varies considerably among the various individuals (7.7 – 15.1 nmol/ml), but no sexual differences have been reported. Therefore, the qualitative analysis of the serum gangliosides in pathological states appears to be more useful that the quantitative one in the diagnostics of various diseases.

Isolation of gangliosides. Several procedures for the isolation of gangliosides mainly from the brain have been described. This variety of techniques is due to significant difficulties of variable nature: in the biological sample the gangliosides are accompanied by substances close to them in their properties exceeding up to a hundred times their amount; the presence of proteolipids soluble in the common solvents for gangliosides makes their purification and isolation dificult; there is a necessity for work in sparing conditions (pH, temperature, airflow, purified reagents, etc.). A problem presents also the isolation of an individual ganglioside type because of their property to get associated among each other and with other polar substances such as the cerebrosides for example. There are two types of techniques for isolation of the gangliosides – analytical and preparative ones. The analytical methods are namely applied in scientific and clinical studies and by the preparative techniques pure products for biological experiments can be obtained [40]. In 1963 Svennerholm proposed a method for an almost total extraction of gangliosides as a modification of the most commonly method used of F o l c h (1957) [14] for extraction of total lipids. I l i n o v et al. [18] have forwarded an original method for isolation of gangliosides from the brain which, compared to the method of Svennerholm, is characterized with an economical usage of reagents and good efficiency [40, 41]. I l i n o v et al. [19] develop also a method for determination of lipid-bound sialic acid after chromatograhic isolation of brain gangliosides, which is particularly useful for some routine diagnostic studies. The commonly used methods for extraction, purification and analyzing of serum gangliosides are the ones of L a d i s h and G i l l a r d [24]; and K u n d u (K u n d u et al., 1985) [22, 23] but, as shown in a number of publications, in the application of these methods there are a lot of additional contaminations (S e n n et al.) [37]. The method for isolation of serum gangliosides of I l i n o v et al. [20] renders an opportunity for a more precise analysis of the serum ganglioside profile in the clinical practice. This method allows for distinct chromatographs good for densitometry. In it a three-stage extraction with cyclohexane and a chloroform-methanol mixture is carried out, which permits the elimination of non-polar lipids. After that, the sample is purified by thin-layer chromatography.

Biological function: Gangliosides are involved in cell recognition during development, in transfer of information in the synapses and in memory formation. They take part in cell growth and differentiation as membrane regulatory factors [4, 16]. Gangliosides participate in the information transfer across the cell membrane [8, 22, 42, 53]. They can serve as tumour markers since a great amount of tumour-associated gangliosides are present in the circulation of tumor bearing patients [9, 10, 17]. Gangliosides have neuritogenic and neuronotrophic effects and can stimulate regeneration in CNS and PNS [7, 15].

Role of gangliosides in autoimmune demyelinating diseases. A number of studies have shown that gangliosides are immunogenic. The inoculation of animals with gangliosides causes chronic relapsing experimental allergic encephalomyelitis (CREAE) – an autoimmune demyelinating disease of the nervous system with clinical and pathological features similar to multiple sclerosis (MS) [6, 26]. Cohen et al. [6] have for the first time induced CREAE in rabbits by inoculation with gangliosides. A lot of very successful animal models of MS have been developed in the recent years, which can be used for all types of investigations concerning the pathogenesis of this disease [48, 49, 54]. It has been demonstrated that there is an increase of GM1 and a decrease of GT1b gangliosides during the first clinical episode and the first remission in the brain and spinal cord of Lewis rats with CREAE. During these stages of the disease light- and electronmicroscopic studies showed a strongly expressed demyelination in CNS [44, 45, 46]. It has been reported that antibodies against GM1 gangliosides cause demyelination in well-myelinated spinal cord cultures [33]. In laboratory animals using various immunization techniques anti-gangliosides antibodies have been obtained [31, 32, 33, 34, 36]. Another proof for the immunogenecity of gangliosides is the presence of antibodies against the gangliosides in the blood serum of patients with autoimmune demyelinating diseases of the nervous system [2, 3, 12, 13, 26, 35, 38, 50, 52]. Using an own modification of ELISA for detecting antiganglioside antibodies an increase of the IgM antibody titer against GM1 in patients during the first MS attack has been established [47, 51].

These results give grounds for the assumption that together with the other myelin antigens gangliosides may also play a role in immune-mediated demyelination. The main requirement for a myelin antigen as a target of antibody-mediated demyelination is to be situated on the extracellular surface of the myelin membrane. Several such antigens have been identified, gangliosides GM1 including [2, 3, 12].

The data obtained in support of the concept of the ganglioside participation in MS pathogenesis represent a basis for continuing the studies in that trend since they are of major importance for the early diagnostics and more efficient treatment of this severe neurological disorder.

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