EFFECT OF IONIC STRENGTH OF THE SOLUTION ON SIZE OF GANGLIOSIDE MICELLES

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The possibility of treating hereditary enzymopathies by administration of deficient enzymes in the form of liposomes is attracting ever-increasing attention in the recent literature [2, 6]. It is considered that oriented transport of liposomes to particular tissues can be carried out by incorporating into the liposome membrane certain compounds with known affinity for receptors of target cells [1, 9]. As compounds of this kind, the writer has previously [3] used native and desialated ceruloplasmin and gangliosides — carbohydrate-containing molecules with terminal residues of galactose, which is known to bind with specific receptors of the hepatocyte plasma membrane [7]. Since ganglioside micelles are considered to differ considerably in size from liposomes, whose diameter is 70-80 nm, like other workers [5] the present writer judged incorporation of gangliocytes into the liposome membrane by the shift of the peak of ganglioside micelles into the region of the liposome peak during chromatography on Sepharose 4B. When these studies were continued it was unexpectedly found that free gangliosides in some cases can form micelles that are indistinguishable in chromatographic behavior on Sepharose 4B from liposomes. It will be evident that such an unusual property of gangliosides could simulate their incorporation into the liposome membrane even if no such membrane was present.

The aim of the investigation described below was accordingly to study the effect of the conditions of solution and chromatography of gangliosides on the size of their micelles.

EXPERIMENTAL METHOD

The following reagents were used: D-glucoso[1-^{14}C]lactose, with specific radioactivity 57.6 Ci/mmole, was from Amersham Corporation, England; a mixture of bovine brain gangliosides containing gangliosides G_{M1}, G_{D1a}, G_{D1b}, and G_{T1}, was from Koch-Light Ltd., England. The gangliosides, in a dose of 1 mg, dissolved in 1.3 ml of the test solution, were chromatographed on a column (2 × 18 cm) with Sepharose 4B (from Pharmacia, Sweden) with an elution rate of 64 ml/h; the volume of the fractions was 2 ml. Elution was carried out with the same solution as that in which the gangliosides were dissolved. Fractions during chromatography were monitored by means of the UV-recording continuous-flow densitometer (LKB, Sweden) at 284 nm. The total content of neuraminic acid in the fractions was determined by the resorcin method [8].

In some experiments the gangliosides were desialated by treatment of the native population with 0.1 N H_2SO_4 at 80°C for 60 min, followed by dialysis for 24 h against H_2O. The effectiveness of desialation was judged from the content of free neuraminic acid in the digest, determined by the reaction with thiobarbituric acid [10].

EXPERIMENTAL RESULTS

It is considered to be firmly established that gangliosides in an aqueous medium, in concentrations above the critical (10^{-5} M), form micelles with mol. wt. of (1-5) × 10^5 daltons [5]. We found that in aqueous solutions and in concentrations above the critical, gangliosides can form micelles of greater molecular weight. The results of gel-filtration of gangliosides, dissolved beforehand in various aqueous media, on Sepharose 4B, are given in Fig. 1. As this figure shows, dissolving the gangliosides in 0.9% NaCl led to their elution from the column with maximum of the peak in fraction 14 (Fig. 1: 7, 8). This corresponds approximate-

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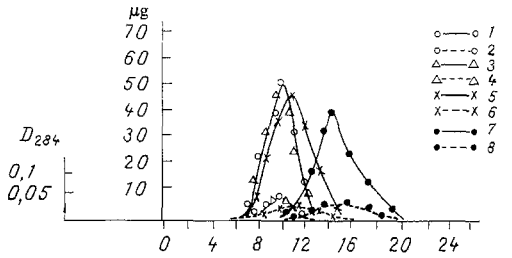

Fig. 1

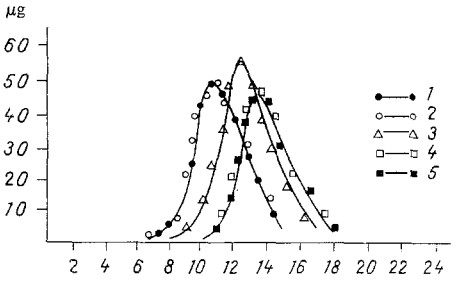

Fig. 2

Fig. 1. Chromatography of gangliosides dissolved in 2 mM Tris-buffer, pH 8.0 (1, 2), in 2 mM Tris-HCl buffer, pH 6.25 (3, 4), in 5 mM phosphate buffer, pH 6.25 (5, 6), and in 0.9% NaCl, pH 6.6 (7, 8), on Sepharose 4B. 1, 3, 5, 7) Content of neuraminic acid in fractions; 2, 4, 6, 8) absorption at 284 nm. Here and in Fig. 2: abscissa, Nos. of fractions; ordinate, content of neuraminic acid (in μg).

Fig. 2. Chromatography of gangliosides dissolved in 2 mM NaCl (1), in 5 mM NaCl (2), in 10 mM NaCl (3), in 20 mM NaCl (4), and in 50 mM NaCl (5) on Sepharose 4B.

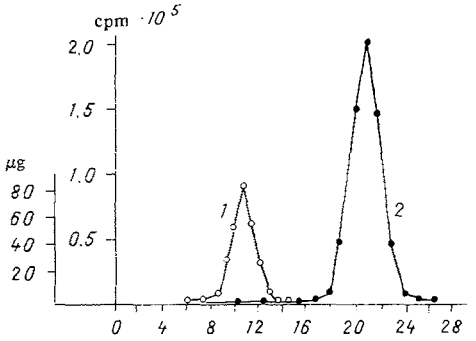

Fig. 3. Chromatography of gangliosides dissolved in 2 mM NaCl containing [^{14}C]lactose on Sepharose 4B. 1) Content of neuraminic acid (in μg), 2) radioactivity of D-glucoso-[1-^{14}C]lactose (in cpm × 10^5). Abscissa, Nos. of fractions.

ly to the molecular weight of the micelles described in the literature: (1-5) × 10^5 daltons. Dissolving gangliosides in 5 mM phosphate buffer, pH 6.25 (Fig. 1: 5, 6) or in 2 mM Tris-HCl buffer, pH 8.0 (Fig. 1: 1, 2) led to much earlier elution from the column. The micelles were virtually completely eluted in the void volume of the column (the maxima of the peaks were in fractions 9 and 10), which corresponds to a molecular weight of $\geqslant 10^7$ daltons.

In the next experiments an attempt was made to discover the causes leading to such marked changes in size of the ganglioside micelles. A change in pH of the Tris-HCl buffer from 8.0 (Fig. 1: 1, 2) to 6.25 (see Fig. 1: 3, 4) was found to have virtually no effect on the size of the micelles. Desialation of the gangliosides (by 70%) caused them, when dissolved in 0.9% NaCl, to begin to be eluted from the column practically together with those dissolved in 2 mM Tris-buffer. Native gangliosides dissolved in 0.9% NaCl (Fig. 1: 7, 8), incidentally, began to be eluted from the column for practical purposes only after elution of gangliosides dissolved in 2 mM Tris-buffer had ended (Fig. 1: 1, 2).

On the basis of these findings the writer postulated that it is the charge of neuraminic acid, removed during desialation, that is responsible for the change in size of the ganglioside micelles under the influence of the different conditions under which they were dissolved. To test this hypothesis, chromatography of the gangliosides was carried out at different ionic strengths, in the hope that under these conditions the charge of the ganglioside molecules on account of neuraminic acid would also change. It will be clear from Fig. 2 that gangliosides dissolved in 2 mM or 5 mM NaCl were eluted from the columns practically within its void vol-

ume. Gangliosides dissolved in 10 mM NaCl were eluted much later, and those dissolved in 20 and 50 mM NaCl later still. A further increase in ionic strength of the solution to 2 M NaCl did not lead to any change in size of the gangliosides micelles. It was concluded from these results that not only the concentration of gangliosides in an aqueous solution, as was previously considered, but also the ionic strength of the solution has some influence on the size of the micelles formed.

The fact that gangliosides in a solution of low ionic strength (2-5 mM NaCl) form micelles with unusually high molecular weight ($\geqslant 10^7$ daltons) necessitated an investigation to determine whether these micelles are not liposomes, i.e., whether they do not contain an internal space. To test this hypothesis, gangliosides were dissolved at low ionic strength in medium containing D-glucoso-[1-^{14}C]lactose and then chromatographed on Sepharose 4B. It will be evident that if the micelles contained an internal space, some at least of the [^{14}C]lactose present in the solution would be eluted from the column in the void volume together with ganglioside micelles. As Fig. 3 shows, all the lactose was eluted during chromatography much later than the ganglioside micelles. It can be concluded from these data that giant ganglioside micelles formed in solutions of low ionic strength do not contain an internal space and, consequently, they are true micelles and not liposomes.

Since gangliosides in an aqueous solution of low ionic strength (2-5 mM NaCl) formed micelles eluted from the column in the void volume, i.e., where liposomes are usually eluted, but in aqueous solution of higher ionic strength ($\geqslant$20 mM NaCl) they form micelles eluted from the column separately from liposomes, conclusions regarding incorporation of gangliosides into the liposome membrane based on coincidence of peaks of gangliosides and liposomes are correct only if ganglioside-containing liposomes are obtained and chromatographed under conditions of high ionic strength ($\geqslant$ 20 mM NaCl).

The results explain a possible cause of the losses of gangliosides found during dialysis. The possibility cannot be ruled out that in these cases passage of gangliosides through the dialysis membrane may be the result of the high ionic strength of the solution, at which the ganglioside micelles, as the present experiments have shown, are considerably reduced in size.

It can be postulated that by analogy with gangliosides the ionic strength of the solution may also affect the dimensions of liposomes obtained from charged phospholipids. Another possibility is that the ionic strength of different biological fluids determines to some degree the size of micelles, formed from different charged molecules (lipoproteins, phospholipids, gangliosides), circulating in them.

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LITERATURE CITED

1. V. Ya. Vidershain, in: Advances in Biological Chemistry [in Russian], Vol. 20, Moscow (1979), p. 46.
2. G. Ya. Vidershain, Vopr. Med. Khim., No. 3, 22 (1982).
3. V. M. Dvorkin, I. L. Galkina, and G. Ya. Vidershain, in: Abstracts of Proceedings of the 7th All-Union Conference on the Chemistry and Biochemistry of Carbohydrates [in Russian], Pushchino (1982), p. 51.
4. Y. Barenholz, B. Certazo, G. Lichtenberg, et al., in: Structure and Function of Gangliosides, New York (1980), p. 104.
5. P. L. Felgner, E. Freire, Y. Barenholz, et al., Biochemistry (Washington), 20, 2168 (1981).
6. G. Gregoriadis, Lancet, 2, 241 (1981).
7. E. F. Neufeld and G. Ashwell, in: The Biochemistry of Glycoproteins and Proteoglycans, New York (1980), p. 241.
8. L. Svennerholm, Biochim. Biophys. Acta, 24, 604 (1957).
9. V. P. Torchilin, B. A. Knaw, V. N. Smirnov, et al., Biochem. Biophys. Res. Commun., 89, 1114 (1979).
10. L. Warren, J. Biol. Chem., 234, 1971 (1959).