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Interaction of $Na_2B_{12}H_{11}SH$ with liposomes: Influence on zeta potential and particle size

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Abstract

The interaction of $Na_2B_{12}H_{11}SH$ (BSH) with liposomes has been studied. BSH is a compound used clinically in boron neutron capture therapy of glioblastoma, and is known to enter tumor cells. Liposomes were used as a model for studying the interaction of BSH with cell membranes. BSH led to changes in the zeta potential of liposomes consisting of DODAB (dioctadecyldimethylammonium bromide) alone or with DOPC (dioleylphosphatidylcholine) or DOPE (dioleylphosphatidylethanolamine). It also led to changes of the size of DODAB liposomes, with a maximum size at small zeta potentials. A firm binding of BSH with the head groups of the lipid must be assumed.

Keywords: BSH; Liposomes; Liposome size; Zeta potential

1. Introduction

BSH ($Na_2B_{12}H_{11}SH$, also known as mercaptoborate sodium) has long been used for therapy of brain tumors with BNCT (boron neutron capture therapy) [1,2]. We have investigated the pharmacokinetics of BSH in patients [3,4] and could determine that the pharmacokinetics is linear. The initial volume of distribution V_1 is 0.3 L/kg [3]. With a dose of 1 g BSH per kg body weight, the initial concentration of BSH in V_1 is about 5 mM. In tumor material of glioma patients who had been infused with BSH, boron persists for long periods of time [5], but is distributed in a heterogeneous manner. Uptake of BSH was mostly in cells of the tumor positively staining for glial fibrillary acidic protein (GFAP) [6]. BSH could not be removed by fixation with aqueous formaldehyde nor by washing during histochemical staining [7]. The subcellular localization of boron in tumor tissue could be determined with both electron energy loss spectroscopy and electron microscopy following immunohistochemical staining [8]; boron was found to be associated with electron-dense material in the intercellular space, with the cell membrane, and with the nuclear membrane and the chromatin. The pathway by which the very hydrophilic boron cluster compound is taken up into the cells, and nature of the binding to structures within the cells, remains unclear. One hypothesis [7] is that the boron cluster initially interacts with quaternary ammonium groups on the cell surface. We have shown that the hydrophilic cluster can dissolve in organic solvents in the presence of phospholipids [9]. Tetramethylammonium ions usually lead to precipitation of cluster compounds from aqueous solutions [10]. The firmly bound BSH could then be internalized, and redistributed within the cell after degradation of the lipids. It is

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interesting to note that the prevalence of choline in brain tumors exceeds those of the healthy brain by a factor of 4 [11].

In order to further explore the hypothesis, we investigated the interaction of BSH with liposomes of different composition, by determining the zeta potential and the size of the particles as a function of the BSH concentration. The zeta potential is a function of the overall charge of a particle, and changes in size reflect aggregation or fusion.

2. Materials and methods

DODAB (dioctadecyldimethylammonium bromide) was a gift of Dr. Radmacher, DOPE (dioleylphosphatidylethanolamine) and DOPC (dioleylphosphatidylcholine) were a gift of Lipoid GmbH. BSH ($Na_2B_{12}H_{11}SH$) was prepared according to the literature procedures [12].

Liposomes were prepared by sonication alone or in combination with extrusion through polycarbonate membranes with a pore diameter of 100 nm (Avestin, Mannheim, Germany). The liposomes consisted of either pure DODAB, or a mixture of 37.5 mol% DODAB and 31.5 mol% each of DOPC and DOPE, or a mixture of 50 mol% DODAB and 50 mol% DOPE and DOPC, respectively. The liposomes were prepared in 1 mM Tris buffer pH 7.4. Lipid contents was determined with the Stewart assay [13], taking into account the different color intensity of each of the lipids. Liposomes were stored up to one week at 4 °C; their size was not found to change during this period.

The size distribution and the zeta potential of the liposomes were measured with a Malvern Zetamaster. For zeta potential measurements, 5–10 individual determinations were averaged. The liposome suspension was 1 mM in Tris–HCl pH 7.4. BSH was added in different concentrations. Measurements were started after a few minutes. The BSH concentration required to achieve a zeta potential of 0 mV was determined by interpolation of the data.

3. Results and discussion

The liposomal suspensions investigated and selected measurements are listed in Table 1. BSH showed a strong and concentration-dependent effect on the average size of the particles in solutions containing DODAB liposome. A maximum size of 5 μm was found when BSH was added to the liposome solution at slightly more than a 1:2 concentration ratio BSH: DODAB (see Fig. 1). At both higher and lower concentrations of BSH, the particle sizes were smaller (in the absence of BSH, 57 nm, in the presence of 500 μM BSH, 105 nm for liposomes present at a lipid concentration of 70 μM). At intermediate concentrations, the solution contained aggregates visible to the naked eye. The size of the liposomes in the presence of high concentrations of BSH was almost twice of that in the absence, indicating that the cluster increases the effective size of the liposome. The same behavior was found for both pure DODAB liposomes and liposomes prepared from DODAB and other lipids (Table 1).

The zeta potential of DODAB liposomes was found to be strongly dependent on the amount of BSH added (Fig. 1). The initially positive value went to neutral and then negative values with increasing BSH concentrations. We interpret this as a firm binding of BSH to the positively charged head groups of the lipid, effectively first neutralizing and then overcompensating the surface charge of the liposome and thereby reducing its electrophoretic mobility. When the zeta potential of the DODAB liposomes passed from positive to neutral values (concentration of BSH around 70 μM, i.e., equimolar to the concentration of the lipid), the colloidal solution turned instable (indicated by the appearance of very large aggregates). This would be expected, when the initial repulsion between the positively charged liposomes is reduced by the firm interaction of the positively charged head groups of the lipid with the negatively charged BSH. With a further decrease of the zeta potential to negative values (−40 mV in the presence of 500 μM BSH), large aggregates were no longer seen, probably by the repulsion of the liposomes now carrying

Table 1
Liposomes prepared and their zeta potentials in the absence or presence of BSH

DODAB (μM)	DOPE (μM)	DOPC (μM)	Zeta potential (mV) w/o BSH	C_{BSH} for zeta potential = 0 (μM)	Zeta potential (mV) at BSH concentration (μM)
35			n.d.	23	−31.0/500
70			43.7	70	−36.9/500
140			n.d.	90	−43.8/1000
200			56.0	120	−34.2/500
500			n.d.	290	−48.1/1000
70	70		48.0	100	−9.8/700
70		70	57.2	130	−19.9/700
53	44	44	41.6	55	−21.5/470

Concentrations are given in μM, zeta potentials in mV. n.d., not determined.

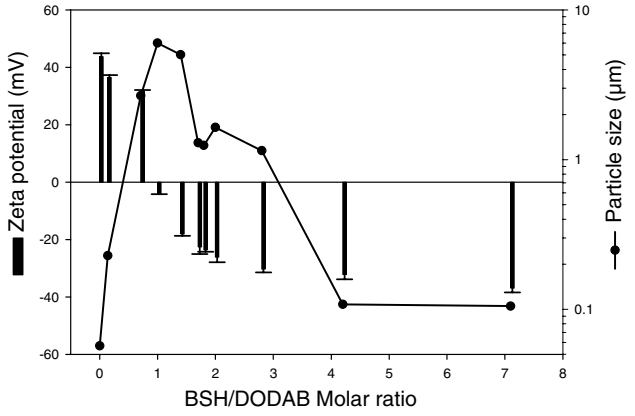

Fig. 1. Zeta potential (including the standard deviation) and particle size for DODAB liposomes as a function of the molar ratio between BSH and the lipid. Lipid concentration was 70 μM in 1mM Tris–HCl pH 7.4.

an overall negative charge, as indicated by the zeta potential.

Maximum particle sizes and minimal absolute values of the zeta potential of DODAB liposomes were observed when BSH was added in concentrations about half of that of the lipid, except for the DODAB/DOPE and DODAB/DOPC liposomes, where more than equimolar amounts of BSH to DODAB yielded the maximum size and a zeta potential of 0 mV (Fig. 2).

As the absolute BSH concentrations were low (between 10 and 500 μM, depending on the lipid concentration used), a rather small dissociation constant (below around 20 μM) for the ion pairs from the quaternary ammonium salt and the cluster would have to be assumed. This assumption is based on the observation that also at low concentrations of lipid and BSH, the concentration ratio for yielding a zeta potential of 0 mV does not change. This is in agreement with the observation that the cluster can be isolated from aqueous solutions by adding tetraalkylammonium ions [10], and indicates that the boron cluster cannot be considered a weakly coordinating anion [14] for quaternary ammonium salts.

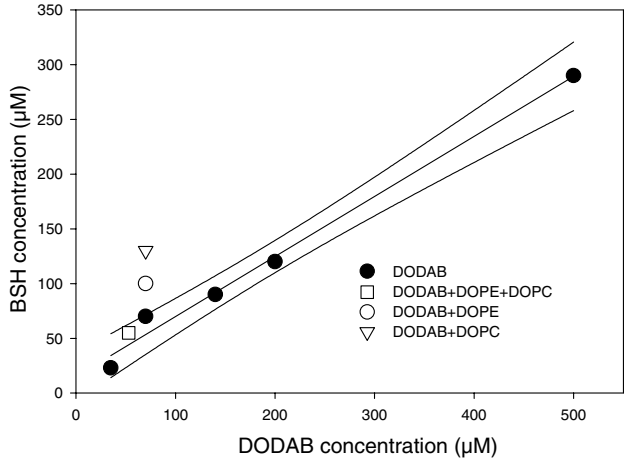

Fig. 2. Concentrations of BSH required for reaching a zeta potential of 0 mV for DODAB liposomes. Liposomes consisted of either pure DODAB (filled circles) or DODAB and additional lipids as indicated. For mixtures of lipids, the DODAB concentration is given.The lines are the linear regression line and the 95% confidence intervals.

Effective neutralization of the charge caused by DODAB would require one molecule of BSH for every two molecules of DODAB on the surface, or every four molecules of DODAB in bigger vesicles (where the curvature of the vesicle and the resulting decrease of the inner surface in comparison to the outer surface can be neglected). It was found that for pure DODAB liposomes, the concentration ratio of BSH to DODAB required for reaching a zeta potential of 0 mV was independent of the concentration of the lipid. The amount of BSH required was 55% of that of the total DODAB concentration; for the lipid molecules on the outside of the liposome, and taking into account the curvature of the membrane, a 1:1 molar ratio between BSH and DODAB present on the surface can be assumed. This represents a formal excess of BSH by a factor of 2.

When the DODAB liposomes contained DOPE or DOPC the concentration of BSH required for a zeta potential of 0 mV were generally higher than those required for the same amount of DODAB of pure DODAB liposomes.

Neutralization of the surface charge of DODAB liposomes was achieved at absolute concentrations of BSH well below the initial concentration reached by BSH following its infusion into patients [3]. The neutralization is an effect associated with BSH and not merily of the presence of divalent anions, as neither a substantial reduction of zeta potential, nor an increase in size, of the liposomes was observed when sulfate anions were added at equal or higher concentrations.

The interaction of negatively charged ions with positively charged liposomes has been observed before [15]. In that case, however, nucleic acids were used as polyanions. To our knowledge, it is the first time that an interaction of liposomes with small divalent anions is observed and quantified.

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