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Binding of GM1 ganglioside to a synthetic peptide derived from the lysosomal sphingolipid activator protein saposin B

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Abstract

Saposin B is a lysosomal sphingolipid activator protein which activates GM1 ganglioside hydrolysis by lysosomal β-galactosidase. To identify the structural elements of saposin B implicated in sphingolipid binding, we studied a synthetic peptide corresponding to a predicted α-helix, sapB-18, spanning residues 52–69 of saposin B. The circular dichroism spectrum of sapB-18 at pH 4.4 was consistent with a 44% α-helix content. As shown by intrinsic Tyr fluorescence studies of sapB-18, this peptide binds the GM1 ganglioside with a K_d of about 7 μM. Thus, we suggest that a putative amphipathic α-helix between residues 52 and 69 of saposin B plays a major role in the recognition and binding of GM1 ganglioside by saposin B.

Key words: Saposin B; GM1 ganglioside; Lysosomal disease

1. Introduction

The four saposins are small dimeric glycoproteins, with subunit M_r's of 8–10 kDa, which activate the hydrolysis of sphingolipids by lysosomal hydrolases [1,2]. They have a common precursor, prosaposin, of M_r 70 kDa [3] containing the saposins as four repeated domains A, B, C and D [4–7]. Saposins A and C are β-glucosidase activator proteins [6,8]; saposin B, also named the sphingolipid activator protein, is the activator of arylsulfatase A, β-galactosidase, α-galactosidase [4] and neuraminidase [9]; and saposin D is a sphingomyelinase activator protein [10]. The physiological function of saposin B is apparently to bring together sphingolipids and lysosomal enzymes for efficient hydrolysis of hydrophobic lipid substrates [11]. Both saposin B and prosaposin bind a variety of sphingolipids and were proposed to be actively involved in intracellular lipid transport [12–16]. A deficiency in saposin B causes a lysosomal storage disease with a clinical presentation similar to that of metachromatic leukodystrophy [17]. A deficiency in saposin C was described in a single patient with a variant form of Gaucher disease [18]. No specific deficiency in saposin A and D have yet been described but two patients with prosaposin deficiency, due to a mutation in the initiation codon of the prosaposin gene, have been reported [19].

The structure of prosaposin and the saposins are still unknown but, in a previous paper, we proposed a structural model of saposin B containing an amphipathic α-helix between residues 56 and 65 [20]. In the present paper, we use circular dichroism (CD) spectroscopy to confirm the helical conformation in solution of a synthetic peptide, sapB-18, including this region of saposin B. Because some amphipathic α-helices have been implicated in protein–lipid interactions [21–23], we analyzed the capacity of sapB-18 to bind to GM1 ganglioside.

2. Materials and methods

2.1. Materials

The peptide sapB-18, NH_2-Ser-Gln-Tyr-Ser-Glu-Ile-Ala-Ile-Gln-Met-Met-Met-His-Met-Gln-Pro-Lys-Glu-NH_2, corresponding to residues Ser^{52}–Glu^{69} of saposin B, was purchased from Multiple Peptide Systems, San Diego, CA. It was purified by reverse-phase HPLC and analyzed by fast-atom bombardment mass spectrometry. The mass of sapB-18 was 2182 Da, within 1 Da of the mass of the peptide computed from monoisotopic elemental composition. GM1 ganglioside was purchased from Calbiochem, San Diego, CA.

2.2. CD spectroscopy

The peptide sapB-18 (91.7 μM in 0.2 M sodium acetate buffer, pH 4.4) was analyzed by CD spectroscopy at room temperature in a Jobin Yvon model CD6 spectrophotometer. The percentage of α-helix was computed from the spectrum according to curve-fitting software developed in the Department of Biochemistry, University of Cambridge.

The amphipathic character of the putative α-helix (residues 56–65) of saposin B was determined by computing its mean hydrophobicity per residue, $<H>$, and hydrophobic moment, $<\mu>$, according to the method of Eisenberg et al. [21].

2.3. Fluorescence measurements

The measurement of intrinsic Tyr^3 fluorescence of the sapB-18 peptide was used to study GM1 ganglioside binding in a Perkin-Elmer model LS 30 spectrofluorometer at an excitation wavelength of 280 nm. The fluorescence spectrum of 5 μM sapB-18 was recorded in the presence of increasing concentrations of GM1 ganglioside between 0.625 and 12.5 μM. The spectra were corrected for the fluorescence associated to GM1 ganglioside itself. GM1 ganglioside was dissolved in chloroform:methanol (2:1) and the solvent was evaporated under a stream of nitrogen. The lipid was resuspended in 0.2 M sodium acetate buffer, pH 4.4, and sonicated for 6 s with an ARTEK sonicator at 60%

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of maximum intensity. The peptide-lipid mixture was incubated for 10 min at 20°C before recording the fluorescence spectrum.

The lipid-peptide interaction was analyzed by means of a Scatchard plot to determine the number of lipid (L) binding sites per mol of peptide (P), n, and the equilibrium dissociation constant, K_d, of the complex.

$$P + L \quad PL \tag{1}$$

$$\frac{r}{[L]} = \frac{-r}{K_d} + \frac{n}{K_d} \tag{2}$$

$$\text{where } r = \frac{[L] \text{ bound}}{[P] \text{ total}} = \frac{I_o - I_L}{I_o} \tag{3}$$

I_o is the fluorescence intensity of the free peptide and I_L is the fluorescence intensity of peptide in presence of the GM1 ganglioside ligand.

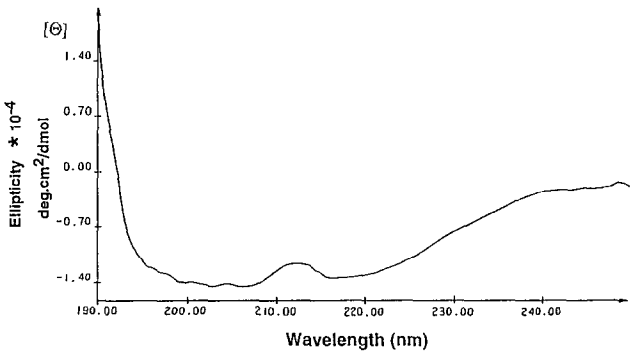

Fig. 1. Circular dichroism spectrum of the synthetic peptide sapB-18.

3. Results and discussion

The predicted α-helix of saposin B (between residues Glu[56] and Met[65]) is an amphipathic helix with a mean hydrophobicity per residue <H> of 0.40 and a hydrophobic moment <μ_H> of 0.28. It has an amphipathic character between that of a transmembrane and a globular protein α-helix in the classification of Eisenberg et al. [21]. Thus, this putative α-helix is a good candidate subdomain of saposin B for interaction with sphingolipids.

Saposin B binds to GM1 ganglioside [4,11]. We have hypothesized that the putative amphipathic α-helix between residues 56 and 65 of saposin B may be implicated in this binding function [20]. To test this hypothesis, the peptide sapB-18, NH_2-Ser-Gln-Tyr-Ser-Glu-Ile-Ala-Ile-Gln-Met-Met-Met-His-Met-Gln-Pro-Lys-Glu-NH_2, corresponding to the sequence Ser[52]–Glu[69] of saposin B, was synthesized. The CD spectrum of sapB-18 is compatible with a 44% α-helix content with typical minima at 208 and 220 nm (Fig. 1). However, it must be pointed out that in the complete saposin B structure, this peptide may adopt a different conformation.

The peptide–lipid interaction was studied by measuring Tyr[3] fluorescence of the sapB-18 peptide as a function of increasing GM1 ganglioside concentration. Determination of the binding constant using a Scatchard plot according to Eq. 2 yielded a K_d value of 7.0 ± 2.1 μM and a n-value of 0.82 ± 0.25 (mean ± S.D. of 3 determinations) (Fig. 2). This result suggests that there is only one GM1 ganglioside binding site per sapB-18 peptide

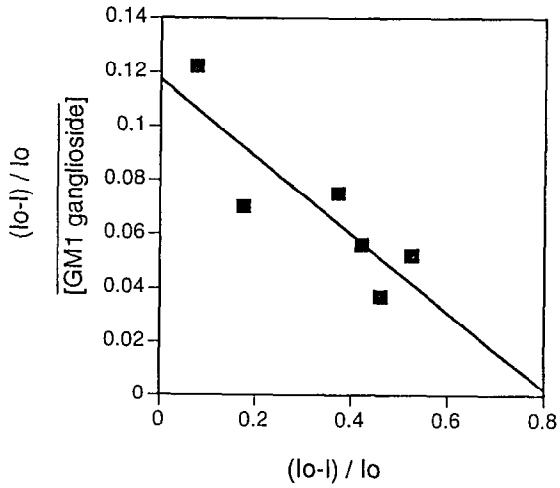

Fig. 2. Scatchard analysis of GM1 ganglioside binding to the synthetic peptide sapB-18.

molecule. The binding constant of GM1 ganglioside for sapB-18 is close to that determined for purified saposin B (around 12 μM) by Hiraiwa et al. [15]. This result suggests that the sapB-18 peptide plays a role in sphingolipid binding and recognition but does not exclude the participation of other structural elements of saposin B. As a control experiment, an 18-residue synthetic peptide of saposin C, derived from the same region as sapB-18 was from saposin B (Fig. 3), did not bind GM1 ganglioside (data not shown).

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          10         20         30         40
      *   *   *        * *    *    *   *   *
A SLPCDICKDVVTAAGDMLKDNATEEEILVYLEKT-CDWLPKPNMSAS
B GDVCQDCIQMVTDIQTAVRTNSTFVQALVEHVKEECDRLGPG-MADI
C DVYCEVCEFLVKEVTKLIDNNKTEKEILDAFDKM-CSKLPK-SLSEE
D GGFCEVCKKLVGYLDRNLEKNSTKQEILAALEKG-CSFLPDP-YQKQ

   50         60         70         80
  *       *              *   *      *
A CKEIVDSYLPVILDIIKGEMSRPGEVCSALNLCESLQKH
B CKNYISQYSEIA-IQ-MMMHMQPKEICALVGFCDEVKEM
C CQEVVDTYGSSILSILLEEVS-PELVCSMLHLCSGTRLP
D CDQFVAEYEPVLIEILVEVM-DPSFVCLKIGACPSAHKP
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Fig. 3. Alignment of saposins A, B, C and D amino acid sequences [4] indicating the synthetic peptides sapB-18 and sapC-18 sequences.

Patthy reported that saposin B is similar to the surfactant protein B (SP-B), another lipid-binding protein [22]. This protein is a major component of the lung surfactant together with other lipid-binding proteins and phospholipids. It is interesting to note that a synthetic peptide (Leu^{49}–Leu^{66} of SP-B), corresponding approximately to the position of sapB-18 in saposin B, also adopts an α-helical conformation and binds lipids [23]. This similarity between SP-B and saposin B suggests that these two similar proteins may bind lipids using similar structural elements and mechanisms.

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