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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 to 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 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]. The deficiency of saposin B causes a lysosomal storage disease with a clinical presentation similar to that of metachromatic leukodystrophy [17]. The deficiency of saposin C was described in a single patient with a variant form of Gaucher disease [18]. No specific deficiency of 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 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 the residues Ser^{52} to 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. The GM1-ganglioside was purchased from Calbiochem, San Diego, CA.

2.2. Circular dichroism spectroscopy

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

The amphipathic character of the putative α-helix (residues 56 to 65) of saposin B was determined by computing its mean hydrophobicity per residue, <H>, and hydrophobic moment, <μ>, 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. The 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% of maximum intensity. The peptide–lipid mixture was incubated for 10 min at 20°C before recording the fluorescence spectrum.

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The lipid–peptide interaction was analyzed by means of a Scatchard plot to determine the number of lipid (L) binding sites per mole 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 GM1-ganglioside ligand.

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 $<\mu_H>$ of 0.28. It has an amphipatic 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 the 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] to Glu[69] of saposin B was synthesized. The circular dichroism 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 Equation (2), yielded a K_d value of 7.0 ± 2.1 μM and an 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 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 derived from the same region of saposin C, as sapB-18 was derived from saposin B (Fig. 3), did not bind the GM1-ganglioside (data not shown).

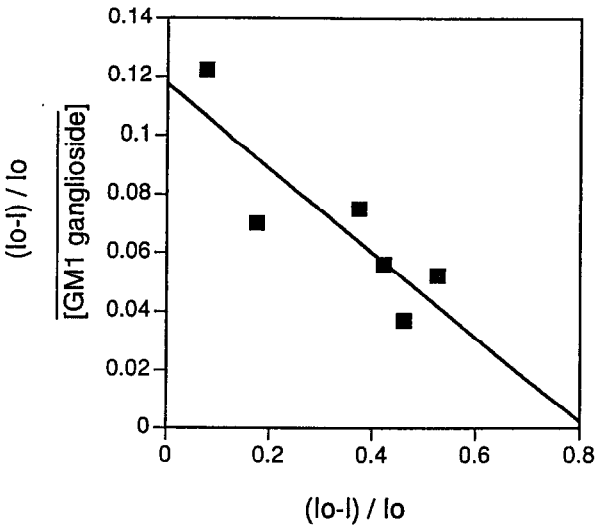

Fig. 2. Scatchard analysis of GM1-ganglioside-binding to the synthetic peptide sapB-18.

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] to 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.

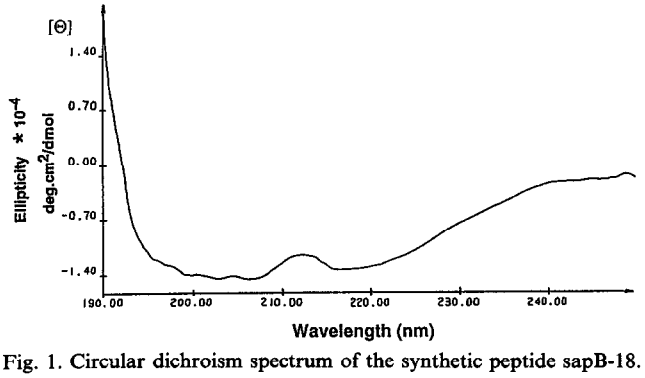

Fig. 1. Circular dichroism spectrum of the synthetic peptide sapB-18.

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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.

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