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FUSION OF SMALL UNILAMELLAR VESICLES INDUCED BY A SERUM ALBUMIN FRAGMENT OF MOLECULAR WEIGHT 9000

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A peptide (P-9) comprising amino acids 307 to 385 of bovine serum albumin induced the fusion of small unilamellar vesicles of phosphatidylcholine at low pH. Upon acidification P-9 exhibited a ultraviolet differential spectrum characteristic of hydrophilic exposure of chromophores. This conformational change, and the structure of P-9 composed of three amphiphilic helixes, suggested a general working hypothesis for the description of protein-induced membrane fusion.

The understanding of the molecular features of membrane fusion, particularly in protein-dependent systems, is acquiring an increasing relevance since proteins play a central role in some fusion processes [1,2]. Protein-induced membrane fusion is being investigated in several model systems including Ca^{2+} synexin [3], clathrin [4], viral protein [5] and albumin [6,7] induced fusion of vesicles. This latter system is characterized by the aggregation and fusion of small unilamellar vesicles of phosphatidylcholine (PC) induced by the F conformation of albumin [8] at low pH [6,7]. Using large fragments from pepsin cleavage of albumin (fragment P-31, $M_r = 31\,000$; fragment P-35, $M_r = 35\,000$) we have recently shown that the nature of aggregates formed prior to fusion determines the size of the fusion products [9]. In this report we show that a peptide of M_r 9000, obtained by pepsin degradation of serum albumin, induces the fusion of small unilamellar vesicles at low pH. This system allows the investigation, in molecular details, of the structure-function relationships in protein-induced membrane fusion.

Controlled pepsin treatment of albumin yields degradation products of molecular weights ranging from 6000 to 35 000 (e.g. fragment P-31 (31 000), fragment P-35 (35 000), fragment P-16 (16 000), fragment P-14 (14 000), fragment P-9 (9000) and fragment P-6 (6000)) [10]. A low-molecular weight fragment was isolated from pepsin-treated bovine serum albumin by Sephadex G-100 gel filtration *. Filtration through Sephadex G-50 gave a single peptide (Fig. 1) characterized as follows. Both Sephadex G-50 filtration and sodium dodecylsulfate (SDS) polyacrylamide [11] electrophoresis indicated that the isolated peptide had a molecular weight lower than that of cytochrome *c* (Fig. 1). The extrapolated molecular weight was 9000 (Fig. 1A, inset). Proof of both purity and nature of the peptide was obtained by determination of the first three N-terminal amino acids.

The N-terminal amino acid was determined by

Abbreviation: PyPC, 2-(10-(1-pyrene)decanoyl)phosphatidylcholine.

* Purified albumin was treated with pepsin in the presence of octanoic acid as described [9]. The fractions containing this fragment are those of the peak immediately following pool I of Fig. 1A in Ref. 9.

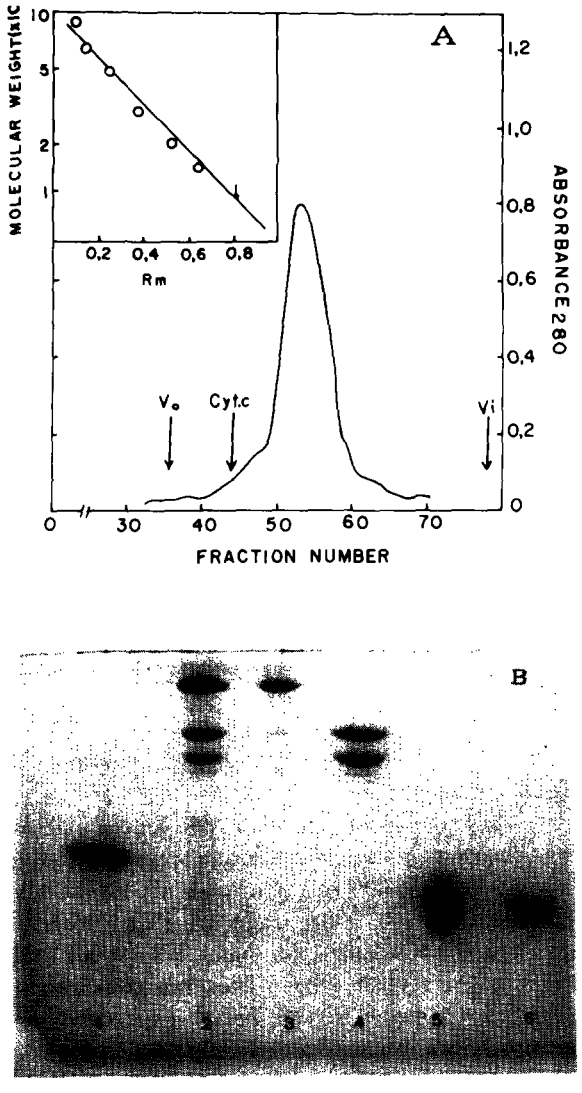

Fig. 1. Purification and molecular weight of P-9. (A) purification of P-9 by Sephadex G-50 filtration. Bovine serum albumin was treated with pepsin in the presence of octanoic acid [9,10] and after Sephadex G-100 filtration of the hydrolyzate the low molecular weight fraction (see footnote on p. 231) was applied to a Sephadex G-50 column. The inset shows the molecular weight determination in SDS-polyacrylamide gel electrophoresis with 15% acrylamide. The standards and respective molecular weights were: phosphorylase B (94000), bovine serum albumin (68 000), ovalbumin (43 000), carbonic anhydrase (30000), soybean trypsin inhibitor (21000) and lysozyme (14300). The arrow indicated the relative migration of P-9. (B) SDS-polyacrylamide gel electrophoresis [11] of: 1, cytochrome *c*; 2, products of the cleavage of albumin; 3, 4 and 5, fractions of the Sephadex G-100 filtration; and 6, a pool of the central fractions of the protein peak of Fig. 1A.

dansylation of the peptide, acid hydrolysis and thin layer cochromatography of the dansylated derivative with standards [12]. The 2nd and 3rd amino acids of the N-terminal sequence were determined by the same technique after one or two Edman degradation [13] cycles. The only N-terminal amino acid was phenylalanine and the next two amino acids in the sequence were alanine and glutamic acid, respectively. On the basis of the molecular weight and N-terminal sequence it is evident that the isolated peptide corresponds to the fragment previously described as P-9, which comprises the amino acids 307 to 385 [10].

Addition of P-9 to small unilamellar vesicles of PC produces: (a) an increase in absorbance due to light-scattering (Fig. 2); (b) a decrease in the excimer to monomer ratio (E/M) of a population of vesicles partially labelled with 2-(10-(1-pyrene)decanoyl)phosphatidylcholine (PyPC) (Fig. 3), which reflects the dilution of the probe in the total pool [7] and (c) an increase in the average hydrodynamic radius of the initial vesicles accompanied by a strict inverse dependence between size and E/M (Fig. 4), showing [7] that P-9 induces fusion of

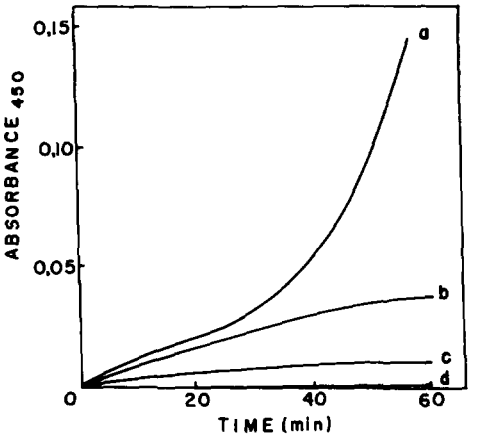

Fig. 2. Effect of P-9 on the absorbance of a solution containing small unilamellar vesicles (SUV) of phosphatidylcholine (PC). PC small unilamellar vesicles were obtained by sonication in the following buffers: K^+/formate, pH 3.6 (a); K^+/succinate, pH 4.0 (b); K^+/succinate, pH 4.5 (c); and K^+/maleate, pH 5.6 (d). All buffers were 10 mM in the corresponding acid and contained 0.15 M KCl. P-9 (24.5 μM) was added to 400 μl of a PC small unilamellar vesicle (12 mM P_i) solution contained in a quartz cell (0.2 cm path length) maintained at 30.0°C. The variation of absorbance due to scattering was registered in a Beckman M-25 spectrophotometer.

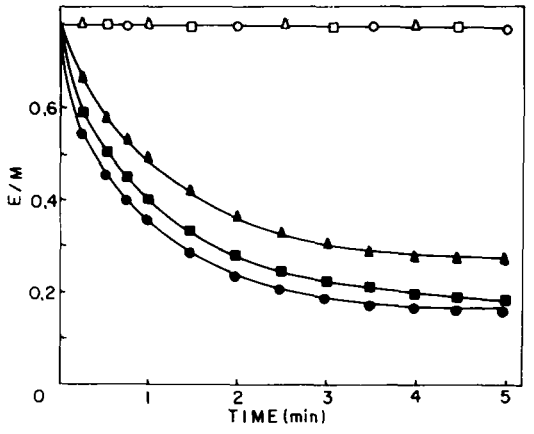

Fig. 3. Effect of albumin fragments (P-31, P-35 and P-9) on the excimer to monomer ratio (E/M) of PC small unilamellar vesicles partially labelled with PyPC. The PC small unilamellar vesicles contained 2% of vesicles labelled with PyPC (7 mol%). 2.7 μM peptide was added to incubation mixtures (30.0°C) containing 0.15 M KCl and 10 mM of either K^+/formate, pH 3.6 (▲, ■, ●) or K^+/maleate, pH 5.6 (△, □, ○). Aliquots (20 μl) were withdrawn, diluted to 2 ml with maleate buffer and the E/M ratio was determined as described previously [9]. P-31 (△, ▲); P-35 (○, ●); P-9 (□, ■).

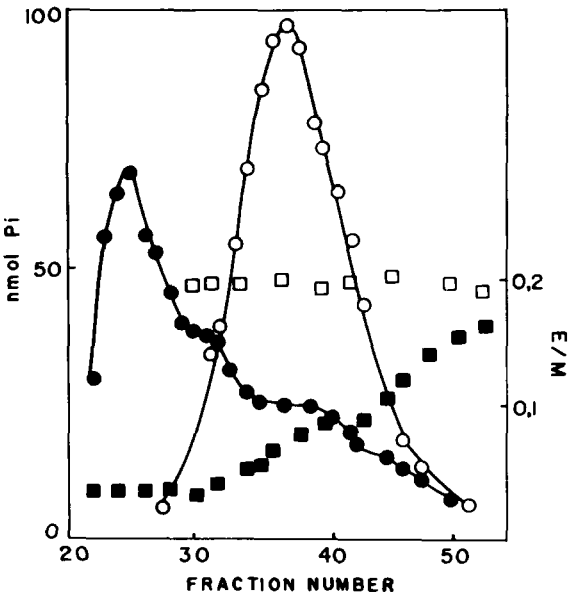

Fig. 4. Sepharose 4-B filtration of the products of incubation of PC small unilamellar vesicles with P-9. PC small unilamellar vesicles (10 mM P_i) containing 2% of vesicles labelled with PyPC (7 mol%) were incubated (30°C, 30 min) at pH 3.6 (K^+/formate 10 mM, 0.15 M KCl) without (○, □) or with (●, ■) P-9 (2.7 μM). The reaction mixture was then applied to a Sepharose 4-B column equilibrated, and developed, with maleate buffer, pH 5.6 (10 mM, 0.15 M KCl). P_i (○, ●); E/M (□, ■).

vesicles. An increase in turbidity can be due to vesicle aggregation or fusion. Turbidity due to aggregation induced by albumin is pH-reversible while that observed after fusion does not decrease upon a pH increase [6]. The increase in turbidity observed upon addition of P-9 at pH 3.5 (Fig. 2, curve a) does not diminish upon raising the pH (data not shown) suggesting vesicle-vesicle fusion. The decrease in the excimer to monomer ratio (Fig. 3), while effectively showing a dilution of the fluorescent probe over the lipid pool, might arise from a P-9-enhanced probe exchange among the vesicles. Although unlikely on a kinetic basis [7], this hypothesis is definitively ruled out by the results shown in Fig. 4. Thus if the decrease in E/M were due to exchange no relationship can be expected between the value of E/M and the relative vesicle size. A correspondence between low E/M values and high hydrodynamic radius (Fig. 4) can only arise if the dilution of the fluorescent probe is due to vesicle fusion. These results, taken together demonstrate that fragment P-9 induces vesicle fusion. Fragment P-9 is fusogenic only at low pH, being totally inactive above pH 4.5 (Figs. 2 and 3).

The structure of albumin is repetitive and large fragments, such as fragment P-31 and fragment P-35, preserve structural and binding properties of the whole protein [14]. It is therefore not surprising that fragment P-31 and fragment P-35, like albumin itself, induce vesicle fusion only at low pH [9]. The preservation of the pH dependence even for the small P-9 fragment led us to investigate conformational changes of this fragment with pH.

The pH-induced N to F conformational transition of albumin [8] can be detected by differential ultraviolet spectroscopy [15]. There are (negative) absorption bands in the 270–288 nm region (Ref. 15, Fig. 5A) which were also observed when a solution of P-9 was acidified (Fig. 5B). The spectral changes were reverted when the pH was returned to 7.2. The differential absorption bands at 280 and 288 nm are characteristic of exposure of tyrosine residues to a more polar environment [15,16]. The differential spectra of P-9 contain an additional broad (positive) band at 260 nm which

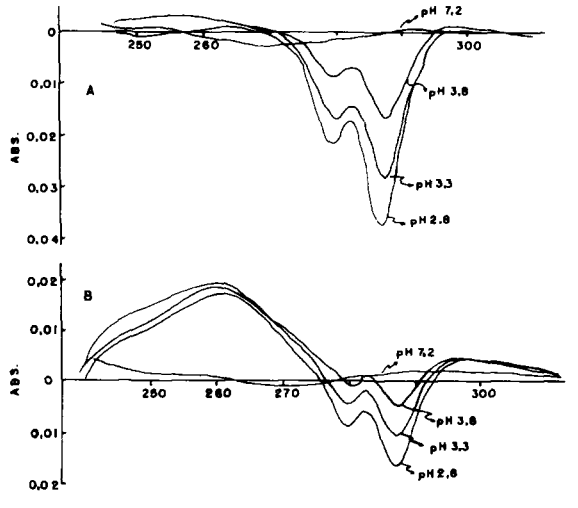

Fig. 5. Differential spectra of albumin (A) and P-9 (B) upon acidification. Protein solutions ($A_{280} = 0.560$) in water were used as a references and the pH change was obtained by adding either (0.1 M) HCl or NaOH to the sample cell. The results are expressed in absorbance using 1 cm cells and wavelength in nm.

is absent in that of albumin (Fig. 5). In other systems [16] a band in this region, and an accompanying band at 293 nm, have been ascribed to polar perturbation of tryptophan. Fragment P-9 contains no tryptophan residue, but aromatic moieties in other amino acids may contribute to the differential absorption at 260 nm.

Arranging the sequence of P-9 according to the proposal of Brown [17] one obtains a structural scheme composed of three amphiphilic helixes (Fig. 6). Since amphiphilic helixes interact favourably with bilayers [18] we consider that the structure proposed in Fig. 6 is a good candidate for inducing fusion. The exposure of this type of hydrophobic domain upon acidification may represent a common feature for albumin, fragment P-31, fragment P-35 and fragment P-9 and be responsible for their fusogenic properties. We can speculate that the exposure of hydrophobic domains determined by amphiphilic helixes might be a general characteristic of proteins capable of inducing membrane fusion.

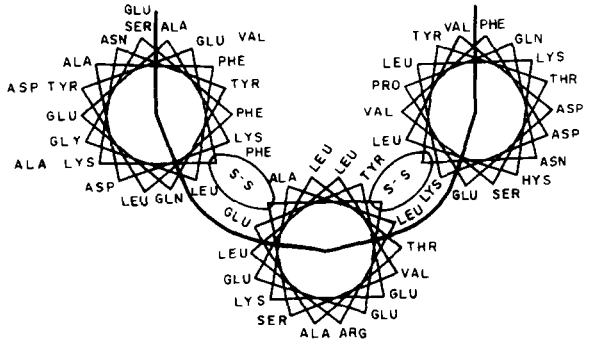

Fig. 6. Structural scheme of P-9 according to the proposal of Brown [17].

Acknowledgements

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