Molecular recognition induced aggregation and fusion between vesicles containing lipids bearing complementary hydrogen bonding head-groups

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Equimolar mixtures of large unilamellar vesicles, obtained from mixtures of egg lecithin and lipids containing complementary hydrogen bonding head-groups (barbituric acid and triaminopyrimidine), aggregate and fuse generating much larger vesicles.

Molecular recognition research has focussed especially on the selective formation of discrete supermolecules from complementary receptor–substrate pairs.[1] Extension to polymolecular assemblies should allow the recognition-directed generation of well-defined, extended supramolecular entities *via* the introduction of suitable recognition features into the molecular components.

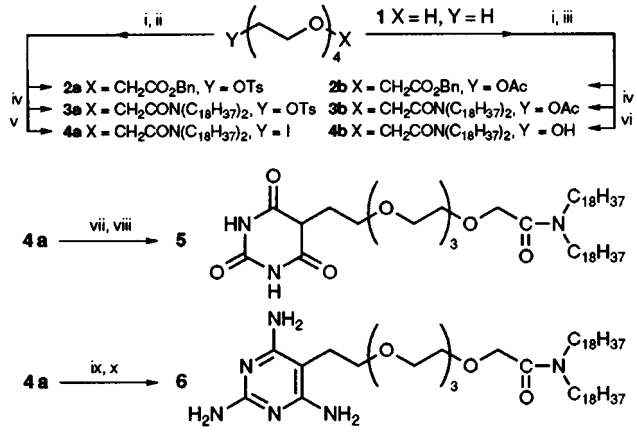
Thus, in previous work from our group, the self-assembly of units bearing complementary hydrogen bonding patterns has been used for generating liquid crystalline phases[2,3] and supramolecular polymers.[3,4] On the other hand, selective interactions take place at molecular layers into which recognition groups have been introduced.[5–7] Further on, molecular recognition may direct processes occuring between discrete polymolecular bodies such as liposomes. Indeed, surface recognition is a basic process controlling the interactions between biological cells.[8] Endowing artificial vesicles with recognition features may provide a means of provoking their interaction and for inducing processes such as aggregation and fusion. In the longer term it should permit the generation of polyvesicular architectures in a controlled fashion, a prerequisite for constructing organized assemblies of vesicles, of interest both as artificial functional nanoarchitectures and as models of biological tissue.

We report here some of our first results on the study of vesicles containing complementary lipids bearing as head groups the barbituric acid (BAR) and triaminopyrimidine (TAP) units which not only interact through three hydrogen bonds, but also, due to their double-faced nature, form self-assembled, extended ribbons.[9] the polymeric character of which is expected to enhance the global adhesion between complementary surfaces. We have previously studied the behavior of electrostatically complementary vesicles bearing opposite charges,[10] and recently the interaction between vesicles containing a biotinylated lipid and streptavidin in solution has been investigated.[11] For this work, we selected the complementary amphiphiles **5** and **6**; they were synthesized according to Scheme 1.‡ In view of the supramolecular organization imposed by hydrogen bonding interaction, a polyoxyethylenic spacer has been introduced in order to provide sufficient orientational freedom to the recognition sites.

Electron microscopy showed that **4b** and 1 : 9 mixtures of **5** or **6** with **4b** form lamellar phases in water under all conditions of vesicle preparation that have been investigated in the present study (detergent removal, sonication or lipid swelling in presence of an alternative electrical field). Large unilamellar vesicles were prepared from 1 : 9 mixtures of **5**, **6** or **4b** and egg lecithin (EPC) either by detergent dialysis[15] or extrusion[16] methods. As indicated by freeze-fracture electron microscopy [Fig. 1(*a*)] and by quasistatic and dynamic light scattering experiments, the size polydispersity of vesicles prepared by extrusion through Nucleopore filters was low, making them suitable to analyse the evolution of the vesicle solution by these techniques. Whereas aqueous suspensions of pure **5**, **6** and **4b** exhibited a sharp signal at 41 ± 1 °C by differential scanning calorimetry (DSC), the 1 : 9 mixtures with EPC did not display any transition, thus suggesting the absence of an extensive lipid segregation within the bilayer.

When identical amounts of weakly light scattering vesicular preparations of **5**-EPC 1 : 9 and **6**-EPC 1 : 9 were mixed, the solution became turbid after a few minutes whereas the appearance of individual preparations remained stable for a week. This observation was quantified by dynamic light scattering measurements (Table 1). The diameters of the starting vesicular suspensions were in the 150 nm range whereas the multimodal mathematical treatment of the data from the final state of the mixture provided an apparent diameter equal to 2000 nm. Considering the relation of size to scattered light intensity, this value can be reasonably assumed to represent the diameter of the largest objects.

In order to define more precisely the nature of the events taking place in these solutions, electron microscopy pictures were recorded at different times after mixing complementary vesicles. First, a rapid aggregation leading to the formation of large clusters of vesicles was observed [Fig. 1(B)]. After 15 min, two different populations of vesicles were visible [Fig. 1(C)]. A few very large vesicles (micrometer range) were surrounded by many smaller ones exhibiting size and morphology close to that of starting preparations. Support for the identification of vesicle fusion as the phenomenon triggering vesicle growth was provided by the observation of singular



Scheme 1 *Reagents and conditions*: i, N_2CHCO_2Bn, $BF_3(OEt_2)$, CH_2Cl_2, room temp., 60%; ii, TsCl, pyridine, 0 °C, 69%; iii, Ac_2O, pyridine, room temp., 80%; iv, (*a*): H_2, Pd/C, CH_2Cl_2, (*b*) *N*-hydroxysuccinimide, DCC, DMAP, (*c*) $HN(C_{18}H_{37})_2$, 30 °C; v, NaI, butanone reflux, 93%; vi, NaOH, EtOH, 96%; vii, $CH_2(CO_2Me)_2$, NaOMe MeOH, reflux, 85%; viii, $OC(NH_2)_2$, NaOMe, MeOH, reflux, 72%; ix, $CH_2(CN)_2$, NaH, Me_2SO, 60%; x, $HNC(NH_2)_2$, NaOMe, MeOH, reflux, 70%

events such as the formation of necks between adhering vesicles and budding on large vesicles [Fig. 1(D)].

In the present system, different interactions may contribute to the observed coalescence phenomena. In view of the acidobasic properties of the BAR and TAP lipids leading to the formation of species of opposite charge, an attractive electrostatic interaction may also play a role as observed previously.[10] Furthermore, ion-pairing reinforced hydrogen bonding between BAR and TAP units has been observed.[17] Since polyoxyethyleneglycol is known to promote vesicle fusion, we established that the spacer itself did not do so, since vesicles prepared from 1 : 9 **4b**-EPC mixtures were stable at least for several days. The present experiments consequently suggest that surface recognition *via* complementary hydrogen bonding patterns plays an important role in the events observed. Studies in progress should provide more information about the nature of the interaction between complementary vesicles and about its role in the vesicle fusion event. The intervesicular processes described here represent a first step towards the controlled, recognition-directed construction of organized, functionally integrated assemblies of vesicles leading towards 'tissue-like' artificial entities.

We thank L'OREAL for a graduate fellowship to V. M. A.

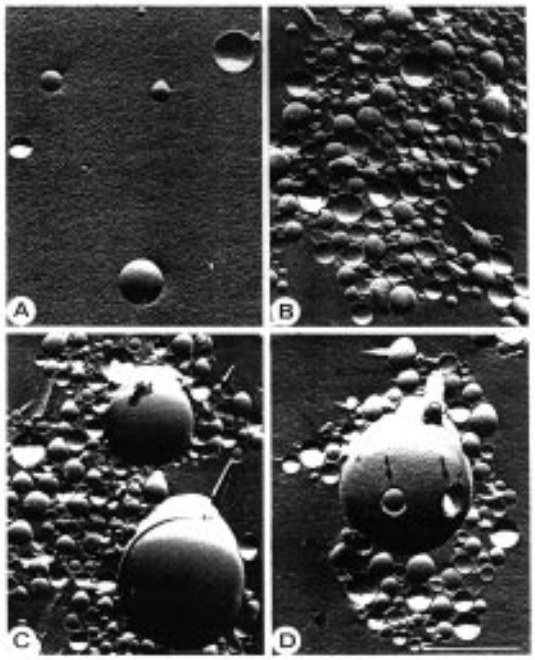


Fig. 1 Freeze-fracture electron micrographs of the vesicles prepared from 9 : 1 mixtures of egg lecithin and lipids **5** or **6** containing either barbituric acid (BAR) or triaminopyrimidine (TAP) head-groups. (A) Typical image of vesicle preparations containing either BAR or TAP lipids. (B) Equimolar mixture of BAR and TAP vesicles immediately after mixing. (C), (D): the same as (B) but after more than 15 min incubation. Notice the aggregation of vesicles after mixing and the appearance of much larger vesicles after incubation, some of which show vesicle fusion [arrows in (D)]. All photographs are at the same scale; the bar on (D) represents 500 nm.

Table 1 Diameters (in nm) of large unilamellar vesicles determined by dynamic light scattering (± 10nm)[a]

Angle(°)	**6**-EPC vesicles	**5**-EPC vesicles	1 : 1 Vesicle mixture
90	137	135	—
70	144	153	—
50	155	156	2000

[a] Vesicles prepared by extrusion from 1 : 9 mixtures of **5** or **6** and EPC at 35 °C in 20 mm glucose; vesicle concentration: 0.5 mg dm^{-3}

Footnotes

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‡ The reaction of an excess of tetraethyleneglycol with benzyldiazoacetate.[12] followed by tosylation of the remaining alcohol group yielded compound **2a** which, *via* hydrogenolysis over Pd/C in CH_2Cl_2 followed by coupling with dioctadecylamine gave the amide **3a** which was converted to **4a**. This led to the barbituric acid derivative **5** by monoalkylation of dimethyl malonate followed by condensation with urea.[13] The substituted triaminopyrimidine **6** was obtained in a similar way *via* monoalkylation of malonodinitrile by **4a** and then condensation with guanidine.[14] The alcohol **4b** was synthesized by a modification of the pathway *via* **2b** and **3b**.

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Received, 16th September 1996; Com. 6/06340C