Polymer Bulletin 24, 545–549 (1990)

Polymer Bulletin

Extraction of cholesterol by non-ionic cholesterol-based polymeric vesicles

Iwhan Cho and Young-Wook Kim

Department of Chemistry, Korea Advanced Institute of Science and Technology, P.O. Box 150, Chongyangni, Seoul, Korea

SUMMARY

In the search for the potential method to extract the free cholesterol from certain biological systems, a nonionic polymeric amphiphile was prepared from cholesterol-based monomer and acrylamide. This copolymer dispersed well forming vesicles when sonicated and was found to be effective for the extraction of cholesterol in water as the experiment with [^{14}C]cholesterol indicated. Extraction became more effective as temperature was raised.

INTRODUCTION

There has been a great interest in synthetic vesicular systems which can lead to various practical applications(1). However, synthetic dialkyl amphiphile vesicles are thermodynamically unstable and undergo fusion on standing. Thus, all the possible applications based on their long-term stability such as drug carriers(2) or models for biological membranes are limited. Recognizing the need for enhanced stability, the preparation of polymerized vesicles have been reported by a number of different laboratories(3-5).

More recent trend of research in this area involves the detailed characterization of polymeric vesicles aimed towards useful and specific applications. In general, these polymers under the investigation are the polymers of rather low molecular weights and also it is common to see the adoption of flexible spacer groups between polar head groups and hydrophobic tails(6-7).

It is commonly observed that natural plasma membranes contain high level of incorporated cholesterol. Cholesterol is a flat, rigid and hydrophobic molecule with a polar hydroxy group. Cholesterol and its esters are found to be included up to the concentration of 150-250 mg/100 ml in normal human blood plasma and its excessive deposition on the veins could cause health problems. Now it is a challenge to find a way to leach out this excess free cholesterol from the blood systems.

We have already reported a novel system in that the cationic cholesterol-containing monomers spontaneously formed highly stable unibilayer vesicles in aqueous system upon polymerization(8-10). We have focused our attention on these points and designed a nonionic copolymeric amphiphile containing cholesterol, in the search for the potential method to extract the free cholesterol from the blood by utilization of our polymers. Using our vesicle systems, we have found interesting results and now wish to report the preliminary results.

EXPERIMENTAL

Synthesis of cholesterol-based monomer

By use of the procedure of Patel(11), 3,6,9-trioxa-octan-1-ol-

cholesteryl-3 ε-ol was prepared from the condensation of cholesteryl p-toluenesulfonate and triethylene glycol in boiling dioxane. Cholesterol-based monomer was prepared with 3,6,9-trioxa-octan-1-ol-cholesteryl-3 -ol (22 mmol), acryloyl chloride (22 mmol), and triethylamine (55 mmol) in methylene chloride at 0°C. The chemical structure of cholesterol-based monomer was confirmed by ^{1}H NMR. ^{1}H NMR ($CDCl_3$): 6.85-5.82 (m, 3H, CH_2=CH-), 5.62 (b, 1H, -C=CH-), 4.55 (t, 2H, -CO-O-CH_2-), 4.15-3.65 (m, 10H, tetraethylene glycol), 3.41 (b, 1H, -O-C-H), 2.5-0.65 (br m, 43H, cholesterol).

Polymerization

1.0g (1.75 mmol) of the cholesterol-based monomer, 0.44g (6.13 mmol) of acrylamide, and 0.01g (0.06 mmol) of azobis(isobutyronitrile) dissolved in 20 ml of 1:1 ethanol-benzene were placed in an ampule, which was sealed upon subjecting to the freeze-pump-thaw cycle. The ampule was kept at 70°C for 5h. The solvent was then removed. The resulting white residue was reprecipitated from dimethyl sulfoxide and acetone.

Gel permeation chromatography

The molecular weight of copolymer was determined in 1,2,4-trichlorobenzene solution (GPC Model: Waters 150C, measured at 135°C, standard: polystyrene).

Vesicle formation and electron microscopy

The nonionic copolymeric amphiphile was dispersed (10 mM in the cholesterol group) in water by sonicating at 70°C for 30 min with a Branson bath-type sonicator (sonic power, 150W). One ml of this vesicle solution was mixed with 1 ml of 2% uranyl acetate. The mixture was applied to carbon-coated grid and then dried in vacuo. A Jeol Jem-100CX electron microscope was used for the measurement.

[^{14}C]Sucrose entrapment

In order to demonstrate that polymeric vesicles were completely sealed spheres, [^{14}C]sucrose was entrapped in the aqueous interior. Using a Sephadex G-50-80 column, gel filtration separation of free [^{14}C]sucrose and [^{14}C]sucrose entrapped in polymeric vesicles was performed.

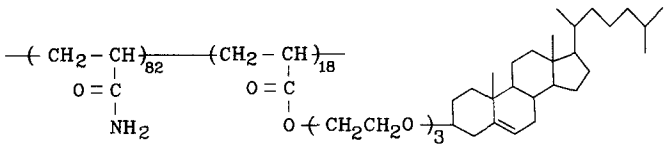
Extraction of free cholesterol by cholesterol-based polymeric vesicles

Labeled monomeric [^{14}C]cholesterol was used as a probe. After injecting 1 μCi (10 μl of toluene solution) of [^{14}C]cholesterol to the 2 ml of vesicle stock solution and the mixture was stirring for 24h at 20, 40, and 60°C. The [^{14}C]cholesterol which was not extracted was then removed by gel filtration on Sephadex G-50-80. One ml of the sample solution was added to a wet 31x1.3 cm column and then eluted with pH 7.2 tris buffer, and each 0.8 ml fractions in test tubes by fraction collector was taken. Extracted [^{14}C]cholesterol was measured by liquid scintillation counter (Beckman Model LS-3133T scintillation spectrometer: cocktail solution, 5 ml).

RESULTS AND DISCUSSION

A nonionic water-soluble copolymer was prepared from cholesterol-based monomer and acrylamide. The molecular weight of copolymer determined in 1,2,4-trichlorobenzene solution was 2 x 10^4. The polymer composition was determined by elemental analysis with the ratio of N/H (N/H: 7.927/8.919).

The nonionic polymeric amphiphile was dispersed in water by sonication at 70°C for 30 min. An electron micrograph of polymeric amphiphile recorded on a JEOL JEM-100CX microscope confirmed the presence of closed spherical vesicles having diameters ranging between 700 and 1200 Å (Figure 1).



Further evidence for the formation of vesicles comes from the entrapment of [^{14}C]sucrose. Sephadex G-50-80 was used for gel filtration. The amount of [^{14}C]sucrose entrapped into vesicles were about 0.2%.

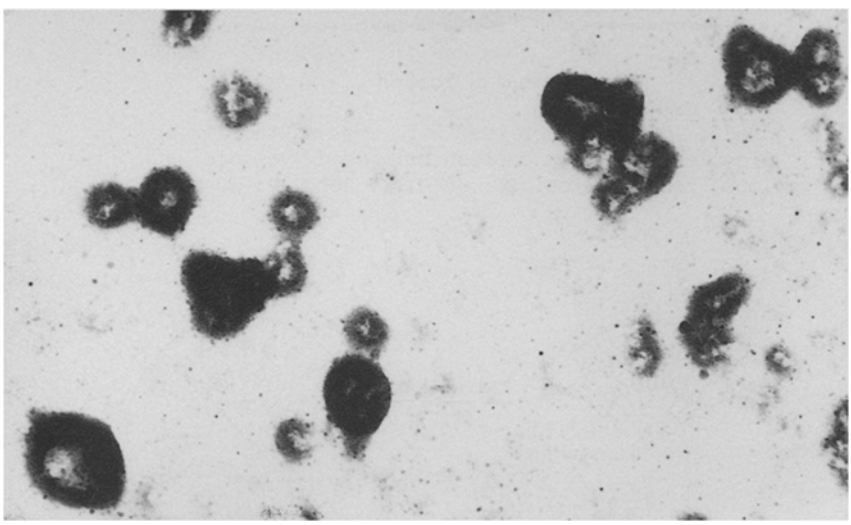


Figure 1. Electron micrograph of the vesicles formed by copolymeric amphiphile. Stainning agent: 2% uranyl acetate, magnification: x 80000.

In order to search for the method to extract cholesterol using our nonionic cholesterol-based vesicles, we prepared the vesicle stock solution (2 mM in the cholesterol group) of the polymeric amphiphile and labeled monomeric [^{14}C]cholesterol was used as a probe. The experimental procedure was described in the above experimental part. Since the solubility of cholesterol in water is 5.2×10^{-9}M/ml, 1 μCi (2×10^{-8}M) of [^{14}C]cholesterol was injected to the 2 ml of vesicle stock solution.

The results are shown in Figure 2. The vesicle fraction of this experiment was in accord with that of entrapment experiment of [^{14}C]sucrose. A pronounced trend is that as temperature was raised, extraction of free cholesterol also increased. 10.8, 25.8, and 48% of the injected [^{14}C]cholesterol was extracted by this vesicle system at 20, 40, and 60°C. It may be possible that at higher temperature the increased fluidity of the hydrophobic layer causes taking up more cholesterol.

As shown in Figure 3, we believe that the free cholesterol is incorporated into the pendent cholesterol bilayer. From the calculation of

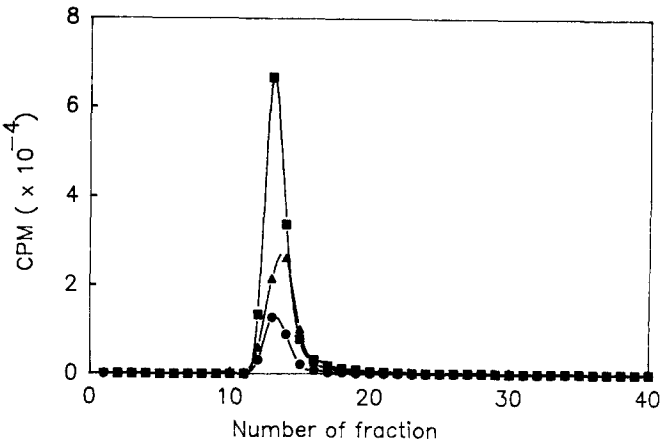


Figure 2. Column profiles for the extraction of [^{14}C]cholesterol by cholesterol-based vesicle at various temperatures. ■: 60°C, ▲: 40°C, ●: 20°C.

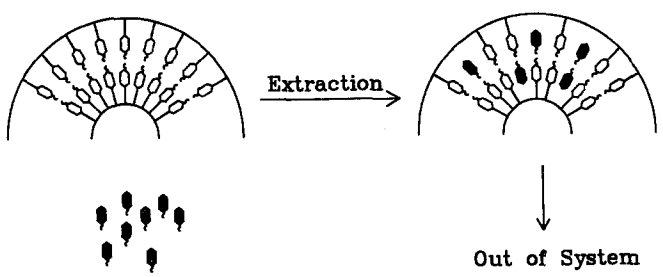


Figure 3. Schematic representation of extraction of free cholesterol by cholesterol-based vesicles. ⚲: free cholesterol, ♀: pendent cholesterol.

the extracted amount (9.6×10^{-6}mM) of free cholesterol at 60°C and the amount of pendent cholesterol (4×10^{-3}mM) in the vesicle solution, we know that the extracting capacity is 1 free cholesterol/400 pendent cholesterol.

This result suggests a possibility of new potential application of cholesterol-containing vesicles. Structural modification and control of the molecular weight of the presently investigated polymer and other related copolymeric vesicles are now in progress. Our interests are also directed towards other cholesterol-containing systems.

REFERENCES

1. J.H.Fendler, "Membrane Mimetic Chemistry", Wiley-Interscience: New York (1982).
2. S.L.Regen, Ann.NY Acad.Sci., 446, 296 (1985).
3. D.Day, et al., Isr.J.Chem., 18, 325 (1979).
4. S.L.Regen, et al., J.Am.Chem.Soc., 102, 6638 (1980).
5. J.H.Fendler, and P.Tundo, Acc.Chem.Res., 17, 3 (1984).

6. T.Kunitake, et al., J.Am.Chem.Soc., 103, 5945 (1981).
7. L.Laschewsky, et al., J.Am.Chem.Soc., 109, 788 (1987).
8. I.Cho and K.-C.Chung, Macromolecules, 17, 2935 (1984).
9. I.Cho and K.-C.Chung, Macromolecules, 21, 565 (1988).
10. I.Cho and J.-G.Park, Chem.Letters, 977 (1987).
11. K.R.Patel, et al., Biochim.Biophys.Acta, 797, 20 (1984).

Accepted August 16, 1990 S