Modes of Interaction of (In^{3+})-8-Hydroxyquinoline with Membrane Bilayer

Karl J. Hwang*

California Institute of Technology, Pasadena, California, and University of Washington, Seattle, Washington

Using the perturbed angular correlation of gamma radiation from indium-111, we have investigated the modes of interaction of $^{111}In^{3+}$-tagged 8-hydroxyquinoline ("In-HOQ") with liposomes of L-α-dipalmityl phosphatidylcholine and of L-α-dipalmityl phosphatidylcholine: cholesterol (2:1, M/M). The study shows that the complex of In-HOQ diffuses rapidly through the lipid bilayer and transfers the $^{111}In^{3+}$ ion to the chelating agent, nitrilotriacetic acid, encapsulated in liposomes. Such transport of metal ions by 8-hydroxyquinoline is governed by the binding constants of the metal ions to the chelating agents on both sides of the lipid bilayer, and by the exchange rates of indium ions from these chelating agents to 8-hydroxyquinoline. Like those of cholesterol, In-HOQ complexes in liposomes are exchanged readily from one liposome to another. A model is proposed in which the complexes of In-HOQ embedded in lipid bilayer are in a dynamic equilibrium between the diffusible and the nondiffusible forms. Most of the nondiffusible complexes are located near the region of the polar head groups on both sides of the bilayer.

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The use of 8-hydroxyquinoline for bactericidal purposes has been known for years (*1*). It has been postulated that this agent can diffuse through the bacterial cell membrane and perform its antibacterial function by sequestering the essential metal ions inside the cells (*2,3*). Recently, the complexes of 8-hydroxyquinoline with several gamma-emitting nuclides, such as indium-111, gallium-68, and technetium-99m have been used to label red blood cells (*4,5*), platelets (*5,6*), and leukocytes (*7,9*). The efficiency of incorporation of radioactivity into these cells can be as high as 95%. These labeled cells have been used successfully in the evaluation of the blood pool, the location of thrombi, and the detection of focal inflammation and abscesses by gamma imaging. The high-yield labeling of the cells is believed to be due to the lipid solubility of 8-hydroxyquinoline, which facilitates the diffusion of the radioactive metal ion through the cell membrane, with subsequent binding to cytoplasmic components. The exact mode of interaction of 8-hydroxyquinoline-metal complex with the cell membrane is not yet known. Experiments designed specifically to investigate the dynamic properties of the 8-hydroxyquinoline-metal complex in membranes will allow the study of such interaction.

The complex of indium-111 with 8-hydroxyquinoline (In-HOQ) is ideally suited for this study, since changes in rotational correlation time of the In-111 can be monitored by the technique of perturbed angular correlation of gamma radiations (PAC) (*10–15*). Two gamma photons are emitted in cascade from the In-111 nucleus and are detected by coincidence counters. The coincidence counting rate depends on the angle between the direction of propagation of the gamma photons. This angular correlation may be perturbed by the interaction of the In-111 with its local environment. Thus, information concerning changes in the tumbling rate of

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For reprints contact: Karl J. Hwang, Dept. of Pharmaceutical Sciences, School of Pharmacy, University of Washington, Seattle, WA 98195.

* Present address: Dept. of Pharmaceutical Sciences, School of Pharmacy, University of Washington, Seattle, WA 98195.

$^{111}In^{3+}$ bound to molecules with different rotational correlation times may be obtained by the technique of PAC.

Liposomes have been extensively studied as models for biologic membranes. The ability to encapsulate materials makes the liposome a simple system for study of the possible interactions of 8-dydroxyquinoline-metal complexes with membrane lipid bilayers as well as the cytoplasmic components of a cell. We have previously shown that the rotational properties of indium bound to materials encapsulated in different types of liposomes can be monitored directly from the measurement of the time-integrated perturbed factor, $<G_{22}(\infty)>$, of the sequentially emitted 173- and 247-keV gamma photons of In-111 (*14*). The present study describes the application of the technique of PAC to study the interaction of In-HOQ with the lipid bilayer of unilamellar liposomes and the entrapped chelating agent, nitrilotriacetic acid, whose ability to bind In^{3+} is analogous to the possible binding of In^{3+} to the cytoplasmic components of a cell.

MATERIALS AND METHODS

The following reagents were procured commercially and used as supplied: L-α-dipalmitoyl phosphatidylcholine (DPPC), soybean phospholipids, cholesterol, 8-hydroxyquinoline, and nitrilotriacetic acid. Carrier-free $^{111}InCl_3$ was purified by chromatography on an AG 1-X8 column, as described previously (*14*). Fresh defibrinated rabbit serum was also prepared as in this reference. Human erythrocytes were obtained from the blood of a normal donor, using 3.8% sodium citrate as an anticoagulant. The erythrocytes were washed with 0.9% NaCl, 5 mM sodium phosphate, pH 7.4 (PBS) and centrifuged at 1000 g for 10 min four times to remove the buffy coat serum protein.

Preparation of ($^{111}In^{3+}$)-8-hydroxyquinoline. Indium-111 was complexed to 8-hydroxyquinoline by a slight modification of the method of Thakur et al. (*6*). Briefly, 16–17 μl of 4 *M* sodium acetate (pH 5.5) were added to 200 μl of 0.002 *N* HCl containing about 300 μCi of $^{111}InCl_3$. Fifty μl of 8-hydroxyquinoline solution in ethanol (1 mg/ml ethanol) was added to the buffered indium solution, mixed thoroughly, and incubated at room temperature for 15 min. The resulting complex was extracted twice with 300 μl chloroform. The combined chloroform was back-extracted once with 1 ml of deionized sterile water. The chloroform was either heated in an oil bath (90°C) to dryness or mixed with appropriate lipids in chloroform for further preparation of liposomes. The dried ($^{111}In^{3+}$)-8-hydroxyquinoline complex was either dissolved in 50 μl of ethanol or suspended in an appropriate aqueous solution.

Preparation of liposomes. Unilamellar liposomes were prepared from DPPC, or soybean phospholipids, or DPPC-cholesterol (2:1, *M/M*), each dissolved in chloroform and dried to a thin film at 42°C under a gentle stream of nitrogen. Dried DPPC (27–54 μmole), or soybean phospholipids (100–200 mg), or DPPC-cholesterol (27 μmole DPPC + 13.5 μmole cholesterol) swelled briefly in 1 ml of PBS in the presence or absence of 1 m*M* nitrilotriacetic acid (NTA), or 10 m*M* sodium citrate (pH 5.2), or 1–2 mCi of $^{111}In^{3+}$, or a combination of them. The mixture was placed into a MPG 100 sonicator* with a titanium microtip, and sonicated for 15 min at high power in a glycerol bath. The liposome suspension was then centrifuged for 15 min at 300 g and 25°C to remove any titanium fragments and highly aggregated material. Untrapped materials were removed by passage of the liposomes over a Sephadex G-50 column, 0.8 × 35 cm, in PBS. For samples containing $^{111}In^{3+}$, the liposome suspension was passed through two Chelex-100 columns, 0.5 × 7 cm, before the gel chromatography on Sephadex G-50.

To prepare DPPC-($^{111}In^{3+}$)-8-hydroxyquinoline (DPPC-In-HOQ) liposomes, or (soybean) phospholipid-In-HOQ liposomes, DPPC (27 μmole), or soybean phospholipids (200 mg), in chloroform were mixed with about 200 μCi of In-HOQ complex (35–50 μg 8-hydroxyquinoline) in chloroform and dried under nitrogen, as described above. A volume of 1 ml PBS was then added to the dried mixture, and the whole sonicated, as above. The resulting liposomes looked exactly like those made without inclusion of the metal complex.

Binding studies. To study the binding of In-HOQ to liposomes, about 15 μCi of the complex in ethanol solution was mixed with an equal volume of twofold concentrated PBS, and then added to an appropriate volume of DPPC liposomes or soybean-phospholipid liposomes. The time-integrated perturbation factor, $<G_{22}(\infty)>$ was measured at room temperature in a perturbed angular-correlation spectrometer as described by Goodwin et al. (*13*). The size of the radioactive source was corrected to the level of a 0.20-ml volume in a 10- × 75-mm glass tube for all data, using an experimental curve established by measuring the $<G_{22}(\infty)>$ values of a 15-μCi sample of ($^{111}In^{3+}$)-NTA solution in various sample sizes and volumes.

To study the binding of liposomes to In-HOQ that was not predissolved in ethanol, 1 ml of clear DPPC liposome suspension in PBS was added to the dried complex and mixed vigorously by a vortex

mixer. Cloudiness usually appeared after mixing. Aliquots of the turbid suspension were taken out and the $<G_{22}(\infty)>$ values measured at various times after mixing.

To study the kinetics of the exchange of In^{3+} ions from In-HOQ complex in lipid bilayer to ethylenediaminetetracecetic acid (EDTA), EDTA was added to a final concentration of 1.0 m*M* to either DPPC-In-HOQ liposomes or DPPC liposomes that had previously been incubated with In-HOQ complex dissolved in 50% ethanol-PBS solution. The $<G_{22}(\infty)>$ values were monitored at various times after the addition of EDTA.

To investigate the exchange of In-HOQ complex between two different types of lipid bilayer, a fresh purified suspension of DPPC-cholesterol liposome entrapping 1 m*M* NTA was added to DPPC-In-HOQ liposomes, and the change in $<G_{22}(\infty)>$ of the suspension was measured at various times after the addition of DPPC-cholesterol liposomes. The effect of temperature on the exchange of In-HOQ was studied by incubating the mixture at various temperatures before the measurement of $<G_{22}(\infty)>$.

To determine the accessibility of $^{111}In^{3+}$ ions to the interior of liposomes, In-HOQ complex dissolved in 50% ethanol-PBS solution was added to DPPC-cholesterol liposomes that had encapsulated 1 m*M* NTA, and the $<G_{22}(\infty)>$ values of the liposome suspension were determined at various times after the mixing. The accessibility of serum proteins to $^{111}In^{3+}$ ions that were either encapsulated in liposomes as ($^{111}In^{3+}$)-NTA complex or incorporated into liposomes via 8-hydroxyquinoline was studied by measuring the change in $<G_{22}(\infty)>$ with time after the addition of fresh rabbit defibrinated serum, or washed human erythrocytes, or Triton X-100, or chloroform, to liposome suspensions.

RESULTS

Diffusion of ($^{111}In^{3+}$)-8-hydroxyquinoline through lipid bilayer. Values of the time-integrated perturbation factor for $^{111}In^{3+}$ in various environments are listed in Table 1. It has been pointed out that the values of $<G_{22}(\infty)>$ can provide a convenient way to estimate the rotational correlation time of $^{111}In^{3+}$ bound to various molecules (*11,15*). Thus $^{111}In^{3+}$ bound to EDTA has the fastest tumbling rate (Table 1), whereas $^{111}In^{3+}$ bound to serum proteins (presumably transferrin) has the slowest tumbling rate among them. We have previously shown that the exchange rate of $^{111}In^{3+}$ ions between the metal-binding molecules (e.g., NTA, lipid head groups, and serum proteins) and water is slow enough so that the percentage of $^{111}In^{3+}$ bound to the components of a binary system can be estimated by the following expression (*14*):

$$<G_{22}(\infty)> = X_1 <G_{22}(\infty)>_1 + X_2 <G_{22}(\infty)>_2, \quad (1)$$

where $X_1 + X_2 = 1$, and where X_i is the mole fraction of $^{111}In^{3+}$ bound to component *i*, which has a characteristic $<G_{22}(\infty)>_i$, and $<G_{22}(\infty)>$ is the observed measurement of the binary system. Using Eq. 1, with $<G_{22}(\infty)> = 0.68$ for $^{111}In^{3+}$ bound to NTA, and $<G_{22}(\infty)> = 0.31$ for all $^{111}In^{3+}$ bound to the inner lipid head group of liposomes, the percentages of $^{111}In^{3+}$ bound to NTA are: 89% for DPPC-cholesterol liposomes entrapping ($^{111}In^{3+}$)-NTA, and 51% for DPPC liposomes entrapping ($^{111}In^{3+}$)-NTA.

As shown in Table 1, the addition of $^{111}InCl_3$ to DPPC liposomes, or to DPPC-cholesterol liposomes entrapping 1 m*M* NTA, consistently resulted in a low $<G_{22}(\infty)>$ value of 0.31. This suggests that almost all the $^{111}In^{3+}$ ions become bound to outer surfaces of the liposomes, which have a rather slow rotational correlation time. Similarly, the addition of In-HOQ in 50% ethanol to DPPC liposomes resulted in a low $<G_{22}(\infty)>$ value similar to the $<G_{22}(\infty)>$ values for DPPC-In-HOQ liposomes, or soybean phospholipid-In-HOQ liposomes, in which the complex of In-HOQ was presumably distributed evenly in the lipid bilayer. In contrast, the addition

TABLE 1. VALUES OF THE TIME-INTEGRATED PERTURBATION FACTORS OF INDIUM-111 IN VARIOUS ENVIRONMENTS

Sample	$<G_{22}(\infty)>$
1. ($^{111}In^{3+}$)-EDTA complex in saline solution	0.75 ± 0.02
2. ($^{111}In^{3+}$)-citrate complex in saline solution	0.71 ± 0.02
3. ($^{111}In^{3+}$)-NTA complex in saline solution	0.68 ± 0.02
4. ($^{111}In^{3+}$)-8-hydroxyquinoline complex in chloroform	0.68 ± 0.02
5. DPPC-cholesterol liposomes entrapping ($^{111}In^{3+}$)-NTA complex	0.64 ± 0.02
6. ($^{111}In^{3+}$)-8-hydroxyquinoline in 50% ethanol	0.56 ± 0.02
7. Suspension of ($^{111}In^{3+}$)-8-hydroxyquinoline powder in PBS containing 11 m*M* DPPC liposome (in the absence of ethanol)	0.50 ± 0.02
8. DPPC liposomes entrapping ($^{111}In^{3+}$)-NTA complex	0.50 ± 0.02
9. DPPC ($^{111}In^{3+}$)-8-hydroxyquinoline liposomes or soybean phospholipid ($^{111}In^{3+}$)-8-hydroxyquinoline liposomes	0.33 ± 0.02
10. $^{111}In^{3+}$-8-hydroxyquinoline in 50% ethanol PBS solution plus DPPC liposomes	0.33 ± 0.02
11. DPPC liposomes entrapping ($^{111}In^{3+}$)-citrate complex	0.31 ± 0.02
12. $^{111}InCl_3$ plus DPPC-cholesterol liposomes entrapping 1 m*M* NTA	0.31 ± 0.02
13. $^{111}InCl_3$, or ($^{111}In^{3+}$)-NTA, or ($^{111}In^{3+}$)-8-hydroxyquinoline plus serum	0.18 ± 0.02

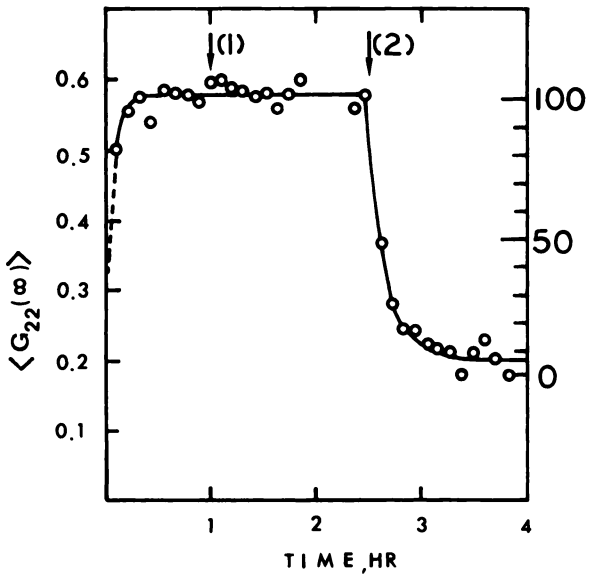

FIG. 1. Loading of indium into liposomes. Loading started at time zero, when In-HOQ (15 μCi) in 50% ethanol-PBS (pH 7.4) was added ot a suspension of DPPC-cholesterol unilamellar liposomes encapsulating 1 mM NTA in (pH 7.4). Addition of rabbit defibrinated serum or washed erythrocytes to liposome suspension (first arrow at t = 1 hr) made no change in $\langle G_{22}(\infty)\rangle$ or tumbling rate of indium ions. Disruption of vesicles by Triton X-100/$CHCl_3$ (second arrow at t = 2.5 hr), and binding of indium ion inside the liposomes to serum transferrin, are indicated by the rapid drop of $\langle G_{22}(\infty)\rangle$ or tumbling rate of indium. Percentage of indium inaccessible to serum proteins as calculated from Eq. (1) is plotted at right.

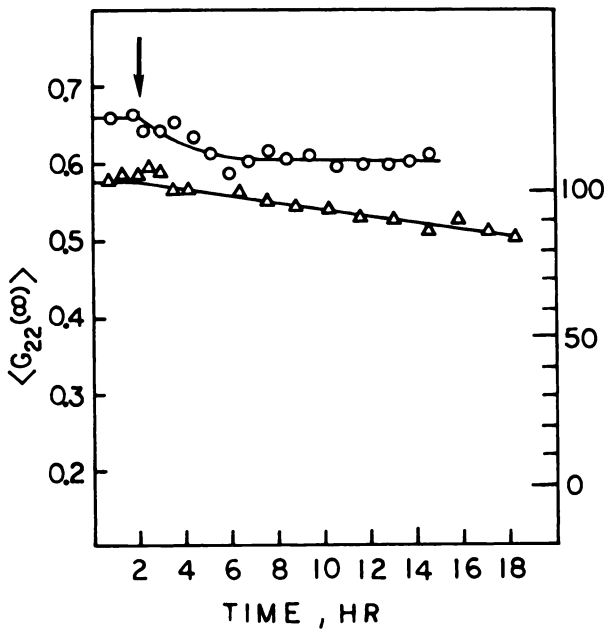

FIG. 2. Release of indium-111 from liposomes. Control DPPC-cholesterol liposomes (o-o) encapsulating ($^{111}In^{3+}$) NTA-, or DPPC-cholesterol liposomes (△-△) that had been loaded with indium by In-HOQ (see legend of Fig. 1) 24 hr before mixing with equal volumes of fresh rabbit defibrinated serum (arrow), were incubated at room temperature, and the $\langle G_{22}(\infty)\rangle$ values of these samples were monitored with time. Percentage of release of indium from DPPC-cholesterol liposomes (△-△) previously loaded with indium by In-HOQ was calculated from Eq. (1) and plotted at the right.

of In-HOQ in 50% ethanol to DPPC-cholesterol liposomes that had encapsulated 1 mM NTA resulted in a relatively high $\langle G_{22}(\infty)\rangle$ value of 0.56 (Table 1, Fig. 1). This implies that $^{111}In^{3+}$ ions are bond to molecules that tumble relatively fast. The high partition coefficient of 8-hydroxyquinoline to hydrophobic solvents—and the fact that the added indium was not accessible to serum proteins (Fig. 1)—strongly suggested that some of the In-HOQ diffused through the lipid bilayer, carrying $^{111}In^{3+}$ ions that then became bound to the fast-tumbling NTA molecules entrapped in liposomes. If one assumes that liposome-bound In-111—either as $^{111}In^{3+}$ ions bound to the inner lipid head groups or as In-HOQ complexes embedded in the lipid bilayer—has a $\langle G_{22}(\infty)\rangle$ of 0.31, an estimate can be made from Eq. 1 that about 68% of the In-111 in the form of ($^{111}In^{3+}$)-NTA complex was encapsulated in DPPC-cholesterol liposomes.

It appears that In-HOQ can diffuse through the lipid bilayer and release $^{111}In^{3+}$ to NTA in a relatively short time (Fig. 1). In contrast, the release of the captured $^{111}In^{3+}$ from liposomes to serum proteins was negligible, as indicated in Fig. 1. This is probably because most of the indium ions are locked in the interior of liposomes by NTA, so that the formation of a proper complex of In-HOQ which might diffuse through the hydrophobic region of lipid bilayer, is hindered or slowed down. In order to test this hypothesis, a long period of incubation of liposomes in serum was carried out (Fig. 2). There was a linear drop of $\langle G_{22}(\infty)\rangle$ with time. The pattern of release was quite different from that of the control DPPC-cholesterol liposomes encapsulating ($^{111}In^{3+}$)-NTA. The control liposomes have previously been shown to release indium at a rate of about 10% for 40 hr in serum. The rate of release of $^{111}In^{3+}$ from DPPC-cholesterol liposomes that had encapsulated 1 m*M* NTA and were subsequently loaded with indium by In-HOQ ethanol was about 4 times as fast as that of the control liposomes.

In addition to the perturbation of serum proteins to liposomes, the release of indium from the above liposomes could be due to the presence of ethanol or 8-hydroxyquinoline. If ethanol could render the liposomes more permeable to the indium, one would expect a release of indium even in the absence of serum. Thus the amount of indium released from liposomes would have been a function of incubation time. The fact that a negligible amount of indium was released from liposomes freshly loaded with indium (Fig. 1)—and from liposomes (Fig. 2) that had been loaded with indium by In-HOQ in 50% ethanol 1 day before the addition of serum—seems to rule out the effect of the presence of a trace

amount of ethanol on the permeability of liposomes. Therefore, the carrier activity of 8-hydroxyquinoline is probably responsible for the release of In-111.

Proximity of ($^{111}In^{3+}$)-8-hydroxyquinoline to lipid bilayer. The incorporation of In-HOQ to lipid bilayer by mixing it with appropriate lipids in organic solvent, before the preparation of liposomes or adding the 50% ethanol solution of the complex to the liposomes, all gave a low $<G_{22}(\infty)>$ value of 0.33 (Table 1). This indicated that most of In-111 ions tumble at a rate characteristic of the liposomes. The exact location of In-HOQ in juxtaposition with other lipid molecules in the bilayer of the liposomes in both cases cannot be determined by these measurements. The strategy adopted for studying this problem was to investigate the accessibility of In-111 embedded in the lipid bilayer as In-HOQ complex, using EDTA as a probe. The strong binding constant of $^{111}In^{3+}$ to EDTA ($K \simeq 10^{25}$) (*16*), the slow exchange rate of $^{111}In^{3+}$ chelated by EDTA (*17*), and the high $<G_{22}()>$ value of ($^{111}In^{3+}$)-EDTA (Table 1), make EDTA a sensitive probe to study the accessibility of indium.

A weak binding constant of $^{111}In^{3+}$ to 8-hydroxyquinoline ($K \simeq 10^{11}$) (*8*) compared with that of $^{111}In^{3+}$ to EDTA, and the ready release of $^{111}In^{3+}$ from 8-hydroxyquinoline to serum proteins (Table 1), imply that if the indium embedded in the lipid bilayer were easily accessible, the addition of EDTA to liposomes that contain In-HOQ in the lipid bilayer would result in an immediate formation of the ($^{111}In^{3+}$)-EDTA complex. The result of the study (Fig. 3) shows that this is indeed the case. However, it appeared that some of the indium was not readily available for binding to EDTA. In the case of the incorporation of indium into lipid bilayer by adding In-HOQ in 50% ethanol solution to DPPC liposomes, about 80% of the indium ions were readily accessible to EDTA. The remaining 20% of the ions could be embedded completely in the lipid bilayer or on the inner surface of liposomes. In the case of DPPC-In-HOQ liposomes, only about 50% of the indium ions were readily accessible to EDTA. The rest of 50% seemed to contain two different populations in terms of their exchange time.

Exchange of ($^{111}In^{+3}$)-8-hydroxyquinoline between liposomes. An alternative approach to study the mode of interaction of In-HOQ with membrane bilayer is to investigate the exchange of In-HOQ between liposomes. The system adopted for such study involved the determination of the amount In-HOQ transferred from DPPC-In-HOQ liposomes to DPPC-cholesterol liposomes. In order to differentiate the In-HOQ complexes in the donor liposomes from those in the acceptor liposomes, the impermeant chelating agent, NTA, was encapsulated in the DPPC-cholesterol liposomes, such that the In-HOQ exchanged to the acceptor liposomes can be locked by the entrapped NTA molecules and determined spectroscopically by Eq. 1.

Immediately after the addition of DPPC-cholesterol, which had encapsulated 1 m*M* NTA to the DPPC-In-HOQ liposomes, the $<G_{22}(\infty)>$ increased steadily with time and leveled off at 0.55 after 2 hr (Fig. 4). When EDTA was added to the liposome suspension there was an initial sharp increase in $<G_{22}(\infty)>$, followed by a slower increase to 0.75. This implies that most of the In-111 ions become bound to the EDTA molecules (Table 1). The percentage of radioactivity associated with liposomes and EDTA was assessed by Sephadex G-50 chromatography at the end of the PAC measurement. All the radioactivity was found in the EDTA fraction (data not shown).

When the exchange experiment was performed at 47°C instead of at room temperature, the resulting $<G_{22}(\infty)>$ was 0.52. The addition of rabbit's defibrinated serum to the liposomes, however, resulted in a rapid drop of $<G_{22}(\infty)>$ to 0.43, after which there followed a similar slow release of $^{111}In^{3+}$ to serum proteins as in the case illustrated in Fig. 2. This indicates that some of In-HOQ can exchange

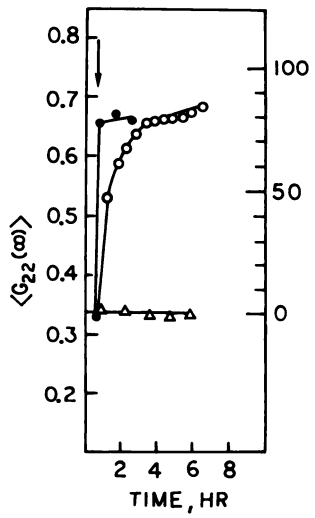

FIG. 3. Accessibility of indium embedded in lipid bilayer. Indium-111 was incorporated into the lipid bilayer by adding 30 μl of In-HOQ (15 μg of 8-hydroxyquinoline, 15 μCi In-111) in 50% ethanol solution of PBS to a solution of 2 ml of DPPC liposomes (15 μmole DPPC) in PBS, and was incubated at room temperature for 1 hr before addition of EDTA (indicated by arrow) to a final concentration of 1 mM (-●-●-). Addition of 1 mM EDTA to 0.2 ml DPPC-In-HOQ liposomes (6 μmole of DPPC, 12 μg of 8-hydroxyquinoline, and 15 μCi In-111) in PBS is also indicated by the arrow (-o-o-). Controls for both cases contained no EDTA (-△-△-). Scale at right is expressed in terms of percentage of ($^{111}In^{3+}$)-EDTA in the solution. To calculate the percentage, we assume that $<G_{22}(\infty)> = 0.75$ for ($^{111}In^{3+}$)-EDTA and 0.33 for liposome-bound In-HOQ.

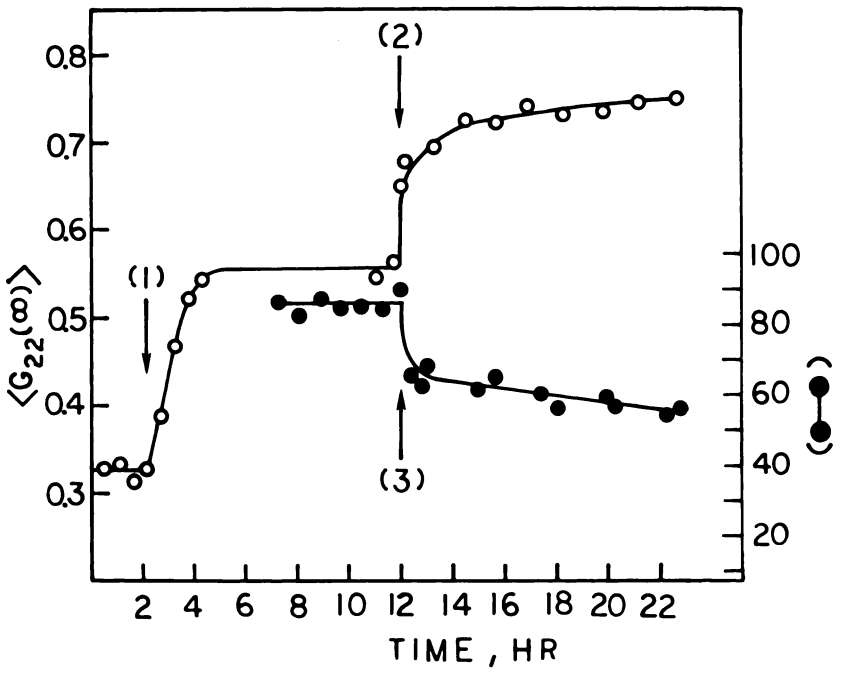

FIG. 4. Exchange of In-HOQ between liposomes (-o-o-). At arrow 1, 0.2 ml fresh DPPC-cholesterol liposomes (3 μmole DPPC and 1.5 μmole cholesterol), which encapsulate 1 mM NTA in PBS, was added to 0.1 ml DPPC-In-HOQ liposomes (3 μmole DPPC, 5 μg 8-hydroxyquinoline, and 15 μCi indium-111) in PBS. At arrow 2, EDTA to a final concentration of 0.7 mM was added to the liposome suspension. Liposomes incubated at 47°C overnight before PAC measurement at room temperature (-●-●-). At arrow 3, 0.5 ml fresh rabbit defibrinated serum was added to the liposome suspension. Scale at right represents percentage of indium-111 inaccessible to serum proteins. To calculate the scale, we assume values of $\langle G_{22}(\infty)\rangle = 0.56$ for indium-111 fully protected by liposomes, and $\langle G_{22}(\infty)\rangle = 0.18$ for indium bound to serum proteins.

from one type of liposome to a different type. Moreover, some of the complexes retained a configuration during the exchange process such that they could diffuse through the lipid bilayer to interact with the entrapped NTA molecules. The rapid drop of $\langle G_{22}(\infty)\rangle$ immediately after the addition of serum may mean that some of the In-HOQ distributed in aqueous phase, and/or that the indium on the outer surface of the liposomes became bound to serum proteins. The exact percentage of In-HOQ that was actually transferred to the acceptor liposomes cannot be determined by the present measurements. We estimated, however, that about two thirds of the In-111 in the donor liposomes was transferred to the acceptor liposomes, assuming that $\langle G_{22}(\infty)\rangle$ is 0.56 for indium inacessible to serum proteins, and that $\langle G_{22}(\infty)\rangle$ is 0.18 for serum-bound indium.

DISCUSSION

The ability of 8-hydroxyquinoline to carry metal ions across cell membranes allows it to sequester metal ions in the cytoplasm of cells as a bactericidal agent and to deliver radioactive metal ions into cells as a radiopharmaceutical. The present study shows that the interaction of the metal complex of the carrier in the membrane with the molecules distributed in both sides of the bilayer membrane plays an important role in the regulation of the net flow of the metal ions. Thus, liposomes encapsulating NTA molecules can efficiently keep the metal ions from diffusing out of liposomes even in the presence of plasma proteins (Fig. 1). However, if a stronger chelating reagent, such as EDTA, is present on the outside of the liposomes, 8-hydroxyquinoline will mediate the transfer of $^{111}In^{3+}$ from the weaker chelating agent, NTA, to the stronger chelating agent, EDTA (Fig. 4). Although serum transferrin can bind $^{111}In^{3+}$ very tightly ($K \approx 10^{31}$), the effect of the transfer of $^{111}In^{3+}$ to serum from NTA is not as efficient as to EDTA (Fig. 2, 4). The behavior is probably due to steric hindrance of the binding sites of the transferrin. In order for indium to get an access to the binding sites of transferrin, the indium has to diffuse from the bilayer to the binding sites of the transferrin. On the other hand, the EDTA molecule can interect directly with the indium embedded in the bilayer.

As is indicated in Fig. 1, the time required for In-HOQ in 50% ethanol to obtain access to the encapsulated NTA molecules was less than 30 minutes. This suggests that In-HOQ diffuses rather fast through the lipid bilayer. In contrast, it took several hours for a stronger chelating reagent, EDTA, to remove all the indium from the lipid bilayer (Fig. 3). Both NTA and EDTA are small-molecule chelating agents. A steric effect would not be the major reason for the large difference in the rate of indium binding. It may be that the rate constant for the formation of a proper diffusible configuration of In-HOQ in the lipid bilayer is the rate-limiting factor in the slow binding of indium to EDTA.

In order for indium to be able to diffuse through the hydrophobic region of a lipid bilayer, the charge on the indium ion has to be camouflaged by appropriate numbers of 8-hydroxyquinoline molecules.

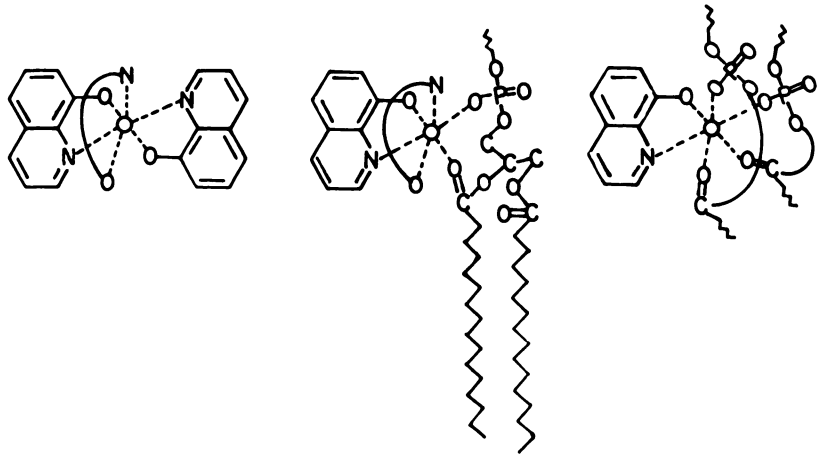

FIG. 5. Possible chemical structure of complex of $^{111}In^{3+}$ to 8-hydroxyquinoline in lipid bilayer. Indium ion could be coordinated by three 8-hydroxyquinoline molecules (left), or two 8-hydroxyquinoline and one phospholipid molecule (center), or one 8-hydroxyquinoline and two phospholipid molecules (right). Long axes of molecules of 8-hydroxyquinoline are perpendicular to plane of lipid bilayer.

The rate of the formation of such a diffusible complex is governed by a) the stability constants for the formation of complexes with various ratios of indium to 8-hydroxyquinoline (*9*), and b) the binding constants of indium to the head groups of the lipids and other chelating agents in the aqueous phase. Thus, In-HOQ in chloroform or in ethanol could be primarily in the lipid-diffusible form. On the other hand, when In-HOQ is embedded in the lipid bilayer, the interference of the polar head groups of the lipids or the encapsulated NTA molecules could shift the dynamic equilibrium between the diffusible and nondiffusible metal complexes. Therefore, some of the indium ions could be locked in a nondiffusible form to the inner sides of the liposomes and might not diffuse out readily, consistent with Fig. 3. The fact that only about one half of the indium ions in DPPC-In-HOQ liposomes were readily available for EDTA might imply that In-HOQ complexes embedded in lipid bilayer are predominantly in a nondiffusible form, locked into regions close to the lipid head groups on both sides of lipid bilayer (by the polar phosphate groups of the lipids). Indium complexes locked to the head groups on the inner side of liposomes could be responsible for the slow phase of indium binding by EDTA, as shown in Fig. 3.

The crystal structure of zinc 8-hydroxyquinoline dihydrate has been determined by the single-crystal x-ray diffractions (*18*). The molecule of 8-hydroxyquinoline is planar, and the metal ion is surrounded octahedrally by two 8-hydroxyquinoline molecules and two water molecules. From the observed bond distances, angles, and an average of 1.1 Å for all C–H bonds (*19*), we estimate that the 8-hydroxyquinoline molecule has a dimension of about 3.0 Å $\times$ 6.3 Å $\times$ 7.4 Å.

In order for 8-hydroxyquinoline to pack in the lipid bilayer and be capable of chelating a metal ion cooperatively with other 8-hydroxyquinoline molecules or the polar head groups of DPPC, the long axis of the 8-hydroxyquinoline is believed to be perpendicular to the surface of the bilayer (Fig. 5). Thus, the 8-hydroxyquinoline molecule would occupy a rectangular area of 3 Å $\times$ 6.3 Å in the plane of a DPPC bilayer. If one assumes that the DPPC molecule is a hydrocarbon cylinder, 4.8 Å in diameter (*20*), and that no indium-oxygen or indium-nitrogen distance is shorter than 2.18 Å (*21,22*), a molecular model of the packing of 8-hydroxyquinoline in the plane of DPPC can be constructed (Fig. 6). In constructing the model, a circle 1.4 Å

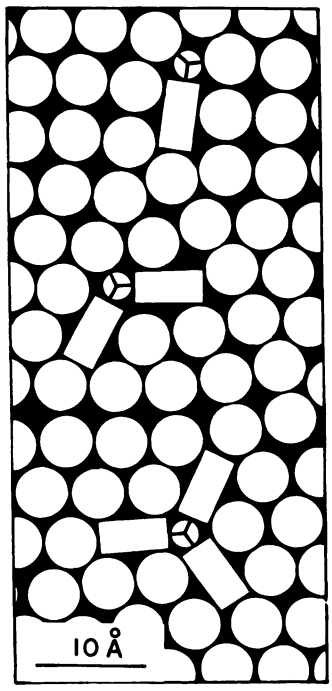

FIG. 6. Part of packing model of In-HOQ complexes in a DPPC liposome viewed perpendicular to plane of lipid bilayer. Small circles represent indium ions chelated by various numbers of 8-hydroxyquinoline (rectangular shapes) and dipalmityl phosphatylcholine (large circles) molecules.

in diameter was used as the minimal contact distance for indium ion. This was calculated from the 2.18 Å In–O (or In–N) distance and the geometry of the Zn-8-hydroxyquinoline complex (*18*). As is suggested in Figs. 5 and 6, the indium ion may be surrounded octahedrally by six nucleophilic atoms donated from three 8-hydroxyquinolines, or from two 8-hydroxyquinolines and one phospholipid molecule, or even from one 8-hydroxyquinoline and two phospholipid molecules. These three different forms of metal complex may be in dynamic equilibrium. The complex of indium:(8-hydroxyquinoline)$_3$ may diffuse through the hydrophobic region of the membrane, or diffuse to the lipid bilayer of a different liposome, upon a transient contact of two liposomes. The other two forms of the complex can diffuse through the hydrophobic region of the bilayer only when the proper diffusible configuration is formed. In DPPC-cholesterol liposomes, a similar packing of 8-hydroxyquinoline in the model proposed by Engleman and Rothman (*20*) can also be constructed. In a more complicated situation where a chelating reagent such as NTA is present in the aqueous phase, the chelating agent may replace one or two of the phosphate head groups of the DPPC and result in an exchange of indium between the aqueous and lipid phases.

In the actual situation of labeling cells, membrane proteins may participate in the binding of indium in a manner like that of the lipid molecules on the surface of a cell. Cytoplasmic proteins could conceivably play a metal-chelating role in stabilizing the indium ions in the cytoplasm. Furthermore, the excess of endogeneous di- or trivalent ions would very well compete with the indium ions inside the labeled cells to form 8-dydroxyquinoline-metal complexes. This would in turn reduce the probability of the release of indium from the labeled cells. Moreover, as in the case of liposomes, the exchange of 8-hydroxyquinoline-metal complexes between cells may also occur. By such an exchange process, the concentration of 8-hydroxyquinoline per labeled cell would be reduced considerably once the labeled cells are transfused back to the recipient. This will further decrease the probability of the release of indium from cells by the ionophoric 8-hydroxyquinoline.

FOOTNOTE

* MSE Model 150 W.

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