Chemistry of Natural Compounds, Vol. 30, No. 1, 1994

INFLUENCE OF THIONIN ON THE STRUCTURE OF A BILAYER AND ON THE INTERMEMBRANE EXCHANGE OF LIPID MATERIAL

S. O. Kolusheva, B. A. Salakhutdinov, T. F. Aripov, and L. P. Vernon

UDC 615.918:577.335:577.336

It has been shown that in lipid dispersions thionin causes the formation of heterogeneous structures with the production of lipid-enriched "clusters" melting at elevated temperatures. This effect of thionin depends on the type of acidic lipid. It has also been established that thionin causes the aggregation and intermembrane exchange of lipid material between two populations of liposomes possessing individual thermodynamic characteristics.

It has been shown previously [1] that the highly basic plant polypeptide thionin isolated from *Pyrularia pubera* Michx. (Santalacea) and possessing a broad spectrum of biological action [2, 3] has a high affinity for membranes bearing a negative surface charge. A negative charge in the lipid matrix is due to the presence of molecules of acidic lipids such as phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid (PA), and cardiolipin (CL). In the presence of these phospholipids, thionin molecules may perturb and change the initial lamellar structure of the membrane with the formation of nonbilayer structures.

The spatial organization of the nonbilayer structures induced by thionin molecules in multilamellar dispersions must depend on the type of acidic lipid. In the individual form, under various physicochemical actions they tend to produce micellar, hexagonal, cubic, etc. formations [4].

In a mixture with the main structure-forming phospholipid, phosphatidylcholine (PC), the probability of the formation of nonbilayer structures depends on the distribution of their acidic lipids in the lamellar phase. The positively charged thionin molecules may be a factor causing the phase segregation of the acidic lipids in the lamellar plane of the lipid matrix. When clusters enriched with acid lipids having the molecular form of a reversed or direct "wedge" are present in the lipid matrix, the tendency to the formation of nonbilayer structures is intensified [4].

The positively charged thionin molecules may cause aggregation of liposomes containing acidic lipids. The appearance of various nonbilayer formations in the region of intermembrane contact may lead to intensive lipid exchange. Aggregation and intermembrane lipid exchange form one of the possible conditions for the process of lipid coalescence.

We have used the method of differential scanning calorimetry (DSC) to investigate the influence of thionin on the thermodynamic parameters of lipid systems formed from mixtures of dimyristoylphosphatidylcholine (DMPC) with PI, PC, and CL, and also on membrane lipid exchange between two populations of liposomes.

Figure 1*a*, shows thermograms of the melting of multilamellar dispersions of DMPC + 2 mole% of CL in the presence of increasing concentrations of thionin. While the initial thermogram of a control sample is represented by a single slightly asymmetic peak with T_m = 23.6°C and the half-width $\Delta T_{1/2}$ = 2°C, a rise in the concentration of thionin in the sample led to a gradual transformation of the shape of the melting curve. At a $C_{thionin}/C_{lipid}$ ratio greater than 1/250 a peak corresponding to lipids with higher melting points was recorded on the high-temperature arm. The peak of the main phase transition shifted into the low-temperature region, and at a concentration ratio of 1/15 this shift amounted to 0.4°C. The appearance of asymmetric high-temperature arm in formations of multilamellar dispersions with 2% of CL is considered as an indication of a lipid phase with T_m = 25°C.

A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, Tashkent, Fax 627071. Brigham Young University, Provo, Utah, USA. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 78-82, January-February, 1994. Original article submitted July 13, 1993.

 0009-3130/94/3001-0068$12.50

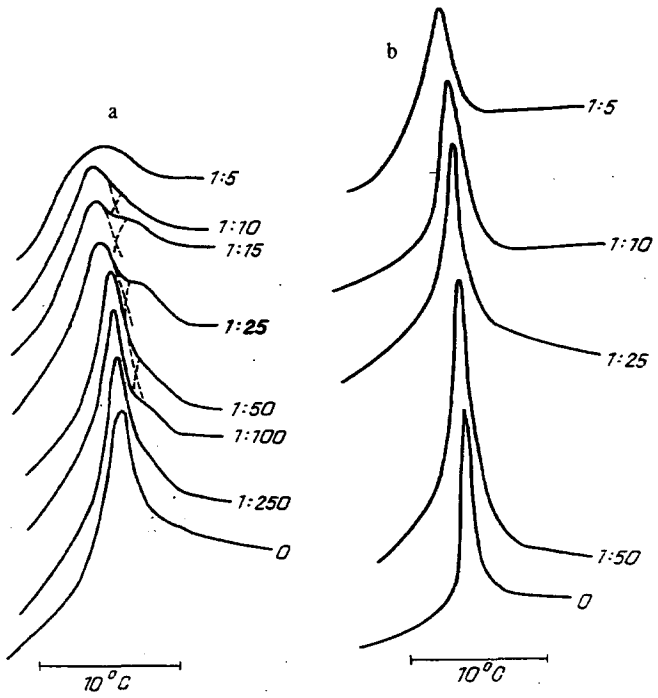

Fig. 1. Thermogram of the melting of a multilamellar dispersion of DMPC + 2 mole% of CL (*a*) and of DMPC + 2 mole% of PS (*b*) in the presence of thionin. The $C_{thionin}/C_{lipid}$ ratios are shown.

The addition of thionin to a mixture of DMPC + 2 mole% of PS led to a broadening of the initial single endothermic peak of the main phase transition and to a shift of T_m into the low-temperature region; thus, for example, at a ratio $C_{thionin}/C_{lipid}$ = 1/10 this shift amounted to 0.5°C (Fig. 1, *b*). The peak in the high temperature region of the thermogram of this sample was not recorded over the whole range of concentrations.

A characteristic change in the thermograms of the multilamellar dispersions of DMPC + 2 mole % of PI on the addition of increasing concentrations of thionin was a shift in the peak of the main phase transition into the low-temperature region with a symbatic increase in the half-width of the peak of the transition. At concentrations greater than 1/10 a small high-temperature shoulder appeared for which lipids with a different packing structure were responsible. The appearance of an asymmetric high-temperature shoulder in samples of multilamellar dispersions with 2 mol. % of PI is considered as an indication of a lipid phase with T_m = 27°C.

In view of the fact that, because of the presence of a large number of unsaturated bonds, the molecules of natural acidic lipids do not participate in a cooperative phase transition, a decrease in the relative value of the the enthalpy ($\Delta H/\Delta H_0$) of the total phase transition will indicate that some of the DMPC molecules are excluded from the process of cooperative melting. In actual fact, an appreciable decrease in $\Delta H/\Delta H_0$ with a rise in the concentration of thionin was observed in dispersions containing CL and CI molecules (Fig. 2), while for samples with PC the value of $\Delta H/\Delta H_0$ scarcely changed over the whole range of concentrations investigated. It follows from this that a change in the temperature of the main phase transition T_m of samples of DMPC + 2 mol. % PS to a value of T_m characteristic for pure DMPCis connected with the phase segregation of the PS under the action of thionin. If the random distribution of PS molecules in the lamellar plane did not change on the addition of thionin, a decrease in the value of T_m should have been observed.

In the case of other samples containing CL and PI, a change in temperatures is also connected with a partial phase segregation of the acidic lipids. However, as experiments showed, this process is accompanied by the formation of a lipid phase with other thermodynamic parameters, different from the parameters of the initial sample.

An analysis of the facts presented permits the conclusion that the nature of the interaction of thionin molecules with lipids in samples containing CL and PI differs in type from samples with PS. The DMPC molecules, most probably directly surrounding molecules of the acidic lipid, are also involved in interaction with the thionin molecules in these systems. The melting of this cluster of lipids requires a high temperature, which is observed in the form of a high-temperature shoulder on

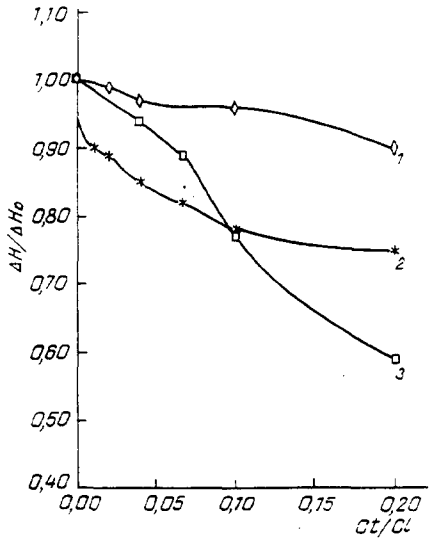

Fig. 2. Graph of the dependence of the change in the relative total enthalpy ($\Delta H/\Delta H_0$) of melting on the concentration of thionin in multilamellar dispersion with various composition: 1) DMPC + 2 mol.% of PS; 2) DMPC + 2 mol.% of CL; 3) DMPC + 2 mol.% of PI.

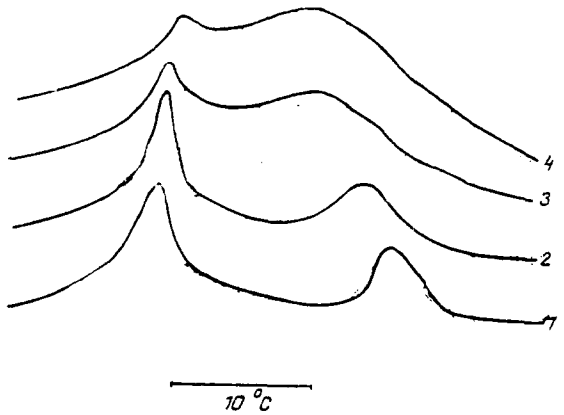

Fig. 3. Thermograms of melting of an equimolar mixture of liposomes with DMPC + 2 mol.% PS and DPPC + 2 mol. % PS in the presence of thionin ($C_{thionin}/C_{lipid}$ = 1/50): 1) control; 2) addition of thionin, first scanning; 3) second scanning; 4) third scanning.

the thermogram. It is most likely that these clusters are uniformly distributed in the plane of the lipid layer and, to a certain extent, perturb the packing of the lipids not subjected to action of thionin. This hypothesis is supported by the fact that the increases in the half widths of the main phase transitions ($\Delta T_{1/2}$) are comparable for all lipid systems.

The slight decrease in ΔH for the transition by 20% in the case of CL and PI-containing dispersions at relative concentrations of thionin not exceeding 1:10 permits the assumption that the thionin molecules are localized practically completely in the surface region of the lipid matrix. In the case of a deeper penetration of these thionin molecules into the bilayer, there would be a considerably greater fall in the value of ΔH [4]. In the samples of multilamellar dispersions the part of the thionin molecules exposed to the aqueous phase may interact with phospholipid molecules of the neighboring lamellar

layer. Then, if defects are present in the sections of localization of the thionin molecules, an interlamellar transfer of lipid molecules may take place. Unfortunately, in monotypical samples the DSC method cannot reveal these processes.

In order to record processes in which lipid material is transferred between two bilayer systems, we have conducted experiments on two populations of liposomes with the aim of determining the influence of thionin on the intermembrane interaction.

The individual populations of liposomes were formed from DMPC + 2 mol. % PS and DPPC + 2 mol.% PS, which have different phase-transition temperatures.

Figure 3 shows thermograms of the melting of a mechanical mixture of these systems, consisting of two individual peaks $T_m^1 = 24.9°C$ and $T_m^2 = 39.3°C$. On repeated scanning, the positions of the peak maxima scarcely changed, although the shape of the line was slightly distorted. The absence of a melting peak with T_m having an intermediate value between T_m^1 and T_m^2 shows that there is no active passage of lipid molecules between the two populations of liposomes [5]. However, the addition of thionin in a molecular ratio of $C_{thionin}/C_{lipid} = 1/50$ leads to a shift in the value of T_m^2 for the high-temperature population of liposomes into the region of low temperatures, which is accompanied by an increase in the half-widths of the melting peaks. On repeated scanning, practically only one broad peak is observed, with a small component for which molecules of the low-temperature population of liposomes is responsible.

According to [6], such a change in the thermograms corresponds to the existence of an intensive process of intermembrane exchange of lipid material and is one of the main factors in the fusion of biomembranes. Consequently, it may be assumed that thionin may possess fusogenic properties.

Thus, from the results obtained it follows that the degree of influence of thionin on the thermodynamic parameters of lipid dispersions and also the formation of heterogeneous structures depend on the type of acidic lipid. The binding of thionin molecules with membranes containing acidic lipids leads to the formation of heterogeneous structures and the appearance of "clusters" enriched with acidic lipids for the melting of which elevated temperatures are necessary.

The highly basic polypeptide thionin causes the aggregation and intermembrane exchange of lipid material between two populations of liposomes possessing individual dynamic characteristics.

EXPERIMENTAL

The thermodynamic parameters of the phase transitions of hydrated lipids were determined on a DASM-4 differential scanning microcalorimeter at a recording rate of 1°C/min. The values of the enthalpies of the melting peaks were determined from the areas of the peaks, which were found by weighing the corresponding paper cut-outs and comparing them with the weights of cut-outs corresponding to the thermal standards of the instrument. The separation of the melting peaks according to their components was made by a graphical method. The accuracy of the determination, the enthalpy of transition was not worse than 8%. The positions of the maxima of the corresponding peaks of heat absorption were determined with an accuracy of 0.2°C.

Multilamellar dispersions and liposomes were formed from a mixture of dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatylcholine (DPPC) with phosphatidylserine (PS) (DMPC + 2 mol. % PS), phosphatidylinositol (PI) (DMPC + 2 mol.% PI), and cardiolipin (CL) (DMPC + 2 mol.% CL) according to [5], the concentration of lipids in the experiment being $3 \cdot 10^{-4}$ M.

A solution of thionin in buffer with a concentration of 1 mg/ml was used. The buffer solution was prepared with Tris-HCl and EDTA from Sigma. The other reagents used were of the kh.ch ["chemically pure"] and ch.d.a ["pure for analysis"] grades.

REFERENCES

1. S. O. Kolusheva, B. A. Salakhutdinov, T. F. Aripov, and L. P. Vernon, Khim. Prir. Soedin., No. 4, 594 (1993).
2. L. P. Vernon, G. E. Evett, R. D. Zeikus, and W. R. Gray, Arch. Biochem. Biophys., **283**, No. 1, 18 (1985).
3. G. E. Evett, D. M. Donaldson, and L. P. Vernon, Toxicon., **24**, No. 6, 622 (1986).
4. V. G. Ivkov and G. N. Berestovskii, The Dynamic Structure of a Lipid Bilayer [in Russian], Nauka, Moscow (1981).

5. S. É. Gasanov, F. G. Kamaev, and I. A. Rozenshtein, Problems and Prospects of the Development of the Chemistry of Natural and Physiologically Active Substances [in Russian], Fan, Tashkent (1988), p. 360.
6. T. F. Aripov, I. A. Rozenshtein, and B. A. Salakhutdinov, in: All-Union Conference on Nitroxyl Radicals: Abstracts of Lectures [in Russian], Chernogolovka (1982), p. 90.