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ASYMMETRY IN THE RENEWAL OF MOLECULAR CLASSES OF PHOSPHATIDYLCHOLINE IN THE RAT-ERYTHROCYTE MEMBRANE

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Summary

1. Rat-blood phospholipids were labeled in vivo with [^{32}P]phosphate. The erythrocytes were treated with phospholipase A_2 plus sphingomyelinase to discriminate between the labeling patterns of the phospholipids from the inner and outer layer of the membrane.

2. The specific activities of the more unsaturated classes of phosphatidylcholine were higher in the outer layer of the erythrocyte membrane than in the inner layer. The disaturated class, however, had the highest specific activity in the inner layer.

3. After incubating ^{32}P-labeled erythrocytes in unlabeled plasma, the labeling pattern recovered in the molecular classes of plasma phosphatidylcholine was very similar to that of the phosphatidylcholines in the outer layer of the erythrocyte membrane.

4. It is proposed that the exchange of phosphatidylcholines between plasma and the outer layer of the erythrocyte is mainly responsible for the renewal of the unsaturated phosphatidylcholines of the erythrocyte, and that the acylation activity of the erythrocyte is directed towards the formation of disaturated phosphatidylcholines at the inside of the membrane.

Introduction

The renewal of phospholipids in the plasma membrane of mature mammalian erythrocytes has been attributed to the following mechanisms: (a) the

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exchange of phospholipids between plasma lipoproteins and the erythrocyte membrane [1—7], and (b) the acylation of lysophospholipids [8—11], which are either formed in the membrane or derived from the surrounding plasma. The relative contribution of these two mechanisms to the renewal of erythrocyte phosphatidylcholine is not well quantified to date, but literature data [11—16] as well as our own observations [17] do suggest, that these contributions are of the same order of magnitude.

We reported in previous studies [18,19] a metabolic asymmetry in the renewal of erythrocyte phospholipids: the exchange process takes place at the outer surface of the membrane, whereas acylation is mainly found in the inner leaflet of the bilayer membrane.

The major phospholipid component in rat plasma and erythrocytes is phosphatidylcholine [20,21]. An intriguing observation is the striking difference in the molecular composition of phosphatidylcholine from plasma and erythrocytes: rat plasma is virtually devoid of disaturated phosphatidylcholines [22], whereas both the outer and the inner layer of the rat-erythrocyte membrane contain considerable amounts of disaturated phosphatidylcholines [19,22].

In the present study the metabolic sidedness in the renewal of rat-erythrocyte phosphatidylcholine has been further elaborated for the renewal of the various molecular classes of this phospholipid.

Methods

Phospholipids in rat blood were labeled in vivo by injecting the rats (female Wistar rats, weighing 150—180 g) intraperitoneally with 1.5 mCi $^{32}P_i$ (specific activity 150—600 Ci/mol) per animal. Blood was obtained from rats by cardiac puncture. The erythrocytes were washed as described before [18]. Samples of 0.50 ml radioactively labeled, packed cells were successively treated with phospholipase A_2 from *Naja naja* venom and sphingomyelinase from *Staphylococcus aureus* to discriminate between the radioactivities in the phospholipid components of the outer and inner layer of the membrane, essentially as described before [18].

Plasma phospholipids were labeled in vitro by incubating ^{32}P-labeled erythrocytes (1.0 ml) in unlabeled plasma (1.5 ml) for 2 h at 37°C. The lecithin-cholesterol acyltransferase activity in plasma had been destroyed prior to this incubation [23].

The lipid extracts of aliquots of erythrocytes and plasma, labeled in vivo and in vitro were chromatographed and assayed for radioactivity and phospholipid phosphorus in the various molecular classes of phosphatidylcholine exactly as described before [19].

Results

The specific activity of plasma phosphatidylcholine reaches a maximum within a few hours after the injection of $^{32}P_i$; thereafter, the specific activity decreases gradually [6,17]. The specific activity of erythrocyte phosphatidylcholine reaches a maximum only after a few days [17]. Therefore, the time intervals chosen for in vivo labeling were 20 h and 96 h. After 20 h the ery-

throcyte phospholipids have become considerably radioactive and after 96 h the plasma phospholipids are still sufficiently labeled.

In this study the distribution of radioactivity among the various molecular classes of plasma phosphatidylcholine is essentially as described before [19] and it hardly changes during the labeling period. The labeling pattern [19] closely follows the mass distribution [22]: the majority of the radioactivity is recovered in the major mass fraction, the polyenoic fraction.

In rat-erythrocyte phosphatidylcholine the major component is disaturated [19]. Yet, the specific activity of the molecular classes becomes higher with increasing degree of unsaturation of the different classes (Table I, data columns 1 and 4). In Table I are also presented the specific activities of the phosphatidylcholine classes from the inner and outer layer of the erythrocyte membrane, 20 h and 96 h after the injection of $^{32}P_i$. The labeling pattern of the molecular classes in the outer layer is distinctly different from that in the inner layer. Whereas the specific activities of the more unsaturated classes in the outer layer are higher than in the inner layer, the reverse situation exists for, in particular, the disaturated class. This suggests that the rates of renewal of the distinct molecular classes of phosphatidylcholine in the outer layer are different from those of the molecular classes in the inner layer of the membrane.

In a previous report [19] the exchange of phosphatidylcholine between plasma and the erythrocyte membrane was studied by incubating unlabeled erythrocytes in ^{32}P-labeled plasma. It was concluded that the rates of exchange of the various molecular classes of phosphatidylcholine were similar. However, a possible preferential exchange of the disaturated molecular class might have been overlooked because of the low content and radioactivity of disaturated phosphatidylcholine in plasma. In the reverse experiment (Table II) labeled erythrocytes were incubated in unlabeled plasma. Assuming that exchange of phospholipids takes place at the outer surface of the cell membrane [18] the labeling patterns of the plasma phosphatidylcholines should be

TABLE I

THE SPECIFIC ACTIVITIES OF THE MOLECULAR CLASSES OF PHOSPHATIDYLCHOLINE FROM RAT ERYTHROCYTES (SPECIFIED FOR INNER AND OUTER LAYER OF THE MEMBRANE) AT DIFFERENT TIMES AFTER THE INJECTION OF $^{32}P_i$

The experimental procedures are mentioned in Methods. In a representative experiment the total radioactivities in phosphatidylcholine per ml packed cells were 32 270 cpm after 20 h and 40 360 cpm after 96 h. The data for the outer layer were calculated from the results obtained from the total membrane and the inner layer. The hydrolysis of erythrocyte phosphatidylcholine by the action of the phospholipases was 58%. The specific activities of the phosphatidylcholine classes in plasma are 45—50 cpm/nmol after 20 h and 8—16 cpm/nmol after 96 h.

Molecular class	Specific activity (cpm/nmol) ± S.D.					
	20 h			96 h		
	Total	Inside	Outside	Total	Inside	Outside
Disaturated	3.4 ± 0.2	6.0 ± 0.2	0.4 ± 0.5	9.8 ± 0.1	15.3 ± 0.5	2.1 ± 0.5
Monoenoic	9.1 ± 0.1	9.3 ± 0.1	5.5 ± 0.3	15.1 ± 0.2	12.6 ± 0.1	16.2 ± 0.4
Dienoic	24.4 ± 0.9	13.9 ± 0.2	33.0 ± 2.1	28.1 ± 0.1	15.5 ± 0.2	40.0 ± 0.3
Polyenoic	41.9 ± 1.1	22.5 ± 0.1	56.5 ± 2.6	44.8 ± 0.1	27.9 ± 0.7	59.0 ± 0.9

TABLE II

THE DISTRIBUTION OF RADIOACTIVITY (% ± S.D.) AMONG THE MOLECULAR CLASSES OF ERYTHROCYTE AND PLASMA PHOSPHATIDYLCHOLINE AFTER THE INCUBATION OF PLASMA WITH ^{32}P-LABELED ERYTHROCYTES

The percentages of radioactivity in the columns under erythrocytes were obtained from the same experimental data on which Table I was based. Part of these in vivo labeled erythrocytes were incubated with unlabeled plasma, after which the radioactivity of the molecular classes of plasma phosphatidylcholine was determined as mentioned in Methods. The total phosphatidylcholine radioactivities were 1863 cpm/ml plasma under A and 2010 cpm/ml plasma under B.

Molecular class	% Radioactivity in molecular classes of phosphatidylcholine					
	Erythrocytes				Plasma incubated with cells from	
	20 h (A)		96 h (B)			
	Inside	Outside	Inside	Outside	A	B
Disaturated	15.8 ± 0.4	0.6 ± 0.7	28.1 ± 0.9	2.7 ± 0.7	1.2 ± 0.2	3.7 ± 0.3
Monoenoic	10.8 ± 0.1	3.7 ± 0.2	10.3 ± 0.1	9.1 ± 0.2	4.0 ± 0.8	5.4 ± 0.1
Dienoic	26.2 ± 0.4	36.2 ± 2.3	20.5 ± 0.3	36.8 ± 0.3	25.7 ± 1.0	30.1 ± 0.1
Polyenoic	47.2 ± 0.1	59.6 ± 2.7	41.1 ± 1.0	51.4 ± 0.8	69.1 ± 1.5	60.8 ± 0.2

compared with those of the outer layer phosphatidylcholines in the corresponding incubations, rather than with those of the total erythrocyte-phosphatidylcholine pool. This comparison shows that the distribution of radioactivity recovered in the plasma-phosphatidylcholine classes largely resembles that of the phosphatidylcholine classes in the outer layer of the membrane, and not that of the classes in the inner layer.

Discussion

The renewal of erythrocyte phospholipids in vivo occurs via exchange with plasma phospholipids, in particular phosphatidylcholine, and via acylation of lysophospholipids. The exchange process takes place at the outer surface of the membrane. The substrates for exchange are highly unsaturated molecular classes of phosphatidylcholine in plasma, whereas the erythrocyte membrane is enriched in the disaturated class. The results from this study extend the conclusion from an earlier observation [19] that the rate of exchange of the various molecular classes of phosphatidylcholine between plasma and the erythrocyte membrane is independent of the degree of unsaturation of the different classes. The overall exchange process is thought to proceed without a net transport of phospholipids [4,6,7,16]. This would imply that unsaturated plasma phosphatidylcholines enter the membrane structure in exchange for both unsaturated and disaturated phosphatidylcholines from the membrane.

In the overall exchange process the supply of disaturated phosphatidylcholine to the membrane is limited by the relative lack of the disaturated molecular class in plasma as a substrate for exchange. It is still an unanswered question to what extent the exchange process might be specifically catalyzed by phospholipid exchange proteins, the existence of which in plasma has been repudiated in the literature [24,25]. Recently, Brewster et al. [26] suggested to

have evidence for a protein component in human plasma, which facilitated the redistribution of phosphatidylcholine between membrane structures. The pattern of specific activities of the outer layer phosphatidylcholines is different from that of the molecular classes in the inner layer (Table I). Also the specific activity of the total outer phosphatidylcholine pool is different from that of the total inner phosphatidylcholine pool. Therefore, rat-erythrocyte phosphatidylcholine does not become homogeneously labeled in vivo after injection of $^{32}P_i$. This is in contrast with studies on rat-liver microsomes [27, 28], in which the phospholipid pool does become homogeneously labeled in vivo. The metabolic sidedness observed in this study suggests that the rate of renewal of the more unsaturated molecular classes of phosphatidylcholine is more pronounced in the outer layer of the rat-erythrocyte membrane, whereas the disaturated class is more rapidly formed in the inner layer. This leads to the following proposal: an exchange mechanism at the outside of the cell might mainly renew the unsaturated phosphatidylcholines of the erythrocyte, because plasma phosphatidylcholine is virtually devoid of disaturated molecular species; the erythrocyte directs its acylation activity towards the formation of disaturated phosphatidylcholines at the inside of the membrane. The final distribution of the radioactivity between the phosphatidylcholines of the outer and inner layer of the erythrocyte membrane would be the result of these two processes and the transposition of phosphatidylcholines across the membrane [18,19,29].

It has to be realized that this study has been performed with mature mammalian erythrocytes, which phospholipid metabolism is virtually limited to exchange with plasma phospholipids and acylation of lysophospholipids. The observed renewal of phospholipids in the membrane presumably takes place without affecting the structural coherence of the membrane. From the relatively long life-span of the erythrocytes it is not to be expected that actual production of new erythrocytes, in which the composition base is laid for the membrane structure, has contributed to an appreciable extent in the labeling. This might explain the apparent discrepancy between the compositional data for the membrane [19], showing that the molecular composition of phosphatidylcholine is identical at both sides of the membrane, and the conclusion from this study that the rates of renewal of the distinct molecular classes of phosphatidylcholine in the outer layer of the rat-erythrocyte membrane differ from those in the inner layer.

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