Phospholipids enhance the binding of peptides to class II major histocompatibility molecules

(immunogenicity/antigen presentation/antigen processing/antigen-presenting cells/lipids)

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ABSTRACT The binding of a lysozyme and ovalbumin peptide to purified class II major histocompatibility molecules in detergents was increased by the addition of certain lipids. Natural lipids from B lymphoma cells enhanced the binding and so did phosphatidylcholine, phosphatidylserine, phosphatidylinositol, and cardiolipin. Phosphatidylethanolamine, sphingomyelin, and cholesterol had no effect. There was no major difference between the effects of a phospholipid and its lyso derivative. As studied with phosphatidylcholine, the increase in peptide binding was also dependent on the fatty acid composition of the lipid. The binding affinity was increased 10- to 50-fold in the presence of lipid as a result of an increase in the association rate while the off-rate remained essentially unchanged. Our results suggest that lipids, directly or indirectly, induce conformational changes in class II molecules that favor their peptide-binding property.

Our laboratory is examining the conditions, *in vitro* or in cells, that may affect the primary binding interaction between peptides and class II major histocompatibility complex (MHC) proteins (1, 2). The extent of binding of peptides to histocompatibility molecules, which can be studied in detergent solutions (1–7), usually correlates with their immunogenicity *in vivo* (2–4). There are, however, indications that the interactions of peptides with class II MHC may be quantitatively different on live antigen-presenting cells. For example, the binding of antigenic peptides to class II MHC molecules on live cells was reported to be much faster than that found in solution (8–10). A similar result was found for the dissociation of peptides bound to the class II MHC molecules (11).

We recently found that the labeling of class II MHC molecules to peptides containing a photoreactive compound was influenced by phospholipids, both in the extent as well as in the relative degree of the α or β chain labeling (12). When tested on cell membranes that contained class II MHC proteins, photoreactive conjugates of the hen egg white lysozyme (HEL) peptide from residues 46 to 61 labeled exclusively the α chain of I-A^k (13). Conversely, I-A^k purified in an equimolar solution of MEGA 8 and MEGA 9 detergents was labeled to similar extent on both chains. However, the addition of certain lysophospholipids resulted in strong binding, with lysophosphatidylcholine predominantly labeling the α chain and lysophosphatidylserine labeling the β chain. We concluded that class II MHC molecules contained dialyzable components required for their binding integrity and that, most likely, these components were lipids.

Lipid-protein interactions are of critical importance in maintaining the biological activity of a number of membrane proteins (for review, see refs. 14–16). These interactions may have different degrees of specificity and often induce con-

formational changes in the protein critical for their function (14, 17–22). Lipids, by interacting directly with the MHC protein or by changing the structure of the detergent micelles, may induce conformational changes reflected in the distal binding site of the protein (23, 24). Consistent with this hypothesis are the observations indicating that class II MHC molecules reconstituted in lipid monolayers present peptides differently, depending on the lipid composition (25). Other studies have shown that treatment of antigen-presenting cells with purified phospholipase A2 or C, or with cerulenin, an antibiotic that interferes with lipid metabolism, greatly diminished their antigen-presenting function (26–28). Perhaps such treatments altered antigen presentation by the class II molecules as a result of changes in the composition of membrane lipids.

The present study represents a detailed analysis of lipid-class II MHC interactions. We show that certain phospholipids strongly enhanced the binding of peptides to purified class II molecules and suggest that the antigen-binding properties of class II molecules in detergents supplemented with lipids more truthfully reflect the events taking place in antigen-presenting cells.

MATERIALS AND METHODS

The basic experimental system consisted of measuring the binding of radioiodinated peptides to purified class II proteins. The two interactions studied were that of (i) the HEL peptide YE52–61 (Tyr-Glu-Asp-Tyr-Gly-Ile-Leu-Gln-Ile-Asn-Ser-Arg) with I-A^k and (ii) the ovalbumin peptide 323–339 (Ile-Ser-Gln-Ala-Val-His-Ala-Ala-His-Ala-Glu-Ile-Asn-Glu-Ala-Gly-Arg) with I-A^d. For binding, HEL(YE52–61) was labeled with I¹²⁵ by the chloramine-T method to a specific activity of $\approx 2.8 \mu\text{Ci}$ per pmol (1 Ci = 37 GBq); ovalbumin (323–339) was labeled with I¹²⁵ with the Bolton-Hunter reagent to a specific activity of $\approx 1 \mu\text{Ci}$ per pmol.

I-A^k and I-A^d molecules were isolated from membranes of B lymphoma cells (either TA3, which bears both H-2^k and H-2^d molecules; or CH27, which bears H-2^k molecules) by a technique described in detail (12). In brief, membranes were obtained from cells grown in the Monsanto Company Cell Biology Facility and solubilized in a detergent mixture containing 40 mM each of MEGA 8 and MEGA 9. The I-A^k or I-A^d molecules were obtained by affinity chromatography by using specific monoclonal antibodies (the monoclonal antibody 10-3.6.2 to obtain I-A^k and MK-D6 for I-A^d). All preparations were devoid of major contaminants except for occasionally small amounts of protein migrating ≈ 66 kDa in SDS/PAGE.

Abbreviations: MHC, major histocompatibility complex; HEL, hen egg white lysozymes.

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Binding of Peptides to Class II MHC Molecules. The peptides were incubated with class II MHC molecules in detergent solution, usually for 48 hr, with different lipids, ranging in concentrations from 1 to 20 mM. The usual incubation mixture consisted of class II MHC molecules at 10–90 pmol, peptides at 0.1–1 pmol, in volumes of 10–30 μ l in a solution containing 20 mM each of MEGA 8 and MEGA 9 in phosphate-buffered saline. Controls consisted of (i) the addition of a few thousand-fold molar excess of unlabeled peptide to the incubation mixture and (ii) the incubation of peptides with or without the lipids in the absence of class II MHC proteins. Extent of binding was quantitated by separating bound and free peptides with Sephadex G-50 chromatography (6). The samples were run on a 1.5 $\times$ 30 cm column containing Sephadex G-50 at a flow rate of 1.33 ml/min, and fractions were collected every 30 sec. The input cpm was $\approx$ 500,000. The free peptide was found in fractions 55–100, whereas the peptide bound to class II MHC molecules was found in the void volume in fractions 25–35 (Fig. 1).

To measure rates of association, HEL(I¹²⁵-labeled YE52–61) and I-A^k were incubated with or without lysophosphatidylserine (final concentration of 5 mM) for up to 96 hr. At different times aliquots were obtained, and free and bound peptide were then measured as described above.

In the results shown in Fig. 4 the association rates of the MHC–peptide interaction were calculated according to the second-order rate equation where

$$k_{at} = \frac{2.303}{[\text{MHC}]_0 - [\text{peptide}]_0} \times \log \frac{[\text{peptide}]_0[\text{MHC}]_t}{[\text{MHC}]_0[\text{peptide}]_t}$$

In Fig. 4 κ corresponds to $2.303/[\text{MHC}]_0 - [\text{peptide}]_0$, and ϕ corresponds to $[\text{peptide}]_0[\text{MHC}]_t/[\text{MHC}]_0[\text{peptide}]_t$. Plotting t vs. $\kappa \log \phi$ gives a line where the slope equals k_a . $[\]_0$ or $[\]_t$ represent the free molar concentrations at time 0 or at the indicated time t , respectively.

To measure the dissociation of peptide bound to the MHC molecules, HEL(I¹²⁵-labeled YE52–61) was first incubated with I-A^k, and after 48 hr the complexes were isolated by Sephadex G-50 fractionation. The complexes were concentrated, incubated with 10,000-fold molar excess of unlabeled

peptide and, at periodic times, aliquots were examined for free and bound peptide.

Lipids. The lipids, obtained from either Avanti Polar Lipids or Sigma, are listed in Table 1.

RESULTS

Lipids extracted from TA3 B lymphoma cells, as well as certain phospholipids, enhanced the binding of peptide by several fold. Details of three representative experiments are shown in Figs. 1 and 2, whereas the summary of all experiments is shown in Table 1. In no instance did we detect significant amounts of peptide eluting in the void volume in the absence of class II MHC molecules. This result indicates that the increase of peptide binding was not due to nonspecific association of the peptide with high-molecular-weight lipid detergent-mixed micelles.

Table 1 indicates that the effects were consistently found in all experiments testing both I-A^k and I-A^d and with various preparations of each. However, the degree of binding varied among the different preparations. The concentration of phospholipids or lysophospholipids that gave optimal enhancement was 5 mM. Over 5 mM and up to 20 mM there was no further increase, and in some experiments a slight decrease was found (data not shown). Concentrations of 1 mM gave about one-third of the enhancement found with the 5 mM dose (data not shown). Phosphatidylserine and phosphatidylcholine and their lyso derivatives enhanced binding; the lyso derivatives were slightly more effective. Phosphatidylinositol, phosphatidic acid, and cardiolipin also enhanced binding. However, phosphatidylethanolamine, diacylglycerols, cholesterol, and sphingomyelin had no enhancing effect, suggesting an influence of the polar head groups for the enhanced binding (Table 1 and Fig. 2).

We tested synthetic phosphatidylcholine or lysophosphatidylcholine containing fatty acid chains ranging from 6 to 18 carbons. Monocaproylphosphatidylcholine was inactive, whereas monolaurylphosphatidylcholine induced some enhancement. Those compounds containing C₁₄- and C₁₆-chain fatty acid (myristic and palmitic acid, respectively) consistently enhanced peptide binding. Dimyristoyl and dipalmi-

FIG. 1. Two experiments showing the effects of lipids on the binding of HEL(YE52–61) to I-A^k. In experiment (Expt 1), I¹²⁵-labeled HEL(YE52–61) and I-A^k were incubated for 48 hr with or without lipids. The solution contained 81 pmol of I-A^k and 0.24 pmol of peptide in 27 μ l without lipids (No Lipid) or with 10 μ g of lipids from B lymphoma cells (Natural Lipids) or with 5 mM lysophosphatidylserine (Lyso PS). Amounts of peptide in the void volume are indicated. In the presence of a 1000-fold excess of unlabeled peptide, amounts in the void ranged from 0.9 to 1.3%. Approximately 500,000-cpm aliquots were passed over the Sephadex G-50 column. In experiment (Expt 2), I¹²⁵-labeled HEL(YE52–61) was incubated with I-A^k (0.3 pmol of peptide with 76 pmol of I-A^k in a 20- μ l volume) in the absence of lipid (No Lipid), or presence of 5 mM lysophosphatidylserine (Lyso PS) or lysophosphatidylcholine (Lyso PC). After 48 hr, aliquots were submitted to G-50 Sephadex chromatography to determine free and I-A^k-bound peptide. Addition of unlabeled peptide in 1000-fold molar excess resulted in no more than 0.6% of labeled cpm in the void volume.

Table 1. Summary of experiments of binding enhancement with phospholipids

Lipid	Experiments, no.	Different preparations of I-A ^k or I-A ^d	Increase in binding, % $\pm$ SD (range of values)
Lysophosphatidylcholine	6	5	296 $\pm$ 137 (131–480)
Lysophosphatidylserine	21	9	254 $\pm$ 156 (92–731)
Lysophosphatidylinositol	2	2	304 (109 and 498)
Lysophosphatidylethanolamine	4	3	23 (0–58)
Phosphatidylcholine	2	2	134 (104 and 163)
Phosphatidylserine	2	2	245 (90 and 400)
Phosphatidylinositol	1	1	108
Lipids from TA3 lymphoma	2	2	309 (180 and 437)
Sphingomyelin	2	2	12 (0 and 23)
Cardiolipin	4	4	576 $\pm$ 280 (275–927)
Phosphatidic acid	2	1	127 (89 and 164)
Cholesterol	1	1	0
Dioleoyl glycerol	1	1	21
Diocanoyl glycerol	1	1	17
Lysophosphatidylcholine stem with			
Caproyl group (6:0)	1	1	0
Lauroyl group (12:0)	2	2	30 (0 and 59)
Myristoyl group (14:0)	4	4	135 (56–255)
Palmitoyl group (16:0)	2	2	150 (50 and 249)
Stearoyl group (18:0)	2	2	87 (0 and 173)
Oleoyl group (18:1)	2	2	140 (56 and 224)
Phosphatidylcholine stem with			
Dimyristoyl group (14:0)	1	1	62
Dipalmitoyl group (16:0)	2	2	89 (67 and 111)
Distearoyl group (18:0)	4	4	9 (0–36)
Dimyristoleoyl group (14:1)	2	2	62 (31 and 92)
Dioleoyl group (18:1)	1	1	82
Dilinoleoyl group (18:2)	2	2	88 (71 and 149)

A composite of all experiments done during a 14-mo time period showing the effects of different lipids on binding of the HEL and ovalbumin peptide to either I-A^k or I-A^d, respectively. Although tested at different concentrations in some experiments, the table shows only the results at optional concentration (generally 5 mM). The following lipids were obtained from Avanti Polar Lipids (catalog number is in parentheses): cardiolipin (830012), phosphatidylcholine (840051), lysophosphatidylcholine (830071), lysophosphatidylethanolamine (860081), lysophosphatidylinositol (850091), lysophosphatidylserine (850092), sphingomyelin (860061), dimyristoyl lecithin (850345), dipalmitoyl lecithin (850355), distearoyl lecithin (850365), dimyristoleoyl lecithin (855475), monopalmitoyl lecithin (855675), monostearoyl lecithin (855775), monooleoyl lecithin (845875). The following lipids were obtained from Sigma: phosphatidylserine (P-7769), phosphatidic acid (P-9511), phosphatidylinositol (P-0639), cholesterol (CH-PL), diocanoyl glycerol (D-5156), dioleoyl glycerol (D-0138).

toylphosphatidylcholine also had a positive effect. However, the addition of mono or distearoylphosphatidylcholine (18:0) gave erratic results with enhancement in only some experiments. Phosphatidylcholine or lysophosphatidylcholine containing unsaturated oleic acid (18:1) resulted in enhancement. Perhaps the more rigid unsaturated chain was not conducive

to the proper interaction with the class II molecule in detergent micelles.

Affinity Measurements. We determined the affinity of I-A^k for I¹²⁵-labeled HEL(YE52–61) with or without 5 mM lysophosphatidylserine (Fig. 3). In agreement with the results of Table 1, the addition in this experiment of 0.4 pmol of

FIG. 2. Another experiment that examines the effect of different lysophospholipids in the binding of HEL peptide (0.2 pmol of peptide with 60 pmol of I-A^k in 16 μ l). All lipids were added to a final concentration of 5 mM. Although lysophosphatidylserine (LysoPS), lysophosphatidylcholine (LysoPC), lysophosphatidylinositol (LysoPI), and cardiolipin markedly increased binding, lysophosphatidylethanolamine (LysoPE) had no effect. Note that the increment is not found when a 1000-fold molar excess of unlabeled peptide is added (example with LysoPS and cardiolipin). Also shown are the effects of different monoacylphosphatidylcholines.

FIG. 3. Binding isotherm of HEL(YE52-61) with I-A^k. Increasing amounts of peptide were incubated with 14 pmol of I-A^k in a total volume of 26 μ l for 48 hr. Values in A and C were calculated by Scatchard analysis (B and D). (A and B) Results without lipid ($K_D = 2.8 \times 10^{-6}$ M). (C and D) Results with 5 mM lysophosphatidylserine ($K_D = 27 \times 10^{-7}$ M).

peptide to I-A^k (14 pmol) resulted in 6.3% bound, which increased to 33.4% with lysophosphatidylserine. Scatchard analysis of the isotherm gave a K_D value of 2.8×10^{-6} M and 2.7×10^{-7} M in the absence or presence of lysophosphatidylserine, respectively. K_D values for binding of HEL(YE52-61) to I-A^k have ranged, in different experiments, from 2.8×10^{-6} M to 4.5×10^{-6} M. The binding affinity for peptide in the presence of phosphatidylserine have ranged from the value shown to 1.01×10^{-7} M. In the experiment of Fig. 3 the degree of occupancy of I-A^k molecules by peptide was calculated to be $\approx 21\%$ for both situations.

We measured the association rate constants of the binding of I¹²⁵-labeled HEL(YE52-61) in the absence and presence of 5 mM lysophosphatidylserine (Fig. 4). In the absence of lipid the 50% saturation was achieved after 2.4×10^5 sec in the absence of lipids and after 8.7×10^4 sec in the presence of the lipid (Fig. 4 A and C). The association rate constant (k_a) was $0.87 \text{ M}^{-1}\text{sec}^{-1}$ and $5.5 \text{ M}^{-1}\text{sec}^{-1}$ in the absence and pres-

ence of lipid, respectively. The corresponding dissociation rate constants (k_d) calculated from k_a and K_D values were $2.4 \text{ (sec}^{-1}\text{)}$ and $1.5 \text{ (sec}^{-1}\text{)}$ in the absence and presence of lipid, respectively. [The influence of phosphatidylserine on off-rate of HEL(YE52-61) bound to I-A^k was directly examined in only one experiment. As before (29), the off-rate was very slow, with 7.9% and 7.5% of the peptide dissociating at 72 hr without and with lipid, respectively.]

DISCUSSION

These studies indicate that the binding of peptides to class II MHC molecules in detergent solution is highly influenced by certain lipids. The enhancement was a consistent finding with many different preparations of I-A^k or I-A^d and three different peptides (the two reported here and a third peptide from bovine ribonuclease in ongoing experiments). Three factors that may account for the variability in the degree of enhance-

FIG. 4. Association rate k_a values. I-A^k at (648 nM) was incubated with I¹²⁵-labeled HEL(YE52-61) (180 nM) in 40 μ l. After incubation for the time periods indicated, the amount of peptide bound to I-A^k was measured. (A and B) Results without lipids. (C and D) Results with 5 mM phosphatidylserine.

ment of binding found with a given lipid (Table 1) are as follows: (i) the extent of endogenous lipids that may be associated with the purified class II molecules, (ii) variations in the amount of inhibitory molecules associated with the class II MHC, and (iii) different degrees of denaturation of the class II molecules.

The experiments indicated that the polar head group of the lipid and, to some extent also, its fatty acid chain played a role. However, our results do not indicate a preference for acidic or basic phospholipids, as reported for many lipid-protein interactions (14, 19–28, 30–40). While phosphatidylserine, phosphatidic acid, and cardiolipin were highly effective, so was phosphatidylcholine. Because class II MHC molecules are built up of two noncovalently associated membrane proteins, the different chains could undergo separate interactions with lipids, which would explain our results.

Several explanations can be offered for these results: (i) lipids may alter the structure of the detergent micelle in a way such that the class II molecule is placed in a suitable conformation; or (ii) the lipids may interact directly with the class II molecule; or (iii) the lipids may affect the conformation of the peptide to one that favors interaction with the class II molecule (29). We favor the first two explanations—that lipids, directly or indirectly, induce conformational changes in the MHC molecule that result in states of higher peptide-binding affinity. Our recent studies of photoaffinity-labeled peptides strongly suggest that class II molecules in detergent solution have considerable conformational flexibility (12). Thus, we found different patterns of labeling of the I-A^k molecules with photoreactive conjugates of HEL peptides (α and/or β chain labeling) on cell membranes or in detergent solution in the absence or presence of phospholipids. The mutual orientation in the membrane of the spanning domains of the α and β chains may be critical for the conformation of the distal peptide-binding site. The optimal binding of the peptide may involve a limited set of conformers of class II MHC molecules in detergents. These conformers could be in equilibrium with others that poorly interact with the peptide. The addition of lipids to the detergent solution may thus stabilize conformers of class II molecule akin to those in antigen-presenting cells. Reconstitution of isolated class II molecules with lipids may be an important step towards a better understanding of their interaction with antigen.

The question still remains whether in the antigen-presenting cell specific lipids directly interact with class II MHC molecules, to create a microenvironment around the membrane-spanning or exposed portions of the α and β chains. Such lipid interactions could change with the lipid composition of the membrane resulting from intracellular trafficking (15, 41) or activation of lipases (36). One can speculate that class II MHC molecules may be found in different conformers within the cell, attaining an active binding state under the influence of component specific to subcellular microenvironments—e.g., in endosomes. Differences in lipid composition among organelles may then reflect in the function of the class II MHC molecule during transport, endocytosis, and recycling.

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