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Peptide Binding to Active Class II MHC Protein on the Cell Surface

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Solution studies have demonstrated the existence of two functionally distinct isomers of empty class II MHC: an active isomer that binds peptide and an inactive isomer that does not. Empty MHC molecules on the surface of APCs can load antigenic peptides directly from the extracellular medium, facilitating the generation of a diverse peptide repertoire for T cell presentation. In this report, we examine I-Ek on the surface of Chinese hamster ovary cells with respect to the active and inactive isomers. As in the case of purified soluble active I-Ek, active I-Ek on the cell surface is unstable, decaying to the inactive form in ~14 min. Evidence is presented suggesting that at steady state <1% of the total cell surface I-Ek is active and that a significant fraction of these active molecules originates from intracellular pools as well as reactivation of inactive cell surface I-Ek. The Journal of Immunology, 2001, 166: 6680–6685.

MHC class II are heterodimeric membrane glycoproteins constitutively expressed on the surface of APCs. Class II MHC bind a diverse array of peptides derived from the endocytic pathway and present them to T cells. APCs have a pool of active class II molecules on their surface that can quickly load peptides from the extracellular milieu for T cell presentation (1). For example, exposure of antigenic peptide to a mixture of T cells and APCs results in a rapid increase in T cell acid release, a class II-restricted and peptide-specific response (2). The kinetics of this T cell response correlate with the kinetics of peptide binding to the surface class II MHC molecules (3–5).

Several laboratories have confirmed the existence of two functionally distinct empty class II MHC isomers in solution (6–8), an active or peptide-receptive isomer that binds peptide rapidly, and an inactive isomer that does not bind peptide. The formation of active MHC and its interconversion to the inactive isomer have been described by the following scheme:

\[
-\frac{p}{M} k_{\text{off}} \quad k_{\text{on}}^M \quad k_{\text{ac}}
\]

\[
p + \frac{p}{M} k_{\text{off}} \quad k_{\text{on}}^i \quad k_{\text{ac}}
\]

(Scheme I)

Here \( p \) is peptide, \( p/M \) is peptide/MHC complex; \( k_{\text{off}} \) and \( k_{\text{on}} \) are the dissociation and association rate constants; \( M_a \) is the active form of MHC; \( M_i \), the inactive form; and \( k_{\text{ac}} \) and \( k_{\text{in}} \) are the activation and inactivation rate constants.

Rabinowitz et al. (6) provided indirect evidence for the existence of the two I-Ek isomers on fixed CH27 B cells. However, it remains unclear whether the behavior and kinetic properties measured for soluble I-Ek are representative of I-Ek expressed on the plasma membrane. This study is aimed at understanding the source of active class II MHC molecules, quantifying their amount and determining their kinetic properties on the surface of cells.

Materials and Methods

Peptides

All peptides were synthesized using standard 9-fluorenylmethoxycarbonyl chemistry, purified by HPLC, and characterized by mass spectroscopy. The peptide sequences used were the following: invariant chain 85-99 mutant peptide (Ii 85-99 M90L M98L) (KPVSQMRMATPLLR); invariant chain 85-99 peptide (Ii 85-99) (KPVSQMRMATPLLMR), and biotinylated moth cytochrome c 82-103 peptide (B-MCC) (FAGLKKANERADIY LLQQATK). Biotin-labeling of Ii 85-99 and MCC 82-103 was conducted while still on resin by reaction of the free amino terminus with biotin N-hydroxysuccinimide ester (Pierce, Rockford, IL) in DMISO with a catalytic amount of diisopropylethylamine.

Cell lines

Chinese hamster ovary cells transfected with native I-Ek (CHO-Ek) (9) cells and CH27 B lymphomas were maintained by passage every 2 days in complete RPMI 1640 medium (Life Technologies, Grand Island, NY) containing 10% FBS (HyClone, Logan, UT), 25 mM HEPES, l-glutamine, 20 \( \mu \)M 2-ME, 900 U/ml penicillin-streptomycin (Life Technologies), and 9 \( \mu \)g/ml gentamicin (Life Technologies) at 37°C and 5% CO2. A subclone of Chinese hamster ovary cells (CHO-K1) was grown as described above and used as negative controls.

Abs and dyes

R-phycocerythrin 14.4.4S Ab was purchased from PharMingen (San Diego, CA) and used to determine the total amount of I-Ek on the cell surface. Briefly, cells were suspended in 100 \( \mu \)l PBS/2% FBS at pH 7.2 and incubated with R-PE 14.4.4S Ab (0.008 ng/cell) for 20 min at 4°C. Cells were washed twice with PBS/2% FBS, resuspended in 500 \( \mu \)l PBS/2% FBS, and immediately analyzed by FACS. Streptavidin-R-PE conjugate was purchased from both Sigma (St. Louis, MO) and PharMingen.

Preloading procedure

CHO-Ek cells were first incubated overnight with 200 \( \mu \)M Ii 85-99 M90L M98L in complete RPMI 1640 at pH 7.2 to generate Ii 85-99 M90L M98L/I-Ek complexes. Unbound Ii 85-99 M90L M98L was then removed by aspirating the supernatant. Next, the Iii 85-99 M90L M98L/I-Ek complexes were allowed to dissociate in the presence of 200 \( \mu \)M Ii 85-99 for a period of 2.5–3 h (~2 half-lives). In this manner, we replaced the Ii 85-99 M90L M98L/I-Ek complexes by Ii 85-99/I-Ek, which dissociates at least 20 times...
faster from I-Ek. This faster dissociation is essential for the experiments that require the rapid generation active MHC.

Flow cytometry

In all experiments, cells were plated on six-well plates at a density of 250,000 cells/well in 2 ml RPMI 1640 and incubated overnight at 37°C and 5% CO₂ in the presence or absence of a preloading peptide. For experiments measuring active I-Ek, cells were exposed to 50 μM B-MCC for varying time periods. For experiments determining the stability of I-Ek, cells were preloaded overnight as described above. The I-Ek/Ii 85-99 complexes were allowed to dissociate for varying time periods, and active I-Ek was measured by exposure to 50 μM B-MCC for 8 min. For peptide association experiments, cells were exposed to varying concentrations of B-MCC for 8 min.

In all experiments, cells were washed once with RPMI 1640 medium following exposure to B-MCC and released from the plate with a cold EDTA solution (0.526 mM). The cells were then centrifuged at 1200 rpm for 5 min, resuspended in 100 μl of PBS/2% FBS, pH 7.2, and stained for 20 min at 4°C with streptavidin-R-PE (0.36 μg). Cells were washed twice with PBS/2% FBS, resuspended in 500 μl PBS/2% FBS, and immediately analyzed.

For peptide dissociation experiments, cells were exposed overnight to 200 μM biotinylated invariant chain 85-99 peptide (B-li 85-99) at pH 7.2, 37°C and 5% CO₂. Cells were released from the plate with EDTA and resuspended in 500 μl PBS/2% FBS. All handling was carried out at 4°C to avoid peptide dissociation. B-li 85-99/I-Ek complexes were allowed to dissociate at pH 7.2, 37°C for various times in the presence of 1 μM MCC 95-103 as a competitor peptide. Then, cells were stained with streptavidin-R-PE as described above. Quantitation of fluorescence intensity was determined with a Quantum R-PE kit (Flow Cytometry Standards, San Juan, PR). Flow cytometry analysis was conducted on Coulter EPICS 753, Altra, and Elite XL flow cytometers. Data were analyzed using either Elite or EXPO Software (Beckman Coulter, Fullerton, CA).

Inhibitors

All inhibitors were purchased from Sigma and used at the following concentrations: berbexcld A, 20 μg/ml; sodium azide, 5 μM; cycloheximide, 5 μg/ml; and chloroquine, 5.2 μg/ml. Experiments with inhibitors involved incubation of cells with the above blockers for 1 hr in RPMI 1640 at 37°C, pH 7.2 and 5% CO₂. Cells were then washed with RPMI 1640 and either exposed to B-MCC to determine active MHC, or lifted off the plate and incubated with R-PE 14.4.4S Ab to determine total MHC.

Inactuation rate constant

The inactuation rate constant of surface-active I-Ek was estimated by fitting the data in Fig. 3B to the analytical solution of the system of differential equations described by Rabinowitz et al. (6). Briefly, the following differential equations were derived from scheme I:

\[
\frac{d[M]}{dt} = k_{on} [p(M)] - k_{off} [M] - k_b \frac{[M]}{[M_0]}
\]

Here \( p/M \) is the I-Ek/Ii 85-99/I-Ek complex with a dissociation constant \( k_{off} \).

Assuming that the conversion from \( M_0 \) to \( M \) is almost zero (\( k_{off} = 0.00064 \) min⁻¹ in solution) (6) and that at steady state \( [M_0] = [M] \), which arises from endogenous peptide dissociation, then we can derive the following analytical solution to the system described above:

\[
[M] = [M_0] \left( 1 - e^{-k_{off} t} \right) + \frac{k_{off} [p(M)]}{k_{on} - k_{off} + 1} (1 - e^{-k_{off} t} - e^{-k_{on} t}),
\]

where \( k_{off} \) for I-li 85-99/I-Ek is 0.2 min⁻¹, \( t \) is the delay time plus 8 min binding time used to measure \( M_0 \), and \( [M_0] \) is 0.3 from Fig. 3B. Then, \([p(M)]\) and \( k_{off} \) were estimated by obtaining the best data fit to Equation 4.

Apparent association rate constant

The apparent association rate constant for B-MCC binding to I-Ek for preloaded cells was calculated by using the following simplified version of scheme I:

\[
-pk_{off} + \frac{p^*}{k_{on}} \frac{k_{on} [p(M)]}{k_{off} [M] - k_{on} [M_0]} \frac{k_{on} [p(M)]}{k_{on} [p(M)] + k_{off} [M] - k_{on} [M_0]},
\]

Here \( p \) represents li 85-99, p/M is li 85-99/I-Ek complex, \( p^* \) is B-MCC, \( p^*/M \) is B-MCC/I-Ek complex, and \( k_{on} \) is the B-MCC association rate constant. Our simplifying assumptions are that the reactivation of \( M_0 \) is negligible on the time scale of our experiments because of its slow activation rate; that reassociation of unlabelled peptide \( p \) does not occur due to its low concentration; and that association of B-MCC is irreversible because of its small off-rate.

The concentration of B-MCC is essentially constant because it is in large excess with respect to cell surface I-Ek (μM range for B-MCC vs pM for I-Ek).

Thus we have the following system of differential equations:

\[
\frac{d[p^*(M)]}{dt} = k_{on} [p^*][M] - k_{off}[p^*(M)]
\]

\[
\frac{d[M]}{dt} = k_{on} [p(M)] - k_{off}[M] - k_{on} [p^*(M)]
\]

\[
\frac{d[M]}{dt} = k_{off}[M] + k_{off}[M] - k_{off}[p^*(M)]
\]

Assuming that at \( t = 0 \) both \([M_0] \) and \( [p^*(M)] \) equal zero, one obtains the analytical solution for \([p^*(M)]\):

\[
[p^*(M)] = \frac{k_{on} [p(M)]}{k_{off} [p^*(M)] + k_{off} [M] - k_{off} [p^*(M)]}
\]

Both \( k_{off} \) and \([p(M)]\) were estimated by obtaining the best data fit of Fig. 3A to this equation using \( k_{off} = 2.6 \) min⁻¹ for li 85-99, an incubation time of 8 min and \( k_{off} = 0.049 \) min⁻¹ ± 0.01 min⁻¹ from Fig. 3B.

Equation 9 is not applicable to the calculation of the association rate constant for untreated cells. Instead, an apparent \( k_{on} \) value was calculated by fitting the data in Fig. 3A to a single exponential curve.

Results

CHO-Ek (9) were used because of their high MHC expression levels. We estimated that CHO-Ek have ~850,000 molecules/cell by comparing the fluorescence of known concentrations of R-PE with the fluorescence of R-PE 14.4.4S Ab bound to cell surface I-Ek (data not shown). B-MCC was used because of its slow dissociation rate from I-Ek (\( t_{1/2} \) at pH 7.0 and 37°C > 200 h) (10). Peptide binding to class II MHC was quantified using FACS together with streptavidin-R-PE conjugate as a marker.

Specificity of the binding of B-MCC to I-Ek was determined using CHO-Ek and CHO-K1 cells. Cells were incubated for 8 min with concentrations of B-MCC ranging from 10 to 400 μM. CHO-K1 cells do not contain I-Ek on their surface and do not exhibit significant peptide binding even at high B-MCC concentrations (Fig. 1). In contrast, CHO-Ek cells show a large increase in fluorescence after peptide incubation, confirming that B-MCC binding is specific to I-Ek molecules on the cell surface.

Peptide dissociation kinetics at the cell surface

The dissociation kinetics of various peptides from solubilized I-Ek have been reported previously (11). To compare the dissociation rate of peptides from solubilized I-Ek to the dissociation rate from cell surface I-Ek, we exposed CHO-Ek cells to 200 μM B-li 85-99 at pH 7.2 and 37°C for 20 h. The cells were released from the plate with cold EDTA and resuspended in PBS/2% FBS in the presence...
of 1 μM competitor peptide (MCC 95-103). The B-Ii 85-99/I-Ek complexes were allowed to dissociate at 37°C for different time periods. Cells were stained with streptavidin-R-PE at 4°C.

Fig. 2 shows the dissociation of B-Ii 85-99 from I-Ek as measured by FACS. A dissociation half-life of 3.5 min for B-Ii 85-99 was calculated by fitting the data to a single exponential curve. This value is slightly lower than that obtained for the dissociation of carboxyfluorescein-labeled Ii 85-99 from water-soluble I-Ek (τ1/2 = 9 min at pH 7.0 and 37°C) (Peter Kasson and J.V., unpublished results).

Dissociation of a prebound peptide generates active I-Ek at the cell surface

Rabinowitz et al. (6) found in experiments with purified soluble I-Ek that dissociation of a preformed peptide/MHC complex resulted in the formation of active I-Ek. The rate of peptide binding to MHC was approximately equal to the rate of dissociation of the preformed complex, suggesting that peptide associated rapidly when the active MHC isomer was formed. Based on these findings, we examined whether preloading CHO-Ek cells with fast dissociating peptides would result in an enhancement of peptide binding to cell surface I-Ek.

Preliminary experiments showed that incubating CHO-Ek cells overnight with either 200 μM Ii 85-99 (dissociation τ1/2 = 3.5 min at pH 7.2 and 37°C), or 200 μM Ii 85-99 M90L M98L (dissociation τ1/2 = 90 min at pH 7.0 and 37°C in solution) (6) increased B-MCC binding to I-Ek by a factor of 2 and 3, respectively, after a 30-min incubation when compared with untreated cells (data not shown). It was also found that Ii 85-99 M90L M98L bound ~10 times more to cell surface I-Ek than Ii 85-99 (data not shown), reflecting its greater affinity for I-Ek (11). On the basis of these results and the need to generate the largest possible amount of active I-Ek quickly, we used the two-step preloading procedure described in Materials and Methods. This procedure was found to be useful in terms of preparing a 5-fold enhanced concentration of I-Ek loaded with Ii 85-99 as compared with no preloading.

The physical chemistry of the two-step preloading procedure is complex. The overall reaction stoichiometry (not mechanism) during the overnight incubation is

\[
M_i + p \rightleftharpoons pM_i
\]

The peptide equilibrium binding constants for this reaction are rather small. The binding constants can be estimated as follows. The rate of inactivation for \(M_i \rightarrow M_s\) is 0.049 min⁻¹, as discussed later, and the rate activation for \(M_i \rightarrow M_s\) is 0.00044 min⁻¹ (6). From these values it can be seen that the equilibrium constants for the reaction \(M_i + p \rightleftharpoons pM_i\) are 2 orders of magnitude smaller than for \(M_s + p \rightleftharpoons pM_s\).

The data given later in this paper enable one to estimate the equilibrium constants for the reaction \(M_i + p \rightleftharpoons pM_i\) at pH 7.0 to be \(3 \times 10^4 \text{ M}^{-1}\) for Ii 85-99 and \(8 \times 10^3 \text{ M}^{-1}\) for Ii 85-99 M90L M98L. Thus, the binding of neither peptide is saturated at the concentration used (200 μM). During the second exchange step, where Ii 85-99 M90L M98L is replaced by Ii 85-99, the system achieves equilibrium by a kinetic pathway distinct from that compared with loading with Ii 85-99 alone. This likely accounts for the modest relative enhancement of 85-99 binding in the two-step procedure.

Fig. 3A shows an ~5-fold enhancement in B-MCC binding following the two-step preloading procedure, whereas total levels of I-Ek (see Materials and Methods) remained constant (data not shown), suggesting that the dissociation of the prebound peptide resulted in the formation of active I-Ek.

To determine whether the kinetic properties of active I-Ek on the cell surface are similar to those in solution, we investigated the stability of I-Ek generated by the preloading procedure. Preloaded CHO-Ek cells were incubated in the absence of peptide for several time periods. Cells were then exposed to 50 μM B-MCC for 8 min to estimate the amount of active I-Ek present. Fig. 3B shows that active I-Ek decreases rapidly when cells are incubated in the absence of exogenous peptide. This decrease in B-MCC binding indicates that active I-Ek rapidly inactivates following peptide dissociation. By fitting the data in Fig. 3B to Equation 4 derived from Scheme I (6) (see Materials and Methods) with a \(k_{in} = 0.2 \text{ min}^{-1}\) for Ii 85-99 (τ1/2 = 3.5 min), one can estimate an inactivation rate constant \(k_{in} = 0.049 \text{ min}^{-1} \pm 0.01 \text{ min}^{-1}\). (τ1/2 = 14 min).

Apparent association rate of MCC to I-Ek

The apparent association rate constant for B-MCC binding to I-Ek on CHO-Ek cells was determined by measuring the concentration dependence of peptide binding following an 8-min incubation. Experiments were performed with both preloaded and untreated cells. The association rate of B-MCC to I-Ek for preloaded cells was calculated by fitting the data in Fig. 3A to Equation 9 (see Materials and Methods), with \(t = 8\) min and peptide concentration variable. Because the rate of dissociation of endogenous peptides

![FIGURE 1. Specificity of B-MCC binding to cell surface I-Ek. Binding of B-MCC, even at high concentrations, only occurs on cells expressing I-Ek. Cells were exposed to 400 μM B-MCC for 8 min followed by streptavidin-R-PE labeling. Fluorescence was measured by FACS. Data represent one of three experiments.](http://www.jimmunol.org/)

![FIGURE 2. Dissociation of peptide from cell surface I-Ek. Dissociation of B-Ii 85-99 from I-Ek on the surface of CHO-Ek cells measured by FACS. B-Ii 85-99/I-Ek complexes were allowed to dissociate for the indicated times in the presence of 1 μM MCC 95-103 competitor peptide. Cells were stained with streptavidin-R-PE after dissociation. Data represent the average of two experiments performed on the same day. Data points were corrected for autofluorescence of 7 U. Error bars show the SD. The solid line indicates a single exponential fit to the plotted data.](http://www.jimmunol.org/)
from I-Ek is unknown, an apparent association rate constant of B-MCC to I-Ek for untreated cells was calculated by fitting the data to a single exponential curve. As a control, the data for preloaded cells were also fit to a single exponential curve. Fig. 3A shows that although the saturation levels are higher for preloaded cells, the apparent association rate constants obtained from the curve fits are comparable, with approximate values of 25 M⁻¹ s⁻¹ for untreated cells, and 35 M⁻¹ s⁻¹ (from single exponential curve) to 60 M⁻¹ s⁻¹ (from Equation 9) for preloaded cells.

**Number of active MHC molecules present on the surface of CHO-Ek cells**

To determine the number of active I-Ek molecules on CHO-Ek cells, cells were exposed to 50 μM B-MCC at pH 7.2 and 37°C for several short time periods. The goal was to expose cells to a sufficient concentration of peptide to quickly bind all active I-Ek molecules. The fluorescence intensity was calibrated as the number of molecules of equivalent soluble fluorochrome (MESF) using a Quantum R-PE kit from Flow Cytometry Standards. The MESF was used as a crude measure of the number of active I-Ek molecules present on the cell surface.

Fig. 4 shows the average of two experiments performed simultaneously (to avoid small day-to-day variations in the levels of active I-Ek). Each data point reflects the number of active I-Ek molecules present. For untreated cells, the data can be fit to a line with a slope giving the rate of formation of active molecules, and an intercept at t = 0 equivalent to the number of active I-Ek molecules at steady state. We estimate a minimum of 2600 active I-Ek molecules present at steady state and a rate of formation of 440 active molecules per minute.

Our finding that there are <1% active I-Ek molecules on CHO-Ek cells is reminiscent of studies showing that splenic and peritoneal B cells and macrophages express little empty active class II MHC on their surface (12).

**Effect of transport and Ag processing inhibitors on I-Ek**

A series of transport and Ag processing inhibitors were used to determine the origin of the active pool of surface MHC on cells. Brefeldin A reversibly disassembles the Golgi complex (13) preventing transport of Golgi-derived vesicles. Sodium azide inhibits mitochondrial respiration preventing energy-requiring processes (14). Chloroquine inhibits Ag processing and presentation of class II MHC on their surface (12).

**FIGURE 3.** Peptide binding properties of active I-Ek on the cell surface correlate with those of purified soluble active I-Ek. A. Association of B-MCC to I-Ek on the surface of CHO-Ek cells. Cells were exposed to the indicated concentrations of B-MCC for 8 min and stained with streptavidin-R-PE. Fluorescence was measured by FACS. The mean fluorescence was divided by the autofluorescence to obtain data points. The solid line represents a data fit to Equation 9 (see Materials and Methods) for preloaded cells, whereas the dashed line represents a single exponential fit to the data for untreated cells. B. Rapid inactivation of active I-Ek on the surface of CHO-Ek cells. Inactivation of active I-Ek measured by FACS. Ii 85-99/I-Ek complexes on the surface of CHO-Ek cells were allowed to dissociate for the indicated “delay times” in the absence of added peptide. The amount of active I-Ek present after the delay was measured by binding of 50 μM B-MCC for 8 min followed by streptavidin-R-PE staining. Data points were corrected for a background fluorescence of 7 U. Data represent the average of three experiments. Error bars indicate the SD. The solid line represents a data fit to Equation 4 (see Materials and Methods).

**FIGURE 4.** Quantitation of active I-Ek and its rate of formation on the cell surface. CHO-Ek cells were incubated with 50 μM B-MCC for the indicated times followed by streptavidin-R-PE labeling. Fluorescence was measured by FACS and converted to MESF with a Quantum-PE kit. Data are the average of two experiments performed on the same day. The solid line is a linear fit to the data for the untreated cells. The dashed line is a linear fit plus a single exponential fit to the data for the preloaded cells because the rate of B-MCC binding is approximately equal to the rate of Ii 85-99 dissociation from I-Ek. Error bars indicate the SD.
II molecules (15) by raising the lysosomal pH, and cycloheximide is a protein synthesis inhibitor (14). We anticipated that each of the inhibitors would decrease the amount of active I-Ek on the cell surface.

CHO-Ek cells were exposed to inhibitors at pH 7.2, 37°C for 1 h. The amount of active I-Ek was quantified by incubation with 40 μM B-MCC for 10 min, and the total surface I-Ek expression was measured by exposure to R-PE 14.4.4S Ab. Fig. 5 shows that although the amount of active I-Ek on the cell surface decreased by an average of 40% following treatment with inhibitors, the total amount of I-Ek remained unchanged. (Similar results were obtained with CH27 B lymphomas, where active I-Ek was undetectable after treatment with inhibitors, whereas the total amount of I-Ek remained constant; data not shown). These results show that MHC molecules are still present at the cell surface after treatment with inhibitors and that a significant part of the active MHC derives from newly synthesized or recycled intracellular MHC. Assuming that the binding of peptide to inhibitor-treated CHO-Ek cells follows a line similar to that in Fig. 4 (open circles), except with a lower slope, we infer that this source of active I-Ek would arise from release of endogenous peptide, or reactivation. Rough calculations indicate that reactivation dominates this source of active I-Ek for inhibitor-treated cells. (We have neglected reactivation in the analysis of experiments involving preloading because this source of active I-Ek is rather small).

To confirm that the ability of MHC to bind peptide was not affected by the treatment with inhibitors, CHO-Ek cells were preloaded as previously described to generate relatively high levels of active I-Ek. Inhibitors were added for the last hour of preloading. Cells were then allowed to bind to 40 μM B-MCC for 10 min. As seen in Fig. 5, B-MCC bound I-Ek normally. The effect of the inhibitors was not detectable when relatively large amounts of active I-Ek were generated by preloading, suggesting that the steady-state number of active I-Ek molecules is a small fraction of the total I-Ek on the cell surface.

Discussion

This report provides evidence for the existence of two isomeric forms of empty I-Ek at the cell surface: an active isomer that binds peptide rapidly and an inactive isomer that does not bind peptide. It is found that many characteristics of soluble active I-Ek are comparable to active I-Ek on the plasma membrane. For example, the peptide association and dissociation kinetics at the cell surface resemble those measured in solution.

The dissociation rates for Ii 85-99/I-Ek in solution and on the cell surface differ by a factor of 3. The dissociation of B-li 85-99 follows a single exponential decay with a rate constant koff of 0.2 min⁻¹ from I-Ek on the cell surface, compared with a koff of 0.077 min⁻¹ for water-soluble I-Ek.

The apparent association rate constants of B-MCC to I-Ek on the cell surface are ~25 M⁻¹ s⁻¹ for untreated cells and 60 M⁻¹ s⁻¹ for preloaded cells. Due to the errors involved in the inactivation and dissociation rate constants used to determine koff, the values of these apparent association rate constants should be taken as rough estimates. Because the koff values for untreated and preloaded cells are comparable, we infer that there is only one form of active I-Ek present on CHO-Ek cells. The cell surface apparent association rate constants are approximately 1 order of magnitude slower than that obtained for water-soluble I-Ek and MCC peptide at pH 7.0 (6, 11).

Analogous to solution studies, we find that in the absence of peptide, active I-Ek is unstable, decaying to the inactive isomer with an approximate half-life of 14 min. One possible role of inactivation might be to maintain a steady but low number of peptide-receptive MHC molecules, facilitating the generation of a diverse repertoire of peptide/MHC complexes for T cell presentation while preventing the binding of a large number of self-peptides for which tolerance is weak or not established.

Consistent with studies indicating that B cells and macrophages express little peptide-receptive MHC on their plasma membrane (12), we find that at steady state ~2600 I-Ek molecules are active, representing <1% of the total MHC present in CHO-Ek cells. A substantial fraction of these active I-Ek molecules must derive from intracellular pools as well as reactivation. From the steady-state number of active I-Ek molecules one estimates the minimum number of peptide/MHC complexes required to trigger T cell activation. Using the cytosensor microphysiometer as a measure of early T cell activation, Beeson et al. (4) demonstrated that a 90-s exposure to 10 nM MCC 88-103 was sufficient to induce a detectable acid release in a mixture of 5CC7 T cells and CHO-Ek cells. However, a 1 μM MCC 88-103 concentration was required for maximal acidification response. From these data and an association rate for B-MCC to I-Ek of 10² M⁻¹ s⁻¹, we estimate that ~0.3 peptide/MHC complexes per APC are required for initiation of detectable signaling and ~30 peptide/MHC complexes are required for maximal early response. Our results agree with previous reports indicating that few peptide/MHC complexes are required to trigger T cell activation (16-21).

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References


