Dimerization kinetics of the IgE-class antibodies by divalent haptens. II. The interactions between intact IgE and haptens

ARTICLE *in* BIOPHYSICAL JOURNAL · SEPTEMBER 1992

Impact Factor: 3.97 · DOI: 10.1016/S0006-3495(92)81610-7 · Source: PubMed

3 AUTHORS, INCLUDING:

Reinhard Schweitzer-Stenner
Drexel University
219 PUBLICATIONS **4,368** CITATIONS

SEE PROFILE

Dimerization kinetics of the IgE-class antibodies by divalent haptens

II. The interactions between intact IgE and haptens

Reinhard Schweitzer-Stenner,* Arieh Licht‡ and Israel Pecht‡

*Institute of Experimental Physics, University of Bremen, 2800 Bremen 33, Germany; and
‡Department of Chemical The Weizmann Institute of Science, Rehovot 76100, Israel

ABSTRACT Interactions between a monoclonal, DNP-specific IgE molecules (hybridoma A2) and divalent DNP-haptens in solution cause aggregation of the former predominantly into closed rings of two IgE and two divalent haptens (Schweitzer-Stenner, R., A. Licht, I. Lüscher, and I. Pecht. 1987. *Biochemistry.* 26:3602–3612). The time course of this process was now investigated by titrating the A2-IgE with divalent DNP-haptens having long and rigid oligoproline spacers (di(N$^{\epsilon}$-2,4-dinitrophenyl)-6-amino-hexanoate-aspartyl-(prolyl)$_n$-L-lysyl; n = 24, 27, 33). Binding was expressed in quenching of the IgE intrinsic tryptophan emission. As shown in the preceding paper, hapten addition to the IgE-A2 at rates faster than a distinct threshold value led to nonequilibrium titrations (NETs) from which kinetic processes slower than 2 s^{-1} can be resolved. Analysis of these titrations shows that the dimeric rings open at rates of $\approx 10^{-2}$ s^{-1}, independent of the divalent hapten's spacer length. The ring closure rate, however, decreases with spacer length. The latter observation was qualitatively rationalized in terms of the diffusion process of a Gaussian chain which relates the ring closure rate constant to the expectation value for the distance between the free ends of the respective open chain.

INTRODUCTION

The primary event initiating the cascade that culminates in mast cells and basophils secretion is the clustering of IgE-class antibodies bound to their type I Fc$_\epsilon$-receptor (Fc$_\epsilon$RI) by multivalent antigens (Siraganian et al., 1975; Segal et al., 1977; Metzger, 1977, Barsumian et al., 1981; Balakrishnan et al., 1982; Ishizaka and Ishizaka, 1984). Our aim is understanding the parameters that determine the efficiency of the stimulatory signal of IgE-Fc$_\epsilon$ RI clusters. One such potential parameter is the lifetime of an Fc$_\epsilon$ RI cluster (DeLisi, 1980), which is a function of its respective formation and disaggregation rate constants. The reaction of divalent DNP-haptens with monoclonal DNP-specific IgE antibodies produced by the hybridoma A2 (IgE-A2; Rudolph et al., 1981) was earlier shown to yield both open and closed dimers (Schweitzer-Stenner et al., 1987). Their respective mole fractions depended on the equilibrium constants of the distinct reaction steps of these processes. The steps leading to IgE dimerization and the labeling of the corresponding equilibrium constants are summarized in Fig. 1.

Some of the kinetic aspects of these reactions were investigated by fluorescence titrations using various hapten addition rates (Schweitzer-Stenner et al., 1992; preceding paper in this issue, herein refered to as paper I). We observe that if one of the IgE-divalent hapten reaction steps is slower than 2 s^{-1}, the titration does not reach a state of equilibrium. From the nonequilibrium titrations (NET) kinetic parameters of the slow processes can be derived. In order to resolve among the distinct reaction steps shown in Fig. 1, we first investigated the intrinsic binding of monovalent haptens to Fab fragments of IgE. It turned out that the reactants in these titrations reach equilibrium independent of the hapten addition rate. This suggested that the corresponding association and dissociation rate constants are larger than 10^7 M^{-1} s^{-1} and 2 s^{-1}, respectively. The binding of divalent haptens to the Fab fragments yields a mixture of monomeric and dimeric complexes. The latter can be regarded as simple models for the open dimers formed in the reaction of intact IgE with divalent haptens. We found that the divalent haptens with short spacers (Γ = 16–21 Å) cause the slow formation of Fab-dimers (i.e., rates between 10^{-2} and 10^{-3} s^{-1}), whereas haptens with longer spacers (Γ > 45 Å) effect relatively fast dimerization with a time constant larger than 2 s^{-1}.

Preliminary NETs of intact IgE with divalent DNP-haptens with long and rigid spacers (i.e., di(N$^{\epsilon}$-2,4-dinitrophenyl)-6-aminohexanoate-aspartyl-(prolyl)$_n$-L-lysyl (bis(DNP)-(pro)$_n$); n = 24, 27, 33; Γ = 110–130 Å) to IgE have shown, however, that a slow step is involved in these interactions (Schweitzer-Stenner et al., 1987). Since the rate of the corresponding Fab-dimerization has been shown to be faster than 2 s^{-1} (paper I) this slow step can be assigned to the closure and opening of dimeric rings rather than to the formation of the corresponding open dimers. Here we report kinetic measurements of the formation of these dimeric rings by NETs of monoclonal DNP-specific A2-IgE by the divalent haptens bis(DNP)-(pro)$_n$ (n = 24, 27, 33). Analysis of the data yielded the rate constants of closure and opening of dimeric rings.

Abbreviations used in this paper: NET, nonequilibrium titration; DNP, 2,4 dinitrophenyl; (pro)$_n$, polypeptide containing n L-prolines; DNP-(pro)$_{21}$, N$^{\epsilon}$-2,4-dinitrophenyl-6-amino-hexanoate-L-aspartate-(prolyl)$_{21}$-OH; bis(DNP)-(pro)$_n$, di(N$^{\epsilon}$-2,4-dinitrophenyl)-6-aminohexanoate-L-aspartate-(prolyl)$_{21}$-lysyl; (DCT)$_2$-cystine, bis[[[(N$^{\epsilon}$-2,4-dinitrophenyl)amino]caproyl-L-tyrosyl]cystine; RMS, root mean square.

MATERIALS AND METHODS

In the preceding paper (paper I) we have described in detail the isolation and characterization of the DNP-specific IgE-A2 antibodies, the

0006-3495/92/08/563/06 $2.00

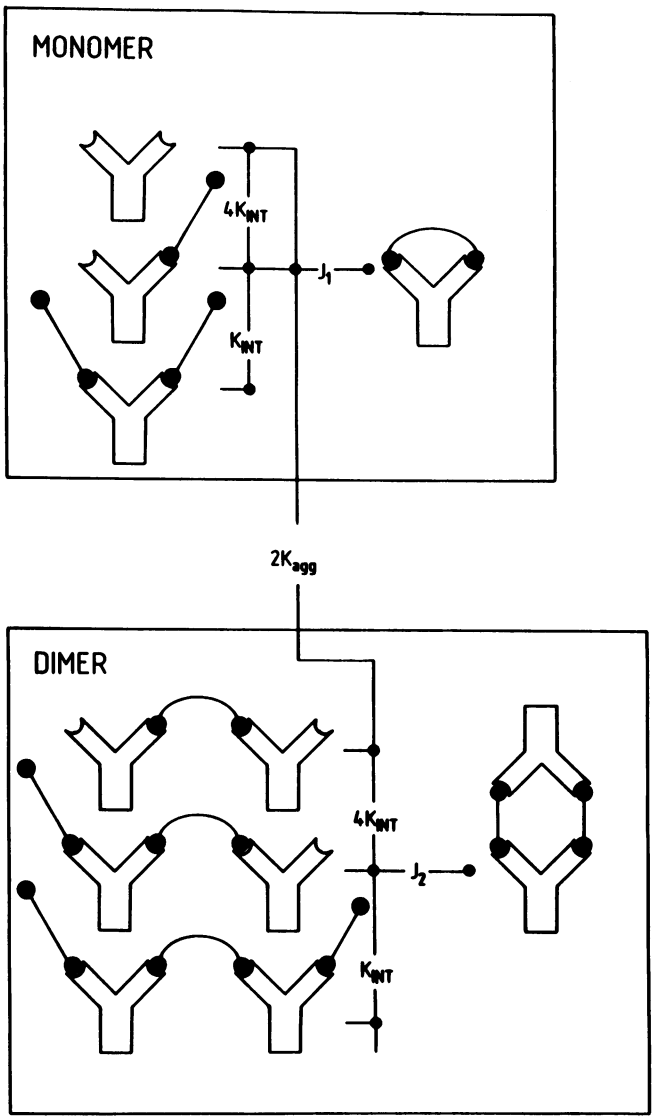

FIGURE 1 Reaction scheme between IgE-class antibodies and divalent haptens. J_1 and J_2 denote the equilibrium constants of the monomeric and dimeric ring closure steps, respectively. The scheme is taken from Schweitzer-Stenner et al. (1987).

preparation of the ligands, and the methodology of employing NETs and equilibrium titrations for the kinetic analysis of divalent hapten-IgE-A2 interaction.

THEORETICAL METHODS

Interactions between intact IgE and divalent haptens

Dembo and Goldstein (1978a) have developed a theory designed to calculate the equilibrium concentrations of the different species produced upon IgE-A2-(divalent) hapten interaction (cf. Fig. 1). A detailed description of our application of that theory has already been reported (Schweitzer-Stenner et al., 1987). Experiments using monovalent Fab fragments provided evidence that the corresponding formation and dissociation of Fab dimers by divalent haptens with spacers longer than 40 Å proceeds at comparatively fast rates ($k_{agg}^- > 2\,s^{-1}$) and cannot be resolved by NET. Consequently, the ring closure remains the only possible candidate for the slow step in the reaction between intact IgE and these divalent haptens. Its time course can be calculated by solving the differential equation:

$$d[D]_r(t)/dt = -j_2^-[D]_c(t) + j_2^+[D]_0(t), \quad (1)$$

where $[D]_r$ and $[D]_0$ are concentrations of closed and open dimer, respectively, and j_2^- and j_2^- denote the rate constants of the formation and opening of closed dimer. If the preceding steps in the hapten-antibody reaction are at equilibrium throughout the titration process, the open dimer concentration can be expressed in terms of the free hapten and antibody concentrations using the theory described before (Schweitzer-Stenner et al., 1987). This leads to:

$$dD_r(t)/dt = -j_2^- D_r(t) + 32 j^+ 2K_{int}^2 K_{agg}[H]_f^2(t)[A]_f^2(t), \quad (2)$$

where K_{int} denotes the equilibrium constant of the binding of one hapten to a Fab site. The equilibrium constant $2 \cdot K_{agg}$ is related to the binding of a divalent hapten already bound at one end to an IgE to another IgE, thus yielding an open dimer (cf. Fig. 1). $[A]_f$ and $[H]_f$ are the concentrations of free IgE and free (divalent) hapten, respectively.

We started the iterative treatment of Eq. 2, assuming that the initial concentrations of free hapten and antibody present at the time t do not change upon closed dimers formation in the interval δt. They are derived by the solving the following nonlinear equations using a two-dimensional version of the Newton-Raphson method (Margenau and Murphy, 1956):

$$[A]_T = \{[A]_f + 16K_{int}K_{agg}[H]_f(t)[A]_f(t)\} \times \{1 + 2K_{int}[H]_f(t)\}^2 + 2[D]_r(t) \quad (3a)$$

$$[H]_T = [H]_f(t)\{1 + 4K_{int}[A]_f(t) + 8K_{int}^2[H]_f(t)[A]_f(t)\} + 8K_{int}K_{agg}[H]_f(t)[A]_f^2(t) \times \{1 + 8K_{int}[H]_f(t) + 12K_{int}^2[H]_f^2(t)\} + 2[D]_r(t). \quad (3b)$$

To obtain a first guess for the concentration of closed dimers $[D]_r$ formed at the end of a time interval δt, the thus calculated $[H]_f$ and $[A]_f(t)$ values must be inserted into:

$$[D]_{r1}(t + \delta t) = 32J_2K_{int}^2K_{agg}[H]_{f0}(t)^2[A]_{f0}^2(t) \times \{1 - \exp(-j_2^-\delta t)\} + [D]_{r0}(t)\exp(-j_2^-\delta t), \quad (4)$$

where J_2 is the equilibrium constant of the ring closure (cf. Fig. 1) and $[D]_{r0}$ is the initial concentration of closed dimers. Subsequently, the calculated value for $[D]_{r1}(t + \delta t)$ was inserted into Eqs. 3a and 3b to recalculate the free reactants concentrations, i.e., $[H]_{f1}$ and $[A]_{f1}$. These were then used in Eq. 4 to recalculate $[D]_{r2}(t + \delta t)$. The iterative procedure was stopped upon convergence. The corresponding criterion was $|[D]_{ri} - [D]_{ri-1}| < 0.01[D]_{ri}$. The fractions X_j of Fab-binding sites occupied by a hapten ($j = 1$), or by a hapten antibody complex in an open ($j = 2$) or closed dimer ($j = 3$) are expressed by:

$$X_1(t + \delta t) = 4K_{int}[H]_f(t + \delta t)[A]_f(t + \delta t) \times \{1 + 2K_{int}[H]_f(t + \delta t) + 32K_{int}^2K_{agg}[H]_f(t + \delta t) \times [A]_f(t + \delta t)^2\}/[A]_T + 64K_{int}^3K_{agg}[H]_f(t + \delta t)^2 \times [A]_f(t + \delta t)^2/[A]_T \quad (5a)$$

$$X_2(t + \delta t) = 16K_{int}K_{agg}[H]_f(t + \delta t)[A]_f^2(t + \delta t)/[A]_T \quad (5b)$$

$$X_3(t + \delta t) = 4[D]_r(t + \delta t)/[A]_T. \quad (5c)$$

To calculate the fluorescence titration curve, one inserts Eqs. 5a–5c into:

TABLE 1 **Best fitting parameters describing the interactions between divalent DNP-haptens and the DNP-specific, monoclonal IgE-A2**

r_H [mol/s]	K_{int} [M^{-1}]	K_{agg} [M^{-1}]	J_2	j_2^+ [s^{-1}]	j_2^- [s^{-1}]	q_1	q_3
I) bis(DNP)-$(pro)_{24}$							
I_1: $1.8 \cdot 10^{-13}$	$1 \pm 0.1 \cdot 10^7$	$3.6 \pm 1.0 \cdot 10^6$	82 ± 10	0.8 ± 0.1	10^{-2}	0.33	0.37
I_2: $8.7 \cdot 10^{-13}$	$1 \pm 0.1 \cdot 10^7$	$3.6 \pm 1.0 \cdot 10^6$	82 ± 10	0.8 ± 0.1	10^{-2}	0.33	0.37
II) bis(DNP)-$(pro)_{27}$							
II_1: $1.8 \cdot 10^{-13}$	$8.8 \pm 1.5 \cdot 10^6$	$3.3 \pm 1.0 \cdot 10^6$	62 ± 10	0.6 ± 0.1	10^{-2}	0.33	0.37
II_2: $8.7 \cdot 10^{-13}$	$8.8 \pm 1.5 \cdot 10^6$	$3.3 \pm 1.0 \cdot 10^6$	62 ± 10	0.6 ± 0.1	10^{-2}	0.33	0.37
III) bis(DNP)-$(pro)_{33}$							
II_1: $1.8 \cdot 10^{-13}$	$1.0 \pm 1.0 \cdot 10^7$	$2.6 \pm 1.0 \cdot 10^6$	7 ± 2	0.07 ± 0.02	10^{-2}	0.32	0.42
II_2: $8.7 \cdot 10^{-13}$	$1.0 \pm 1.0 \cdot 10^6$	$2.6 \pm 1.0 \cdot 10^6$	7 ± 2	0.07 ± 0.02	10^{-2}	0.33	0.37

Two different addition rates were employed in titrations using each hapten. The stock hapten ($[H]_s$) and antibody concentrations ($[A]_r$) were $5.9 \cdot 10^{-6}$ M and $1.25 \cdot 10^{-7}$ M for each experiment, respectively.

$$I([H]_T, [A]_T, t + \delta t) = I_{max}\left(1 - \sum_j \{q_j X_j(t + \delta t)\}\right), \quad (6)$$

where the sum Σ_j includes all hapten-IgE complexes and q_j are the respective quenching coefficients.

Fitting procedure

The rate constants of the closure (j_2^+) and the opening (j_2^-) of dimeric rings and the quenching coefficients q_i (i = 1, 2, 3) are used as free parameters in a fit to the experimental titration curves. The intrinsic binding constant K_{int} has been derived from titrations with the monovalent hapten N$^{\alpha}$-2,4-dinitrophenyl-6-aminohexanoate-L-aspartyl-$(prolyl)_{21}$-OH (DNP-$(pro)_{21}$).

The aggregation equilibrium constants K_{agg} were taken from the respective equilibrium titration reported earlier (Schweitzer-Stenner et al., 1987). Slight variations in both, K_{int} and K_{agg} were allowed for fine tuning the fits to the experimental data. For the fitting procedure we used a program called MINUITL obtained from the CERN library (James, 1972). It contains three different minimization subroutines, SEEK, SIMPLX, and MIGRAD designed to search a local minimum in the corresponding χ^2 function.

The procedure employed for the error analysis of the obtained parameter values is described in paper I.

RESULTS AND DISCUSSION

Brief description of the NETs

The intrinsic binding constant K_{int} of the A2-IgE interactions with the divalent haptens bis(DNP)-$(pro)_n$ was derived by titrating A2-IgE with the monovalent hapten DNP-$(pro)_{21}$. Analysis of the data yields $K_{int} = 1.2 \cdot 10^7$ M^{-1}, in good agreement with our earlier value (Schweitzer-Stenner et al., 1987). The titration curve could be fitted assuming that the binding capacity of the antibodies is practically 100% (data not shown).

NETs of IgE-A2 by the divalent haptens bis(DNP)-$(pro)_n$ (n = 24, 27, 33) were carried out at two different rates of hapten addition. The experimental parameters, i.e., total IgE-A2 concentration $[A]_T$, stock concentration $[H]_s$ of the employed haptens, and rate of hapten addition r_H, are listed in Table 1. The NETs by bis(DNP)-$(pro)_{27}$ and bis(DNP)-$(pro)_{33}$ are shown in Fig. 2. The ring opening rate constants, i.e., $j_2^- = 10^{-2}$ s^{-1}, derived from the fits (solid lines) were within the limit of accuracy, the same for all the haptens employed.

The ring closure constant j_2^+ was found to decrease with spacer length from 0.8 ± 0.1 s^{-1} for bis(DNP)-$(pro)_{24}$ to $7 \pm 2 \cdot 10^{-2}$ s^{-1} for bis(DNP-$(pro)_{33}$, thus causing a decrease in the respective equilibrium constant J_2, in full agreement with our earlier study (Schweitzer-Stenner et al., 1987). The values of the rate and equilibrium constants, the quenching coefficients, and their respective statistical errors, as derived from the fitting of the NETs, are presented in Table 1. It should be emphasized that the corresponding NETs performed with the same haptens could be fitted in terms of the very same parameter values.

Kinetics of IgE-ring closure

Open dimer formation by the divalent bis(DNP)-$(pro)_n$-haptens proceeds at a rate faster than 2 s^{-1}, probably due to its being free of orientational constraints (cf. paper I). In contrast, the corresponding ring closure rates are apparently slower than 2 s^{-1}. The rate constant of the latter decreases with increasing spacer length, whereas the corresponding dissociation rate constant remains nearly constant. This observation is consistent with the expectations from an intramolecular ring closure process. Provided that the process is diffusion controlled, the ring closure rate constant decreases with increasing distance between the free Fab site and the free DNP group of a bound divalent hapten. The opening rate constant, however, depends mainly on the properties of the encounter complex. It is reasonable to assume, that these parameters do not depend significantly on the spacer length of the haptens. In order to quantitatively assess the relation between the end-to-end distances r of the IgE-open dimer and the ring closure rate constants, we first calculated the RMS value of the latter in terms of the equilibrium constant of the ring closure process using the expression:

$$\langle r^2 \rangle_{dim}^{1/2} = \sqrt{(1.5\pi)(K_{agg}/\rho J_2)^{1/3}}, \quad (7)$$

derived by Schumaker et al. (1980). The factor $\rho = 0.6$ nm^{-3} M^{-1} has to be inserted in order to take into account the particle density in the standard state. Using the

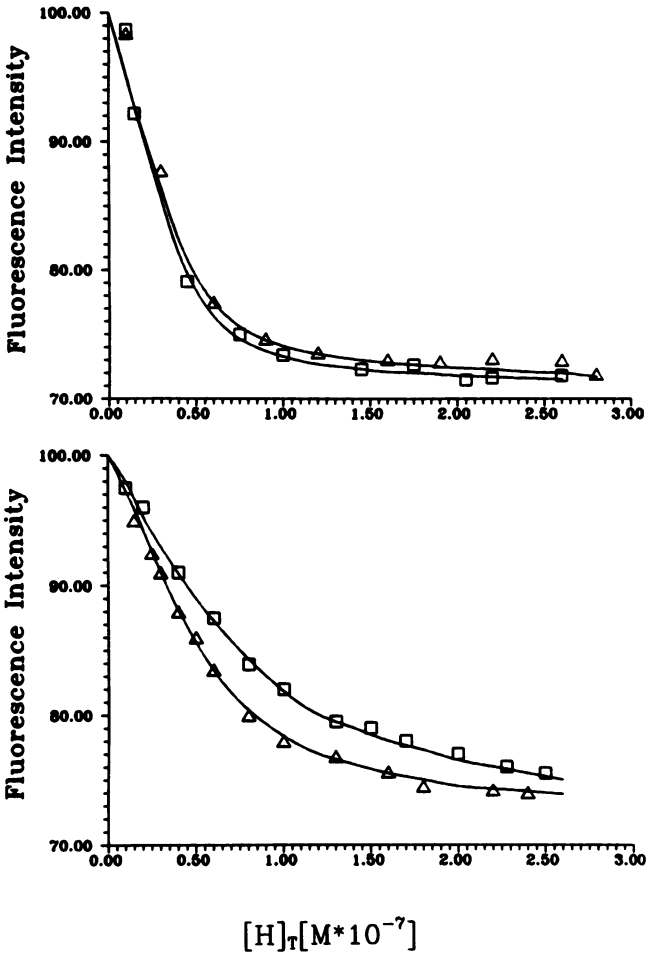

FIGURE 2 Fluorescence titrations of the IgE-A2 antibodies by the divalent hapten bis(DNP)-$(pro)_{27}$ (*upper panel;* $r_Q = 1.75 * 10^{-13}$ mol/s (□) and $r_Q = 8.7 * 10^{-13}$ mol/s (△)) and bis(DNP)-$(pro)_{37}$ (*lower panel;* $r_Q = 1.3 * 10^{-13}$ mol/s (△) and $r_Q = 8.7 * 10^{-13}$ mol/s (□)). The solid lines are calculated using the parameters derived from the fitting procedure and are listed in Table 1 (II_1, II_2, III_1, III_2). The representative data points (12 of 1,200) are taken from the fluorescence titrations.

parameter values listed in Table 1, one calculates $\langle r^2 \rangle_{dim}^{1/2}$ as 280, 340, and 480 Å for bis(DNP)-$(pro)_n$ with n = 24, 27, and 33, respectively.

The decrease in the ring closure rates with increasing spacer length can be rationalized in terms of a reaction theory developed by Szabo et al. (1980), which analyzes diffusion controlled intrachain reactions of polymers. The quantity of interest in the applied model is the function $\Sigma(t)$, the fraction of polymers yet unreacted at time t (i.e., the fraction of open dimers; cf. Fig. 1). It depends on the probability distribution $p(r, t)$ of finding the system at position r at time t:

$$\Sigma(t) = \int drp(r, t), \tag{8}$$

where the integral extends over the whole diffusion space and $p(r, t)$ obeys the Smoluchowski equation (Smoluchowski, 1917). In the following we choose $\Sigma(t)$ to be normalized so that $\Sigma(t = 0) = 1$.

As shown earlier by Archer and Krakauer (1977), polymers formed by antigen–antibody complexes can be represented by Gaussian chains. Assuming that this also holds for the open IgE dimers, their equilibrium end-to-end distribution $p_{eq}(r, t)$ is given by:

$$p_{eq}(|r|) = cr^2 \cdot \exp(-dr^2/2\langle r^2 \rangle_{dim}), \tag{9}$$

where c is a normalization constant and $d = 3$ for a three-dimensional polymer.

$\Sigma(t)$ can now be calculated by inserting Eq. 9 into Eq. 8 and by evaluating the thus derived integral. In principle this must be done by numerical methods for $d > 1$. If, however, the radius a of the reaction sphere is significantly smaller than the respective expectation value of the end-to-end distance (Eq. 7), the following approximation holds (Szabo et al., 1980):

$$\sum(t) = \exp(-t/\tau), \tag{10}$$

where the time constant τ is given by:

$$\tau = (j_2^+)^{-1}$$
$$= \{\sqrt{\pi}/2\alpha + (\ln 2 - 1) - \alpha\sqrt{\pi}/2 + 4\alpha^2/3)\}\langle r^2 \rangle_{dim}/3D, \tag{11}$$

and the factor α is written as:

$$\alpha = \sqrt{(d/2)}a/\langle r^2 \rangle_{dim}. \tag{12}$$

The explicit calculation of $\Sigma(t)$ requires knowledge of the diffusion coefficient D. This parameter, however, is physically ill-defined because the ring closure of an IgE dimer is subject to coupled translational and rotational diffusion processes of the distinct IgE domains and other connecting elements. We can estimate, however, the ratio R of the association rate constants j_2^+ for the different bis(DNP)-$(pro)_n$-IgE dimers. For this purpose we use Eqs. 10–12 to calculate:

$$R_{\tau n} = j_2^+(n)/j_2^+ \quad (n = 24), \tag{13}$$

for n = 27 and 33. Inserting the corresponding end-to-end distances calculated above (Eq. 46) yields $R_{\tau 27}$ = 0.60 and $R_{\tau 33}$ = 0.19. The respective ratios of the j_2^+ parameters obtained from the fluorescence titrations are $R_{\tau 27}$ = 0.75 and $R_{\tau 33}$ = 0.08. This shows that the employed model provides a qualitative rationale for our data even though a Gaussian chain is only a crude approximation to our system.

Comparison with other studies

The results presented in this and the preceding paper are in good agreement with results of the recent kinetic studies of IgE-divalent hapten interactions reported by Posner et al. (1991). These authors have measured and analyzed the dissociation of the complex formed be-

tween the divalent hapten bis[[{N$^{\epsilon}$-2,4-dinitrophenyl)-amino}caproyl]-L-tyrosyl]cystine ((DCT)$_2$-cystine)) and intact IgE-H1-26.82 in solution and derived two rate constants, namely $k_{-1} = 2.5 \cdot 10^{-2}$ s^{-1} and $k_{-2} = 1.3 \cdot 10^{-3}$ s^{-1}. Whereas k_{-1} was assigned to the dissociation of monomeric hapten-IgE complexes (in accordance with experiments of Goldstein et al., 1989), k_{-2} was attributed either to the dissociation of an open dimer or to the opening of a closed dimer.

In view of our results we would assign k_{-2} to the opening of a closed dimer rather than to the dissaggregation of an open dimer, because the spacer length of (DCT)$_2$-cystine was reported to be 49 Å, and our studies on the kinetics of Fab-divalent hapten interaction have suggested that the dimerization with haptens having $\Gamma > 40$ Å proceeds comparatively fast.

Possible biological relevance

The interest in a physico-chemical understanding of the oligomerization processes of antibodies by divalent haptens serving as well defined and simplest model antigens, is based primarily on their capacity to produce cellular response. In this case, the Fc$_\epsilon$RI-IgE clustering by the divalent haptens and the resultant mast cells secretory response were investigated rather early (Siraganian et al., 1975). The efforts to further extend this approach and determine its quantitative aspects in more rigorous fashion using the currently available monoclonal IgE class mAbs and the range of divalent haptens, met so far only with limited success. Thus, using the above oligoproline spaced divalent haptens to stimulate RBL-2H3 cells has shown that only very limited extent of mediator secretion could be obtained and even that only with the bis(DNP)-(pro)$_{33}$ hapten (Reck et al., 1985). Significantly, Kane et al. (1986) using the bivalent (DTC)$_2$-cystine hapten ($\Gamma \approx 50$ Å) have also observed a rather low secretory response of RBL-2H3-cells ($\approx$33%). One possible rationale for this low stimulatory capacity of divalent haptens may be provided by experiments (Fewtrell and Metzger, 1980), suggesting that Fc$_\epsilon$RI-dimers are inefficient in triggering RBL-2H3 secretory response. Ortega et al. (1988) have shown, however, that distinct monoclonal antibodies specific to the α-subunit of the Fc$_\epsilon$RI can be efficient in triggering release, even though the maximal aggregate formed upon their binding to Fc$_\epsilon$RI is a dimer. Thus, the low degranulation capacity of hapten-IgE complexes cannot be explained solely by their dimeric size.

The results presented in this and the preceding study provide a hint for an alternative parameter that determines the stimulatory efficiency of Fc$_\epsilon$RI oligomers, namely their lifetime. Even though the IgE-dimers dissociate much slower than the corresponding monomeric hapten complexes, their dissociation rates are still faster than those of the Fc$_\epsilon$RI-dimers formed upon binding of the Fc$_\epsilon$RI by specific monoclonal antibodies (F4 and J17), which are both considerably more effective in inducing RBL cell secretion (Ortega et al., 1988, 1991; Schweitzer-Stenner et al., 1991). Further investigations of the kinetics of open Fc$_\epsilon$RI-IgE dimer formation and ring closure by divalent haptens are necessary in order to clarify which parameters determine the efficiency of the secretory response. Such studies could provide insights into the dynamics of IgE-oligomerization in the two dimension of the cell surface in comparison to those in the homogenous solution.

The authors are indebted to Professor Wolfgang Dreybrodt, Dr. Ulrich Kubitscheck, Dr. Ulrich Pilatus, and Diplom-Physiker Martin Kircheis for intensive, critical, and illuminating discussions.

The experimental part of this work was carried out while Reinhard Schweitzer-Stenner was a recipient of a short term MINERVA fellowship. The generous support of the research reported in this paper by the government of Lower Saxony, FRG, and by the Council of Tobacco Research Council USA Inc. is gratefully acknowledged.

Received for publication 9 January 1992 and in final form 26 March 1992.

REFERENCES

Archer, B. G., and H. Krakauer. 1977. Thermodynamics of antibody-antigen reactions. 2. The binding of bivalent synthetic random coil antigens to antibodies having different antigen precipitating properties. *Biochemistry.* 16:618–627.

Balakrishnan, K., T. J. Hsu, A. D. Cooper, and H. M. McConnel. 1982. Lipid hapten containing membrane targets can trigger specific immunoglobulin E-dependent degranulation of rat basophilic leukemia cells. *J. Biol. Chem.* 257:6427–6433.

Barsumian, E. L., C. Isersky, M. G. Petrino, and R. Siraganian. 1981. IgE induces histamine release from rat basophilic leukemia cell lines. *Eur. J. Immunol.* 11:317–323.

DeLisi, C. 1980. The biophysics of ligand-receptor interactions. *Q. Rev. Biophys.* 13:201–230.

Dembo, M., and B. Goldstein. 1978. Theory of equilibrium binding of symmetric bivalent haptens to cell surface antibody: application of histamine release from basophils. *J. Immunol.* 121:345–353.

Goldstein, B., R. G. Posner, D. C. Torney, J. Erickson, D. Holowka, and B. Baird. 1989. Competition between solution and cell surface receptors for ligand. Dissociation of hapten bound to surface antibody in the presence of solution antibody. *Biophys. J.* 56:955–966.

Ishizaka, T. and K. Ishizaka. 1975. Cell surface IgE on human basophil granulocytes. *Ann. NY. Acad. Sci.* 254:462–475.

James, F. 1972. Function minimization: Proceedings of the 1972 Cern-Computing and Data-Processing School. Pertisau, Austria. 72–121.

Kane, P., J. W. Erickson, C. Fewtrell, B. Baird, and D. Holowka. 1986. Cross-linking of IgE-receptor complexes at the cell surface: synthesis and characterization of a long bivalent hapten that is capable of triggering mast cells and rat basophilic leukemia cells. *Mol. Immunol.* 23:783–790.

Margenau, H., and G. M. Murphy. 1956. The Mathematics of Physics and Chemistry, Van Nostrand, New York.

Metzger, H. 1977. Receptors and Recognition. A. Series, P. Cuatrceasas, and M. F. Greaves, editors. Chapman and Hall Ltd., London. Vol. 4: 75–102.

Ortega, E., R. Schweitzer-Stenner, and I. Pecht. 1988. Possible orientational constraints determine secretory signals induced by aggregation of IgE receptors on mast cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:4101–4109.

Ortega, E., R. Schweitzer-Stenner, and I. Pecht. 1991. Kinetics of li-

gand binding to the type I Fc_ϵ receptor on mast cells. *Biochemistry.* 30:3473–3483.

Posner, R. G., J. W. Erickson, D. Holowka, B. Baird, and B. Goldstein. 1991. Dissociation kinetics of bivalent ligand-immunoglobulin E aggregation in solution. *Biochemistry.* 30:2348–2356.

Reck, B., R. Sagi-Eisenberg, and I. Pecht. 1985. Bytosolic free Ca^{2+} in mast cells and their mediators release. Proceedings of the XII International Congress of Allergology and Clinical Immunology, Mosby Company. 164–169.

Rudolph, A. K., P. D. Wahl, and M. R. Wahl. 1981. Thirteen hybridomas secreting hapten-specific immunoglobulin E from mice with Ig^a and Ig^b heavy chain haplotype. *Eur. J. Immunol.* 11:527–529.

Schumaker, V. N., G. W. Seegan, C. A. Smith, K. Ma, J. D. Rodwell, and M. F. Schumaker. 1980. The free energy of angular position of the Fab-arms of IgG-antibody. *Mol. Immunol.* 17:413–423.

Schweitzer-Stenner, R., A. Licht, I. Lüscher, and I. Pecht. 1987. Oligomerization and ring closure of immunoglobulin E class antibodies by divalent haptens. *Biochemistry.* 26:3602–3612.

Schweitzer-Stenner, R., A. Licht, and I. Pecht. 1992. Dimerization kinetics of the IgE-class antibodies by divalent haptens. I. The Fab-hapten interactions. *Biophys. J.* 63:550–561.

Schweitzer-Stenner, R., E. Ortega, and I. Pecht. 1991. Kinetics of the formation of the $Fc_\epsilon RI$-dimers on mast cells as caused by the binding of monoclonal receptor specific antibodies. *Biophys. J.* 351a. (Abstr.)

Segal, D. M., J. D. Taurog, and H. Metzger. 1977. Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. *Proc. Natl. Acad. Sci. USA.* 74:2993–2997.

Siraganian, R. P., W. A. Hook, and B. B. Levine. 1975. Specific in vitro histamine release from basophils by bivalent haptens: evidence for activation by simple bridging of membrane bound antibody. *Immunochemistry.* 12:149–154.

Smoluchowski, M. V. 1917. Versuch einer mathematischen Theorie der Koagulationskinetik kolloider Lösungen. *Z. Phys. Chem.* 92:129–168.

Szabo, A., K. Schulten, and Z. Schulten. 1980. First passage time approach to diffusion controlled reactions. *J. Chem. Phys.* 72:4351–4357.