Proc. Natl. Acad. Sci. USA
Vol. 74, No. 3, pp. 869–872, March 1977
Biochemistry

Molecular weight and valence of the cell-surface receptor for immunoglobulin E

(mast cell/basophil/Fc receptor/membrane receptor/nonionic detergent)

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Communicated by C. B. Anfinsen, December 2, 1976

ABSTRACT **The molecular weight of the active solubilized cell-surface receptor for immunoglobulin E (IgE) was measured in nonionic detergent. The diffusion coefficient was estimated by gel filtration, the partial specific volume was estimated from the differential sedimentation in sucrose gradients prepared from H_2O and D_2O, and the sedimentation constant was estimated from the same centrifugation experiments. The receptor has an apparent molecular weight of 130,000. Its high partial specific volume (0.81 cm^3/g) suggests that bound detergent contributes significantly to the mass. The molecular weights of the receptor–IgE complex and of unbound IgE determined similarly were 310,000 and 200,000 respectively, clearly showing that the receptor is univalent. The implications of these results for the subunit structure of the receptor, receptor–membrane integration, and a possible mechanism of receptor triggering are discussed.**

The surface membrane of normal and certain tumor mast cells and basophils contains a component that specifically binds IgE, and which putatively acts as a receptor for antigen-induced, IgE-mediated cell degranulation (1). That the receptor can be iodinated (2), that radioactive amino acids and sugars can be incorporated into it (3), that it is sensitive to proteases (2, 3) and moderate changes in temperature and pH (4)—all suggest it is a glycoprotein. Its activity is unchanged by exposure to a variety of lipases (G. Rossi, unpublished observations). The denatured receptor in sodium dodecyl sulfate exhibits a single band of apparent molecular weight 50–60,000 on polyacrylamide gel electrophoresis (refs. 3 and 5; J. Taurog and H. Metzger, unpublished observations) but gel filtration experiments on the solubilized active receptor in nonionic detergents suggested a substantially higher molecular weight (6). This paper describes experiments designed to determine the molecular weight and valence of the active receptor.

MATERIALS AND METHODS

Receptor Preparation. Receptor-containing extracts were prepared by suspending washed, pelleted rat basophilic leukemia cells (7) in 0.5% or 1% Nonidet P-40 (Particle Data, Inc. Elmhurst, Ill.) in 0.2 M borate-buffered saline, pH 8.0, as described previously (2, 6). When not used immediately, preparations were stored at −90°.

Marker Proteins. For the sedimentation analyses two marker proteins were used: IgG_{War}, a human γ_1 myeloma protein with sedimentation coefficient $s_{20,w}$ of 6.7 *S* and partial specific volume $\bar{v}$ of 0.74 cm^3/g (8) and equine cytochrome *c* (Calbiochem, San Diego, Calif.) with $s_{20,w}$ 1.83 and $\bar{v}$ of 0.725 (9). For the gel filtration experiments the markers were human IgM (purified from the serum of a patient with Waldenstrom's macroglobulinemia) (8), its cysteine-reduced subunit, IgMs (10), bovine thyroglobulin (Sigma Chemical Co., St. Louis, Mo.), equine apoferritin (Miles Laboratories, Inc., Kankakee, Ill.), human secretory IgA dimer (a gift from H. Reynolds, National Institute of Allergy and Infectious Disease), and the IgG referred to above. The diffusion coefficients ($D_{20,w} \times 10^7$, cm^2/sec) of these proteins were taken as 1.75 (8), 3.4 (8), 2.4 (11), 3.6 (12), 2.7 (13), and 4.0 (8), respectively. All proteins were iodinated with either ^{125}I or ^{131}I carrier-free preparations (New England Nuclear Corp., Boston, Mass.) by the chloramine-T method (14).

Receptor Assay. The binding activities of the free, solubilized receptors were assessed using the $(NH_4)_2SO_4$ assay described previously (6).

Gel Filtration. Gel exclusion chromatography was performed on 92 × 1.4 cm columns of Sepharose 6B (Pharmacia, Piscataway, N.J.) equilibrated at 4° with borate buffer containing 0.5% or 1% Nonidet P-40 and 0.05% bovine serum albumin. Column fractions of 1% bed volume were collected and flow rates were ≤1 bed volume/5 hr. The exclusion volume (V_0) was marked with ^{131}I-labeled tobacco mosaic virus (a gift from T. Triche, National Cancer Institute) after we observed that dextran blue appeared to interact with the solubilized receptor. A standard included volume (V_G) was marked with the IgG and the R_F for the unknowns and standards was calculated from their elution volumes (V_e) using the formula

$$R_F = (V_e - V_0)/(V_G - V_0) \quad [1]$$

The R_F of each standard was plotted versus $1/D_{20,w}$ and the line fit by least squares was used to convert the R_F values of the unknowns to their $D_{20,w}$ values (15).

Sucrose Density Gradient Centrifugation. Centrifugation experiments were performed with an L265B-G ultracentrifuge using an SW 40 rotor (Beckman Instrument Co., Palo Alto, Calif.). Pairs of 14.2 ml, 2% to 8% (wt/vol) linear sucrose gradients in borate buffer containing 1% Nonidet P-40 and 0.05% bovine serum albumin were prepared by pumping through mixing chambers into nitrocellulose tubes. After storage for ≤2 hr at 4°, 50–250 μl of sample in a solvent which was identical to that of the gradient except for the sucrose was layered on the gradient and the tubes were centrifuged at 40,000 rpm for 14–20 hr at 4°. Fractions of approximately 250 μl (7 drops) were collected automatically. There was less than 5% variation in sample size between the second and last fraction collected and refractive index measurements on unspun gradients showed excellent linearity. For the IgE, IgE–receptor complex, and marker proteins the radioactivity of the samples could be counted directly. For the free receptor 75–140 μl portions of the fractions were diluted at least 2:1, incubated with labeled IgE, and assayed with the $(NH_4)_2SO_4$ technique. The densities of the starting solvents were measured directly in a 10 or 25 ml pycnometer and the refractive index for each was determined

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on a Bausch and Lomb (Rochester, N.Y.) Abbé-type refractometer. The refractive index of individual fractions was likewise measured and converted to density relative to the starting solution. Relative viscosities of the starting solvents were measured in an Ostwald viscometer (flow time of H_2O at 20°, about 80 sec) at 4°.

Calculation of $s_{20,w}$ and $\bar{v}$. In order to improve the separation between the sedimenting species we used larger volumes (longer tubes) and shallower gradients than those employed by Martin and Ames (16). Because we could not assume a constant velocity of the components, on which their method of calculation is based, we determined the $s_{20,w}$ for the unknowns without this assumption. For any component in a centrifugal field (17):

$$s_{20,w} = \frac{dx/dt}{\omega^2 x}\left(\frac{(1-\bar{v}\rho_{20,w})\eta_{T,m}}{(1-\bar{v}\rho_{T,m})\eta_{20,w}}\right) \quad [2]$$

$$= \frac{1-\bar{v}\rho_{20,w}}{k}\int_{x_0}^{x}\frac{\eta_{T,m}dx}{x(1-\bar{v}\rho_{T,m})} \quad [3]$$

in which $\bar{v}$ is the partial specific volume, ρ is the density, η is the viscosity, x_0 and x are the positions of the particle with respect to the axis of rotation at time t_0 and some other time t, ω^2 is the angular velocity, the subscripts 20 or T refer to the temperature, w or m refers to the medium (H_2O or otherwise), $s_{20,w}$ is the sedimentation constant, and $k = \omega^2(t - t_0)\eta_{20,w}$. If $\rho_{T,m}$ is a linear function of x, i.e., $\rho_{T,m} = ex + u$ and likewise if $\eta_{T,m}$ can be represented by $fx + z$, as is approximately true for the shallow gradients we employed, then Eq. 2 may be integrated (ref. 18, integrals 29 and 40) and yields

$$s_{20,w} = \frac{1-\bar{v}\rho_{20,w}}{k}\Phi$$

in which

$$a = z/(1-\bar{v}u),\ b = (f/\bar{v}e) + a$$

and

$$\Phi = \ln\frac{x^a[(1-\bar{v}u) - \bar{v}ex_0]^b}{x_0^a[(1-\bar{v}u) - \bar{v}ex]^b} \quad [4]$$

or

$$k = \frac{1-\bar{v}\rho_{20,w}}{s_{20,w}}\Phi \quad [5]$$

If the system is ideal, then for two markers run simultaneously in the same gradient, k should be the same. Since the k values are likely not to be the same because of a variety of factors, including simply experimental error, the values of k for a component of unknown $s_{20,w}$ run in the same gradient must be taken as either an interpolated value (which assumes $k = jx + i$) or the average for the standards (which assumes the variation in k is primarily due to random errors). We have performed both calculations. Providing $\bar{v}$ for the unknowns can be estimated, its $s_{20,w}$ can then be calculated.

To estimate $\bar{v}$ we used the method of Edelstein and Schachman (19) as modified by Meunier *et al.* (20), in which the relative migrations in two solvents of different densities (H_2O and D_2O) are compared under otherwise identical conditions. Then $(\phi/k)H_2O = (\phi/k)D_2O$ where ϕ is a function of $\bar{v}$. By computer-assisted reiteration procedures, $\bar{v}$ can then be solved for. This method assumes that both $\bar{v}$ and $s_{20,w}$ are constant. As discussed elsewhere, these assumptions are not likely in general to lead to gross errors (15).

Molecular Weights. The masses of the components were calculated by use of the Svedberg equations $M = s_{20,w}RT/D_{20,w}(1-\bar{v}\rho_{20,w})$. The molecular weight of the detergent-free receptor was calculated from the measured $\bar{v}$ and molecular weight of the receptor in detergent, the literature value for the $\bar{v}$ of the detergent [0.94 (ref. 21; manufacturer's specifications)], an assumed $\bar{v}$ for the detergent-free receptor, and the relationship (15)

$$(M_r)_R = (M_r)_{RD}(\bar{v}_{RD} - \bar{v}_D)/(\bar{v}_R - \bar{v}_D) \quad [6]$$

in which M_r is molecular weight and R, D, and RD refer to the receptor, detergent, and receptor–detergent complex, respectively.

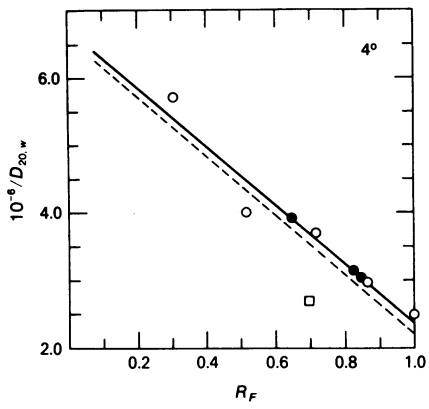

FIG. 1. Determination of $D_{20,w}$ by gel filtration. ○, marker proteins, from left to right: IgM, thyroglobulin, IgA, IgMs, IgG. □, apoferritin. ●, from left to right: IgE–receptor complex, receptor, IgE. The solid line was drawn through the data for the open circles only and was used to determine the $D_{20,w}$ of the unknowns. The broken line is drawn using the R_F of apoferritin also.

RESULTS

Diffusion Coefficients. Fig. 1 presents the data from which the diffusion coefficients were calculated. Each unknown (and most of the standards) was run at least three times, and the R_F was highly reproducible (±4%). The R_F of the receptor–IgE complex was unaltered by the presence of large excesses of IgE. The half-height widths of the elution peaks of the unknowns were entirely comparable to those of the standards and the peaks were symmetrical. Fig. 10 in ref. 6 shows a typical pattern. It can be seen that apoferritin is some distance from the line but whether the value for this standard was included in the calculations or not made little difference (±3%) in the values obtained for the unknowns.

Partial Specific Volumes. Fig. 2 presents data from a single centrifugation run in which the receptor and receptor–IgE complex were compared in H_2O and D_2O and is typical of the quality of the data from all of the experiments. The partial specific volume estimates for each species are derived from two separate runs, each performed in duplicate. The values calculated on the basis of an average k (see *Discussion*) for the free receptor, the complexed receptor, and IgE were, respectively: 0.815, 0.810; 0.774, 0.781; 0.743 (only one run in duplicate), and the means 0.81, 0.78, and 0.74 were used.

Sedimentation Constants. The data used for the determination of $\bar{v}$ as well as other data were used to determine the sedimentation constants. The values were: free receptor, 3.3 (0.13, 10); complex, 7.3 (0.11, 7); and IgE, 7.0 (0.31, 7), where the standard deviations and the number of experimental values are given in the parentheses.

Molecular Weights. On the basis of the values of $D_{20,w}$, $\bar{v}$, and $s_{20,w}$ the molecular weights of the receptor, receptor–IgE complex, and unbound IgE in the nonionic detergent were 130,000, 310,000, and 200,000, respectively. Assuming that the

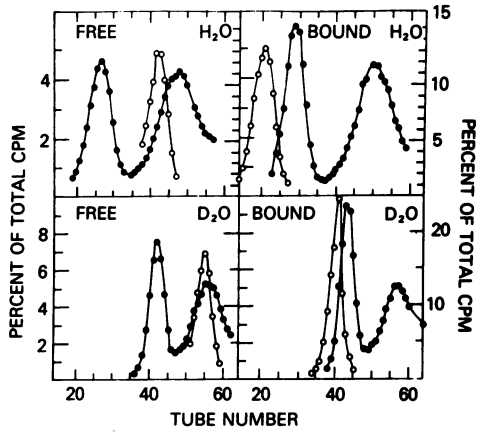

FIG. 2. Typical sucrose density gradient patterns. *Top*, H_2O solvent; *bottom*, D_2O solvent; *left*, free receptor; *right*, IgE–receptor complex. ●, (left ordinate): IgG and cytochrome markers (^{131}I-labeled). ○, (right ordinate): unknowns, measured directly (complex) or assayed after incubation with ^{131}I-labeled IgE (free receptor). All solvents contained 0.5% detergent.

receptor has a $\bar{v}$ typical of an average glycoprotein (0.72) a detergent-free molecular weight of 77,000 can be calculated. The results are collected in Table 1.

DISCUSSION

Methodological considerations

In these experiments we were faced with the common problem of trying to determine molecular weights on tiny amounts of material in an impure mixture by indirect methods. A variety of assumptions had to be made, which in general do not cause major errors but which in any particular case may (15).

(*1*) In the determination of $D_{20,w}$ it is assumed that the R_F is strictly proportional to the Stokes radius. The aberrant value for apoferritin (Fig. 1) shows that this may only be approximately true. If any of the unknowns behaved as aberrantly as apoferritin appeared to, our estimate of $D_{20,w}$ might be significantly (about 25%) in error. Nozaki *et al.* (23) recently reported aberrant apparent Stokes radii when large asymmetric proteins were studied on columns calibrated with globular proteins. The apparent values were as much as 30% too low. It is not possible to determine to what extent such a systematic inaccuracy exists in our results. To the extent it does our molecular weight estimates are falsely low.

(*2*) In the determination of $\bar{v}$ we assume that changes due to deuteration of the components are insignificant and that there is no significant change in detergent binding due to the sucrose, substituting D_2O for H_2O, or the increased pressure during centrifugation. It should be emphasized that given the high values of $\bar{v}$, an error of 1% in $\bar{v}$ can lead to a 4–5% error in the term $1 - \bar{v}\rho$ used in the calculation of $s_{20,w}$ and the molecular weights. This becomes particularly prominent when one attempts to calculate (below) the detergent-free molecular weight of the receptor utilizing Eq. 6 (*Materials and Methods*). The calculation of $\bar{v}$ also assumes no local solvent effects of the bound detergent so that a true $\bar{v}$ is indeed being measured by the method used. To what extent this may be a problem has not been discussed for systems such as the present one, but the agreement between molecular weights determined by these techniques and independent measurements (15) suggests no major problems.

(*3*) Our analysis of the sedimentation data is similar to that employed by others (15) with the difference that any nonconstancy in the velocity of migration [expected because our conditions were different from those of Martin and Ames (16)] was explicitly accounted for by integrating the sedimentation equation. The data in Table 1 are based on calculations in which we used a value of k (see *Materials and Methods*) that was the average of the values determined for the two standards. This seemed to us the more conservative approach and the one least likely to cause a major error. If instead we use the interpolated values, the $\bar{v}$ values are not substantially different: 0.73 versus 0.74, 0.82 versus 0.81, and 0.78 versus 0.78 for the IgE, receptior, and receptor–IgE complex, respectively. The sedimentation constants show a larger differential: 8.7 versus 7.0, 3.2 versus 3.3, and 9.3 versus 7.3 for the same components. The difference for the IgE–receptor complex is approximately 25% and leads to an estimated molecular weight of 400,000 versus 310,000. That the estimates for the complex show the largest differential is not surprising because this component runs substantially ahead of both standards (Fig. 2) while the IgE is close to the IgG standard (not shown) and the free receptor is between the two standards. The two methods of calculating the molecular weight of the free receptor lead to almost identical results: 133,000 versus 134,000. The molecular weight determined for IgE (200,000) is close to the value that is calculated from the weight estimates for the separated polypeptide chains of rat IgE myelomas by polyacrylamide gel electrophoresis: 183,000[7% gel (24)]; 196,000 [12.5% gel (J. Kanellopoulos and H. Metzger, unpublished observations)]. This agreement as well as direct determinations of the R_F of the IgE on gel filtration in the presence and absence of detergent (unpublished observations) suggests that the IgE does not bind nonionic detergent appreciably. The moderately high frictional coefficient for the receptor (Table 1) is similar to the value observed for several other membrane receptors (15).

Table 1. Molecular parameters of IgE receptor, receptor–IgE complex, and IgE in nonionic detergent

	$D_{20,w} \times 10^7$, cm²/sec	$\bar{v}$, cm³/g	$s_{20,w}$, S	M_r	f/f_0*
Receptor	3.2	0.81	3.3	130,000	1.9
Complex	2.5	0.78	7.3	310,000	1.9
IgE	3.3	0.74	7.0	200,000	1.7
Detergent-free receptor		0.72†		77,000	

* From frictional coefficient $f/f_0 = kT/D_{20,w}\,\eta(162{\cdot}\pi^2{\cdot}M_r{\cdot}\bar{v}/N)^{1/3}$ in which k is Boltzmann's constant and N is Avogadro's number (ref. 22).

† Assumed.

Model building

While we are well aware of many of the possible errors that can make our results inaccurate, certain conclusions seem reasonable.

(*1*) The receptor is made up of one or two polypeptide chains. This is based on the following: surface (2) or internal (3) labeling of the receptor with lactoperoxidase-catalyzed iodination or radioactive sugars and amino acids, respectively, yields a single band on polyacrylamide gel electrophoresis in sodium dodecyl sulfate. This band travels like a component with a molecular weight of 60,000 [low cross-linked gels (2)] to 50,000 [high cross-linked gels (ref. 3; J. Taurog and H. Metzger, unpublished observations)]. Assuming a $\bar{v}$ of 0.72 for the detergent-free receptor and a molecular weight of 130,000 for the receptor in detergent, a molecular weight of 77,000 is calculated

for the detergent-free receptor. If the carbohydrate content of the receptor is very high, the true $\bar{v}$ might be as low as 0.68 (25), giving a molecular weight of only 65,000. However, the molecular weight estimated by gel electrophoresis might then also be significantly in error (too high) and the monomer polypeptide chain might be as low as 30,000 (25). If, on the other hand, there is very little carbohydrate, the electrophoretic results might be approximately correct. But then the $\bar{v}$ might be as high as 0.76, yielding a molecular weight of 94,000. Thus, in either case a dimer seems somewhat more probable. We think it useless to juggle the numbers further. It is clear that the methodology has limitations and different rather than more experiments seem appropriate. Sufficient receptor to obtain a composition and perhaps to allow a direct estimation of detergent binding (15) is required. Such material could also be subjected to (internal) cross-linking reagents and the multiplicity of the polypeptide chains could be assessed directly (26, 27).

(2) The data suggest that the receptor binds a significant amount of detergent. It is likely that the amount of detergent bound is proportional to the hydrophobic surface area and calculations of such areas have been performed on other membrane proteins on that basis (15). We think that, despite their precision, the accuracy of our values of $\bar{v}$ and molecular weight are too uncertain to make such an estimate meaningful. Furthermore, we have preliminary evidence that the detergent binding is temperature dependent and we are sceptical about the validity of using measurements taken at 4° to assess the physiological condition of a membrane component. Nevertheless, because we observe an apparent increase in binding of detergent (an increase in $\bar{v}$) at higher temperatures, it appears that the receptor has an extensive hydrophobic surface and is likely to be significantly embedded in the surface membrane. These are other indirect data that suggest the same: (*a*) the receptor *in situ* can be iodinated only in the absence of IgE (5), suggesting limited surface exposure; (*b*) the receptor *in situ* is resistant to a variety of proteases (4); and (*c*) high detergent concentrations appear to interfere with the binding of antireceptor antibodies to determinants distal to the IgE-binding site (28). It is true, of course, that each of these observations could have alternative explanations.

(3) The valence of the solubilized receptor for IgE is unity. This agrees with our assessment of the valence of the receptor *in situ* by entirely different methods (29). If we accept those results, the present data indicate that the receptor does not aggregate when solubilized with nonionic detergents. It must be recognized that this valence is an effective valence. It does not preclude that each receptor is a dimeric structure (above) with two identical IgE binding sites, only one of which can be occupied at a time. One is reminded of the case of immunoglobulin *M*, where, at least with regard to large antigens, the effective valence is ½ of the actual number of combining sites (30).

The univalence has implications with regard to the potential role of the receptor in IgE-mediated triggering of mast-cell exocytosis. Triggering requires IgE aggregation (reviewed in ref. 1). It has been suggested that this system may behave like the classical pathway of complement fixation by immunoglobulins in which the Fc receptor (C1q) is itself multivalent and responsive to the state of immunoglobulin aggregation (31). Thus, it was speculated that the cell receptor for Fc_ϵ was an analogous multivalent molecule (32). This study as well as the study of the receptor *in situ* now clearly eliminates this possibility and suggests that if the aggregation of the receptor generates the signal, it must be *via* an inter- rather than an intramolecular aggregation.

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