Unexpected Findings from Target Analysis of Immunoglobulin E and Its Receptor†

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ABSTRACT: The membrane receptor for immunoglobulin E (IgE) and its ligand, IgE, were irradiated with high-energy electrons. Loss of binding activity was measured for each, and the size of the functional targets was assessed. In both cases, the target size was substantially smaller than the covalent structure of the molecule. The direction of this discrepancy is unprecedented on the basis of experience with loss of enzymatic activity by irradiation; indeed, two enzymes which were present in the receptor preparations gave expected values when measured simultaneously. We suggest that in instances where a function such as ligand binding resides in a conformationally stable domain, radiation inactivation may be capable of revealing this.

Mast cells and basophils have a surface membrane protein which binds monomeric immunoglobulin E (IgE)[1] with high specificity and avidity. This protein consists of two noncovalently attached polypeptides: a $\simeq$50 000 molecular weight "α" chain which contains the binding site for IgE and has about 30% carbohydrate (Kanellopoulos et al., 1980), and a $\simeq$30 000 molecular weight "β" chain (Holowka et al., 1980; D. Holowka and H. Metzger, unpublished experiments). Although α chains continue to bind IgE firmly in the absence of β chains, it remains possible that the latter influence the binding properties of the receptor.

Surface aggregation of the receptor stimulates degranulation of the cells, but the immediate molecular consequences of receptor aggregation are completely unknown (Metzger et al., 1981). A possible consequence is the formation of new molecular associations; however, there are only limited ways by which such transient interactions can be discovered. One approach to this problem is to use cross-linking reagents. Preliminary efforts by our group using this stratagem failed to discover such new molecular interactions between aggregated receptors and other cellular components (Holowka et al., 1980).

An alternative approach is to use radiation inactivation (Kempner & Schlegel, 1979). With this method, it is potentially possible to demonstrate new interactions of a macromolecule in unfractionated biological material, e.g., whole cells, on the basis of a change in the target size associated with the function of the molecule (Houslay et al., 1977; Schlegel et al., 1979; Martin et al., 1979; Harmon et al., 1980). We decided to explore this approach and began by examining the target size of the receptor for IgE from rat basophilic leukemia (RBL) cells and of IgE itself, using their binding to each other as the functional parameter. It was of interest to see if the target size of the receptor might be larger than the IgE-binding α chain because of the close association between α and β (D. Holowka and H. Metzger, unpublished experiments). Surprisingly, the target size both for the receptor and for IgE was smaller than the smallest covalently bonded structure for each molecule. Such findings are at variance with previous reports concerning the relationship between the covalent structure of enzymes and the minimal target size of their function as determined by radiation inactivation (Kempner & Schlegel, 1979).

Materials and Methods

Preparation of RBL Cell Extracts. RBL cells grown as a solid tumor in rats (Kanellopoulos et al., 1979) or as a monolayer in stationary flasks (Taurog et al., 1979) were solubilized in the cold at a concentration of 2×10^8 or 0.5×10^8 cell equivalents (respectively) per mL of 0.5% Triton X-100 in BBS (borate-buffered saline: 0.2 M $NaBO_3$ and 0.16 M NaCl, pH 8) containing the protease inhibitors aprotinin (1 trypsin inhibitor unit/mL) and phenylmethanesulfonyl fluoride (1 mM). The suspension was centrifuged at 50000*g* for 1 h at 4 °C, and the supernatant containing solubilized receptors for IgE was diluted with an equal volume of BBS. In experiments where results with irradiated cell extracts were to be compared with those obtained with irradiated cells, this dilution was made with Hepes-buffered salt solution [135 mM NaCl, 5 mM KCl, 0.05% gelatin, and 10 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (Hepes), pH 7.4] and was done after irradiation. Aliquots (0.5 mL) of the solubilized material were dispensed into 2-mL vials. The vials were then sealed and the contents frozen in dry ice/acetone and stored below −60 °C.

Preparation of RBL Cells for Irradiation and Subsequent Solubilization. RBL cells from monolayer cultures were harvested (Taurog et al., 1979) and suspended at 5×10^7 cells/mL of Hepes-buffered salt solution, and 0.5-mL aliquots were dispensed into vials as for the cell extracts. After irradiation, the frozen cells were thawed and solubilized by the addition of 0.5 mL of 0.5% Triton X-100 in BBS containing protease inhibitors (above), and the supernatants, after centrifugation at 50000*g* for 1 h at 4 °C, were frozen and stored below −60 °C. Full recovery of material was assessed in two ways. In one instance, the cells had been reacted with a trace of [^{125}I]IgE prior to freezing, and so the recovery of ^{125}I counts in the extract was determined. In a second experiment, a small number of cells with [^{125}I]IgE bound to them was added to the irradiated cells prior to solubilization of the latter. In both instances, essentially complete recovery of radioactivity was obtained from all the specimens.

Preparation of IgE for Irradiation. Rat monoclonal IgE, from tumor IR 162, prepared as described previously (Kulczycki & Metzger, 1974), was made up to a final concentration of 2 mg/mL in BBS. In a preliminary experiment, a gradual loss of recovered activity was observed when irradiated IgE

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[1] Abbreviations used: IgE, immunoglobulin E; RBL, rat basophilic leukemia; BBS, borate-buffered saline (0.2 M $NaBO_3$ and 0.16 M NaCl, pH 8); Hepes, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid; SE, standard error.

was stored at 4 °C, presumably due to nonspecific adsorption. Cytochrome *c* (0.5 mg/mL), which because of its low molecular weight is relatively resistant to radiation inactivation, was therefore added as a carrier in subsequent experiments. Aliquots (0.5 mL) were dispensed into vials and stored frozen. When these precautions were taken, recovery of IgE (determined by adding a trace of ^{125}I-labeled IgE prior to irradiation) was complete in all cases regardless of the degree of irradiation.

Irradiation. A beam of 13-MeV electrons produced by a linear accelerator (Armed Forces Radiobiology Research Institute, Bethesda, MD) was spread with a water scatterer to provide a uniform dose rate (typically 13 Mrad/h) with less than 10% variation over a 225-cm^2 exposure area at 90 cm from the beam port. Thermoluminescent dosimeters were used before and after each series of radiations. Samples in sealed glass vials were maintained during exposure between −110 and −150 °C by a stream of cold nitrogen gas. Temperature was monitored continuously with a platinum resistance probe (YSI, Yellow Springs, OH). In each case, two vials were kept as unirradiated controls.

Assays. (1) β-Glucuronidase and β-Hexosaminidase. The residual activity of these enzymes in detergent extracts of RBL cells (after irradiation of whole cells or cell extracts) was measured by the rate of release of 4-methylumbelliferone from synthetic substrates (1 mM) in 0.12 M acetate buffer at pH 4.4 (Brot et al., 1974). The substrate for β-glucuronidase was 4-methylumbelliferyl β-D-glucuronide (Sigma, St. Louis, MO 63178), and that for β-hexosaminidase was 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside (Koch Light, Colnbrook, England). The reaction was allowed to proceed for 30 min at 37 °C and was quenched by the addition of 10 volumes of 0.35 M glycine and 0.44 M sodium carbonate, pH 10. Fluorescence emission above 420 nm was read by using excitation at 365 nm.

(2) Binding Activity of Soluble Receptors. The capacity of solubilized receptors to bind ^{125}I-labeled IgE was measured as described previously (Rossi et al., 1977) with minor modifications. Briefly, [^{125}I]IgE at a concentration of 0.7 μg/mL or higher was incubated at 4 °C for 90 min with solubilized receptors for IgE in BBS containing 0.25% Triton X-100 and 0.2% human serum albumin (0.1% cytochrome *c* in later experiments). A 2-fold or greater molar excess of IgE over receptor was used in all cases. We calculated from the kinetic parameters of binding [see Rossi et al. (1977)] that under these conditions 99% saturation should be reached in 75 min or less. IgE–receptor complexes were separated from free IgE by precipitation from 40% saturated $(NH_4)_2SO_4$ in BBS containing 0.25% human serum albumin as described previously (Rossi et al., 1977).

In this assay, the residual binding capacity of the receptors is measured directly. A number of trivial explanations which could potentially account for an overestimate of residual activity and thus be interpreted as a falsely low target size (see Results) were excluded. Incomplete equilibration of IgE binding was excluded by demonstrating no effect on the estimated activity by altering the concentration product of the reactants over 200-fold. This also reconfirms the linearity of the assay (Rossi et al., 1977). Induction of nonspecific binding by the irradiation was excluded by showing that excess unlabeled IgE was as effective in blocking the binding of labeled IgE in the irradiated samples as in the unirradiated ones. Finally, we demonstrated that a mixture of irradiated material with unirradiated material had the activity anticipated from simple additivity of the activities assessed on the extracts individually.

(3) Binding Activity of IgE. The binding activity of IgE was measured by using an inhibition assay (Fewtrell & Metzger, 1980). On the basis of a preliminary estimate, samples were diluted such that they would contain an amount of active IgE approximately equivalent to the native [^{125}I]IgE which was added to it. The binding of the [^{125}I]IgE was then assessed with a constant amount of a detergent extract of tumor cells by using the soluble receptor assay (above). The [^{125}I]IgE was in 1.5-fold excess over the predetermined binding capacity of the extract. The exact amount of inhibition was determined for each sample, and the amount of active unlabeled IgE which would account for the inhibition was calculated.

In this assay, we measured the residual binding capacity of IgE indirectly. Thus, the target size of the IgE might be underestimated (see Results) by any factor which would nonspecifically cause less [^{125}I]IgE to be precipitated in the ammonium sulfate assay. To test for such an effect, we added 5 times the usual concentration of IgE which had been irradiated with 48 Mrad to a mixture containing extract and [^{125}I]IgE which had been preincubated for 1 h at 0 °C. The counts precipitated were within experimental error of those found for the control incubation mixture to which only buffer had been added.

Target Analysis of Inactivation Data. The fraction of surviving activity was plotted logarithmically as a function of radiation dose. The data for each inactivation curve were fitted to a single straight line by using unconstrained least-squares analysis. Points were equally weighted except for the unirradiated controls which were weighted times 2 since duplicate samples of these were measured in each assay.

The slope of the line = $1/D_{37}$, where D_{37} is the dose of radiation required to reduce the activity to 37% of that of the unirradiated control. This is related to the target size (mass) of the functional unit. When the dose is given in megarads

$$\text{target size} = \frac{6.4 \times 10^5}{D_{37}} S_t$$

where S_t is a temperature factor, experimentally determined to be 2.8 for irradiation carried out at −130 °C (Schlegel et al., 1979).

Extrapolation from data obtained for low-temperature irradiation of a wide variety of molecules including proteins, glycoproteins, nucleic acids, and certain plastics gave a similar value for the temperature factor (Kempner & Schlegel, 1979; E. S. Kempner, unpublished observations).

Gel Electrophoresis. Irradiated IgE was analyzed by gradient gel electrophoresis in sodium dodecyl sulfate on Pharmacia PAA 4/30 gradient slab gels (Plotz et al., 1979).

Results

Enzyme Markers. As an internal control, we measured the inactivation of two acid hydrolases which are present in RBL cells. β-Glucuronidase (EC 3.2.1.31) is a tetrameric glycoprotein composed of identical subunits with molecular weights of about 75 000 (Tulsiani et al., 1978) while β-hexosaminidase (EC 3.2.1.52) is a 100 000 molecular weight enzyme consisting of two major glycoprotein subunits which have molecular weights of about 55 000 (Hasilik & Neufeld, 1980; Puchalski & Neufeld, 1981). Figure 1 and Table I show the results from eight different experiments in which the enzymes from both irradiated cells and irradiated detergent extracts of cells obtained from five separate irradiations were measured. For both enzymes, the target sizes observed closely approximated the molecular weights of the subunits of the enzymes as determined by structural analyses. The average value of 85 000

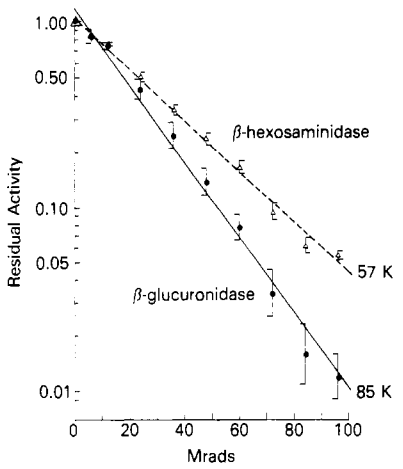

FIGURE 1: Radiation inactivation of β-hexosaminidase and β-glucuronidase from RBL cells. The combined results obtained from eight different experiments using irradiated RBL cells or irradiated cell extracts are shown. The residual activity observed after increasing doses of irradiation is expressed as a fraction of the activity observed in unirradiated controls. Bars show SE.

Table I: Radiation Inactivation of IgE, Its Cellular Receptor, and Enzyme Controls

activity measured	specimen	no. of irradiations	functional target (±SE)	smallest covalent structure
β-glucuronidase	extract	5	85 800 ± 3 800	75 000[a]
	cells	3	83 500 ± 5 200	
β-hexosaminidase	extract	5	57 600 ± 1 500	54 000[b]
	cells	3	55 500 ± 1 600	
receptor for IgE	extract	5	27 900 ± 1 300	50 000[c]
	cells	3	37 300 ± 2 200	
IgE	IgE	3	116 700 ± 5 800	200 000[d]

[a] Tulsiani et al. (1978). [b] Puchalski & Neufeld (1981). [c] Kanellopoulos et al. (1980). [d] Bazin & Beckers (1976).

obtained for β-glucuronidase was somewhat higher than the expected value of about 75 000. This was because in three out of the eight experiments, values closer to 95 000 were obtained. The reason for this does not appear to be related to errors in the dosage of irradiation since a corresponding increase in the target size of β-hexosaminidase was not seen in these experiments. Indeed, there was much less variation in the values observed for β-hexosaminidase, and the mean target size of 57 000 agrees well with the 54 000 molecular weight subunit of this enzyme from RBL cells (Puchalski & Neufeld, 1981). There was no significant difference between the mean target sizes of the two enzymes obtained when whole cells, as opposed to cell extracts, were irradiated (Table I).

Solubilized Receptors for IgE. When solubilized receptors for IgE were exposed to increasing doses of high-energy radiation, there was a simple exponential loss of activity (Figure 2). Although least-squares fits of the experimental points were not constrained to give a value 1.0 for the activity of the unirradiated material, it is clear that the lines pass very close to this point. The slopes of the lines obtained for the extracts prepared from solid tumors and cultured cells were virtually indistinguishable. However the calculated target sizes, 29 000 and 27 000, respectively, are only a little over half of the molecular weight expected (50 000) for the α chain of the receptor which binds IgE (Table I, see Discussion).

A number of trivial explanations for the high residual activity (which results in the unexpectedly low estimate of the molecular weight of the receptor) were excluded (see Materials and Methods). One nontrivial explanation was that because of the very high affinity of IgE for its receptor [$K_D = <10^{-10}$ M (Kulczycki & Metzger, 1974; Rossi et al., 1977)] the binding assay could be insensitive to major changes in the affinity of the receptor. To test for partial inactivation, unirradiated tumor extract and extract in which the apparent IgE binding activity had been reduced approximately 50% by irradiation were individually incubated with [^{125}I]IgE for 90 min to saturate the active receptors. A 20-fold molar excess of nonradioactive IgE was then added, and the rate of dissociation of [^{125}I]IgE from the receptors was measured. Figure 3 shows that with both irradiated and unirradiated material a value close to 3×10^6 s was observed for the half-time of dissociation of IgE, which is in good agreement with the previously published value of 2.7×10^6 s (Rossi et al., 1977). This clearly showed that irradiation was not generating partially inactivated receptor molecules with substantially reduced affinities for IgE.

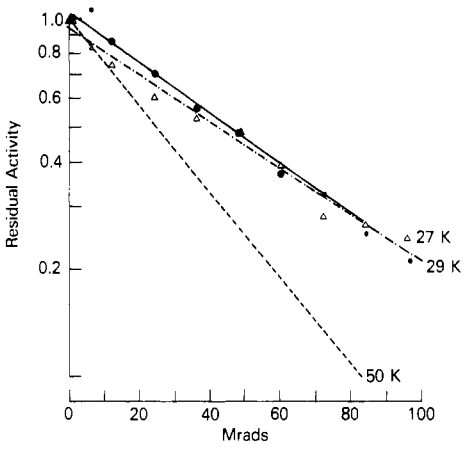

FIGURE 2: Radiation inactivation of Triton X-100 solubilized receptors for IgE. (●) Receptors from RBL cell tumors grown in rats. (△) Receptors from RBL cells grown in tissue culture. (---) Theoretical inactivation curve for a target size of 50 000.

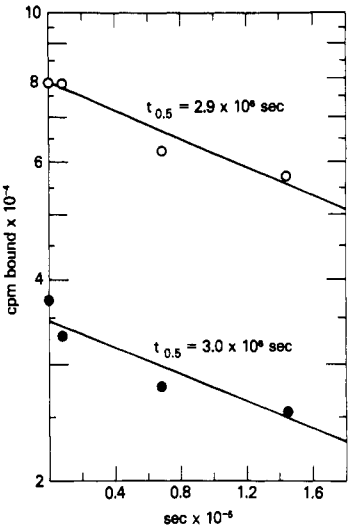

FIGURE 3: Rate of dissociation of [^{125}I]IgE from irradiated (●) and unirradiated (○) receptors for IgE.

Membrane-Bound Receptors. The effect of irradiating the native receptor for IgE in the cell membrane was also examined. Cells were frozen, irradiated, and then solubilized, and the residual IgE binding activity was determined.

The pooled results from three experiments in which whole RBL cells were irradiated prior to solubilization are shown in Table I and by the triangles in Figure 4. Again, the target size observed was substantially smaller than the molecular weight of the IgE binding α chain of the receptor. However, the value obtained (37 000) was somewhat larger than the

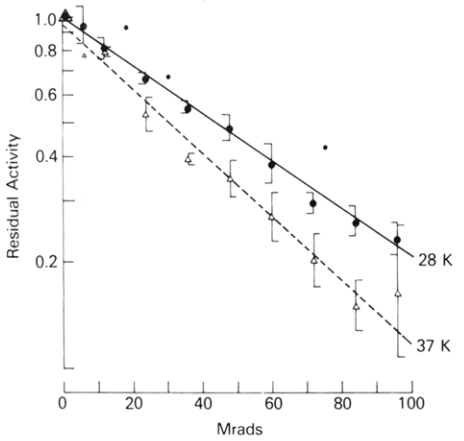

FIGURE 4: Radiation inactivation of the receptor for IgE. (●) Irradiated Triton X-100 extracts of RBL cells (five experiments). (△) Irradiated RBL cells (three experiments). In both cases, residual binding activity was measured after solubilization in Triton X-100. Bars show SE. Small symbols without bars show values obtained in one experiment only.

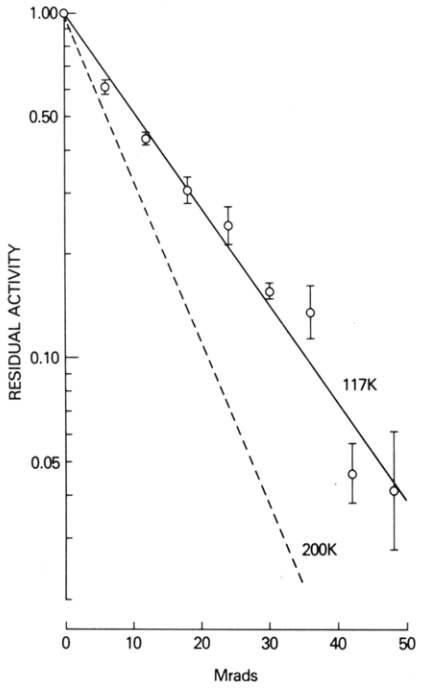

FIGURE 5: Radiation inactivation of IgE as determined by its ability to bind to the receptor for IgE. The combined results obtained from three separate irradiations are shown (○). Bars show SE. (---) Theoretical inactivation curve for a target size of 200 000.

average value of 28 000 obtained with receptors irradiated in solution. This difference was observed in each of three paired experiments and is statistically significant (p = <0.0005).

IgE. The radiation-induced loss of the ability of IgE to bind to its receptor is shown in Figure 5. IgE is composed of four disulfide-linked polypeptide chains with an aggregate molecular weight of 200 000 (Bazin & Beckers, 1976), while target analysis clearly yields a substantially lower value for its receptor-binding function.

Again, we tested the possibility that we were overestimating the residual activity because our assay would not distinguish between fully active and substantially inactivated molecules. However, the rate of dissociation of the residual "active" IgE in an irradiated sample was indistinguishable from that of native IgE.

In the case of IgE, we were able to examine the effect of increasing irradiation on the gross structure of the molecule.

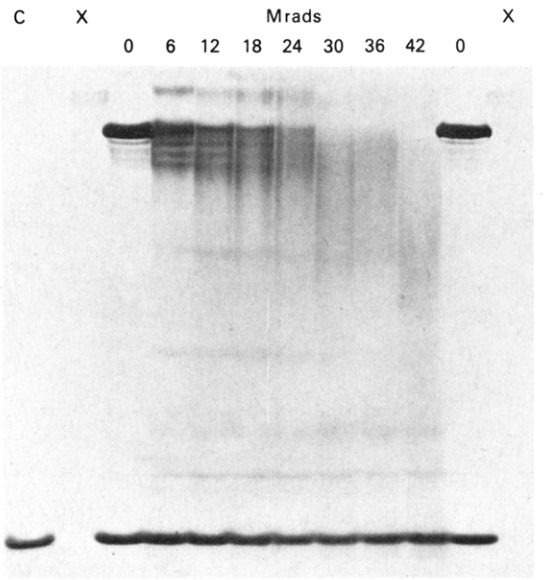

FIGURE 6: Sodium dodecyl sulfate gradient gel electrophoresis of irradiated IgE. Each lane contains the equivalent of 20 μg of IgE and 5 μg of cytochrome *c*. (X) 2 μg of chemically cross-linked dimers and trimers of IgE (Fewtrell & Metzger, 1980). (C) 5 μg of cytochrome *c* alone. Staining was with Coomassie Blue.

Figure 6 shows a representative analysis of the size of irradiated IgE by gradient gel electrophoresis in sodium dodecyl sulfate. A range of quite large breakdown products is seen with the pattern becoming more diffuse with increasing irradiation. A number of discrete bands is also seen which suggests that certain regions of the molecule may be more susceptible to chain cleavage. Small amounts of species having a higher molecular weight than that of native IgE were also generated. The latter should not have affected the assessment of binding activity since the assay should be insensitive to the polymeric state of the IgE (Fewtrell & Metzger, 1980).

We considered target analysis of a quantitative scan of the gel shown in Figure 6. However, without consideration of the technical limitations, there are theoretical problems since not every hit would be expected to produce an appreciable change in molecular weight. We therefore felt that such an analysis was beyond the scope of this paper.

Discussion

A recent reanalysis of older studies (Kempner & Schlegel, 1979) as well as newer data (Kempner et al., 1980) suggests that when a protein is subjected to high-energy irradiation its activity is completely and irreversibly destroyed by a single hit. In many cases, the target size corresponds to the molecular weight of the entire protein molecule, but with multisubunit proteins, the target size often corresponds to the molecular weight of one subunit. This suggests that a subunit within a protein molecule can be hit without necessarily affecting the activity of the other subunit(s). However, we can find no well-defined example in which the target size was *smaller* than the smallest covalent structure as we have now found for IgE and its cellular receptor.

Several possible explanations for our unusual results must be considered. Since simultaneous assessment of two relatively well-characterized enzymes gave appropriate values, our basic procedures and analyses appear to be valid. We also explored a variety of possibilities by which our binding assay could have led to incorrect assessments of residual activity but could find no evidence for this. It should be emphasized that although the same assay was used to assess the receptor and the IgE, the assays were in effect complementary. As an explanation

of the smaller target sizes found, the assay would have had to generate falsely high values for binding of [^{125}I]IgE in one instance (assay of receptors) and falsely low estimates of binding in the other (assay of IgE).

A second possibility is that the estimates of the covalent molecular weights of the IgE and its receptor from physicochemical studies are incorrect. This is clearly not the case for the IgE used since its structure is well documented (Bazin & Beckers, 1976). The molecular weight of the α chain of the receptor for IgE, to which the IgE binds, is not as well-defined. Structural analyses have led to a best estimate of 45 000–50 000 (Kanellopoulos et al., 1980), which is still considerably larger than the target size we determined for the solubilized receptor.

We propose, therefore, that our findings are valid and that the difference between these and previous observations results from the nature of the molecules studied or of the activity measured, or both. In the majority of radiation inactivation studies, the function measured has been enzymatic activity (Kempner & Schlegel, 1979), and this is likely to be extremely sensitive to relatively minor changes in protein conformation. In contrast, the simple binding of IgE to its receptor may not have such strict conformational requirements, and so a molecule that is hit might still retain full activity. It is, however, unlikely that our results are due to the generation of partially inactivated molecules with substantially reduced binding abilities since the rate of dissociation of IgE–receptor complexes was unaffected by prior irradiation of either species.

Since 30% of the α chain of the receptor for IgE is carbohydrate (Kanellopoulos et al., 1980), one possibility is that hits in this region [which is probably not required for IgE binding (Pecoud et al., 1980; Hempstead et al., 1981)] have no effect on the binding activity of the rest of the molecule. However, this cannot explain the low target size observed for IgE. This would require a carbohydrate content of about 40% whereas human IgE contains only about 12% carbohydrate (Bennich & Johansson, 1971) and rat IgE is unlikely to be very different (H. Bennich, unpublished experiments).

Previous studies on the radiation inactivation of IgG[2] also gave a target size that was smaller than the molecular weight of the whole molecule (Rosse et al., 1967). No correction for irradiation at low temperature (Kempner & Schlegel, 1979) was made, but even when this is done, the value obtained is a partial molecular weight. This may be related to the fact that the ligand binding activities of immunoglobulins are localized in structurally distinct domains which are covalently bonded to each other, and these regions show unimpaired activity when the molecules are fragmented (Porter, 1959; Metzger, 1978). In contrast, enzymes usually do not have their catalytic sites localized to discrete structurally independent regions within a continuous polypeptide chain. It is not clear how many discrete regions of the IgG molecule are necessary for the activity (complement fixation) measured in the earlier study (Rosse et al., 1967). However, in our experiments, we measured the ability of IgE to bind to its receptor which only requires an intact Fc region of the molecule.

Our knowledge of the α chain of the receptor for IgE is much less complete. However, it is interesting that recent experiments with proteases suggest that the α chain contains two relatively stable domains of approximately equal size connected by a protease-sensitive region (Goetze et al., 1981). The difference between the susceptibility of the receptor to radiation inactivation in the cell membrane or after detergent solubilization is intriguing. We cannot exclude the possibility that interactions with other components in the membrane, such as lipids or other proteins, may contribute to the larger target size of the membrane-bound receptor. However, another plausible interpretation is that changes in the relationship between domains after solubilization may be responsible for the observed reduction in target size. This raises the interesting possibility that radiation inactivation is able to detect conformational changes in proteins.

[2] Inactivation data obtained with IgM are difficult to interpret since nonlinear inactivation curves were obtained (Rosse et al., 1967).

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Role of Disulfide Interchange Enzyme in Immunoglobulin Synthesis†

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ABSTRACT: The role of disulfide interchange enzyme in protein biosynthesis was evaluated by studying the enzyme from mouse lymphoid tissue. The enzyme isolated from lymphoid cells was shown to have no tissue-specific characteristics. It was identical with the enzyme synthesized by mouse liver in its biochemical and immunological properties and its capacity to promote both disulfide bond formation and insulin degradation. In contrast to liver, the levels of enzyme in lymphoid tissues were found to vary with immunoglobulin secretory activity. Assays of lymphoid cells and their transformed counterparts showed that the enzyme contents of cells actively secreting immunoglobulin were 1–2 orders of magnitude higher than that of unstimulated B cells or non-immunoglobulin-producing T cells. The increase in enzyme levels paralleled the increase in immunoglobulin synthesis after antigen or mitogen stimulation and was independent of the class of immunoglobulin produced. This correlation indicated that the enzyme plays a critical role in the formation of intramonomer bonds common to all immunoglobulin molecules. Supporting data were obtained by assaying the ability of the enzyme to promote the polymerization of mouse pentamer IgM in vitro. The enzyme was found to catalyze the formation of the interchain bonds required for monomer IgM assembly but not the formation of the intermonomer bonds required for pentamer assembly. The sum of these results provides strong evidence that disulfide interchange enzyme functions in the in vivo synthesis of protein disulfide bonds.

The physiological function of disulfide interchange enzyme (thiol:protein disulfide oxidoreductase) remains to be established. The enzyme has been implicated in the formation of protein disulfide bonds on the basis of considerable indirect evidence: its wide tissue distribution, its localization in the microsomal fraction, and its ability to convert inactive, randomly reoxidized proteins to their native forms (De Lorenzo & Molea, 1967; Fuchs et al., 1967; Goldberger et al., 1963, 1964; Steiner et al., 1965). As yet, however, there is little direct evidence that the enzyme performs such a function in vivo. One problem has been the purity of the preparations used in the investigations. Most previous studies have been carried out with partially purified enzyme so that it was not possible to distinguish catalytic from nonspecific primer effects (Murkofsky & Lamm, 1979; Della Corte & Parkhouse, 1973; Teale & Benjamin, 1976; Wilde & Koshland, 1978). Moreover, preparations of disulfide interchange enzyme have been found to exhibit a second thiol oxidoreductase activity (EC 1.8.4.2), the reductive cleavage of insulin disulfide bonds (Ansorge et al., 1973; Varandani et al., 1975; Morin et al., 1978). Whether the two activities are mediated by a single enzyme of broad specificity or by distinct enzyme species with overlapping specificities is a question that has yet to be resolved (Varandani, 1978; Freedman, 1979; Hillson & Freedman, 1980).

Investigations of the role of disulfide interchange enzyme have also been hampered by the lack of definitive assays for physiological activity. In the past, studies have been generally confined to systems such as liver protein biosynthesis, which have proved too complex to give clear-cut data. Only recently have studies been initiated on tissues that are metabolically more restricted and synthesize a single major disulfide-linked protein. By analyzing collagen and bone synthesis in chick embryos, Brockway et al. (1980) have been able to correlate the levels of disulfide interchange enzyme with the synthesis of the disulfide-linked procollagen.

We have undertaken to obtain definitive evidence for the role of disulfide interchange enzyme by using the assembly of mouse pentamer IgM as the functional assay. To address the problem of enzyme heterogeneity, disulfide interchange enzyme was purified not only from the homologous IgM-secreting lymphoid tissue but also from mouse liver, and the two preparations were compared with respect to substrate specificity and to biochemical and immunological properties. A radioimmunoassay was developed to follow the intracellular levels of disulfide interchange enzyme as a function of the successive differentiative steps in Ig[1] synthesis. Finally, the enzyme preparations were tested for their ability to promote the repolymerization of mouse IgM in vitro.

This system was chosen because the in vitro assembly of immunoglobulin (Petersen & Dorrington, 1974; Sears et al., 1977) has been shown to be representative of the in vivo process. Within the cell, the intrachain and interchain S–S bonds of Ig heavy and light chains are formed extremely rapidly, even in some cases as the polypeptides are being synthesized on membrane-bound ribosomes (Bergman & Kuehl, 1979a,b). Shortly after the completion of the synthesis of the chains, the remaining disulfide bonds are formed and the disulfide-linked pairs, either H–L, or L–L and H–H, are then disulfide bonded to assemble the basic monomeric unit of H_2L_2.[1] In the case of IgM, the monomers are transported

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[1] Abbreviations used: H, heavy chain; L, light chain; Ig, immunoglobulin; $NaDodSO_4$, sodium dodecyl sulfate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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