Radiation inactivation of oligomeric enzyme systems: Theoretical considerations

(target analysis)

A. S. VERKMAN*, K. SKORECKI†, AND D. A. AUSIELLO†‡

†Renal Unit, Massachusetts General Hospital and the *Department of Medicine and the Biophysical Laboratory, Harvard Medical School, Boston, MA 02114

Communicated by Alexander Leaf, August 18, 1983

ABSTRACT Radiation inactivation has been used as a tool to explore the size, structure, and function of soluble and membrane-bound enzymes. Enzyme systems consisting of a single functional unit yield a single exponential dependence of enzyme activity on radiation dose. Complex, nonexponential, inactivation curves suggest the presence of a multiunit enzyme system. A concave-upward inactivation curve suggests the presence of multiple independent functional units of distinct size and activity that do not interact with each other. An oligomeric enzyme, consisting of n identical subunits in equilibrium with monomers, can give simple exponential decay curves or more complex inactivation curves with various degrees of upward or downward concavity, depending upon the extent of oligomer–monomer equilibration among subunits after radiation, oligomer-to-monomer size and activity ratios, and multi-hit requirements for oligomer inactivation. For each of these possibilities, equations for the inactivation curves are derived, calculated numerically, and discussed in qualitative terms. A systematic approach to the evaluation of complex radiation inactivation curves is proposed and limitations of the radiation inactivation method are reviewed.

Target theory was developed to explain observed activity–dose relationships for biologically active molecules exposed to high-energy radiation (1). Subsequently, target theory has been used to determine the molecular weight and functional arrangement of enzyme subunits in a variety of complex receptor, polyenzyme, and oligomeric enzyme systems (2). Such complex systems have yielded nonlinear activity–dose relationships. The interpretation of nonlinear radiation inactivation data depends critically upon the physical and mathematical assumptions used in formulating a model; it becomes necessary to explore several types of models and values of model parameters before tentative conclusions of biological significance can be proposed. The oligomeric enzyme system represents a well-defined model system to evaluate the assumptions made in interpreting nonlinear activity–dose curves.

In general, an oligomeric enzyme consisting of n identical subunits, A_n , is an equilibrium initially with monomers, A , with an equilibrium constant, K .

$$A_n \rightleftharpoons nA; K = \frac{[A]^n}{[A_n]} \quad [1]$$

The system is exposed to radiation dose, D , and enzyme activity is assayed. The measured activity–dose relationship provides information about the size, structure, and function of enzyme A_n .

Although the equilibrium in Eq. 1 consists of a simple two-component system prior to radiation exposure, a number of physical and chemical factors determine the detailed shape

of the activity–dose curve. Equilibrium between A_n and A molecules surviving radiation exposure may or may not be reestablished, depending upon enzyme association and dissociation rates and assay conditions. Enzyme activity may be present in A_n or A or both species. Inactivation of the oligomer may result in its dissociation into subunits. This inactivation may require one or more subunits to be “hit” for completion. Finally, there may be energy transfer among oligomer subunits; a single oligomer hit may irreversibly inactivate some or all n subunits in A_n .

Each set of conditions describes a particular model for radiation inactivation and is associated with a unique activity–dose relationship. For example, analysis of the activity–dose data for calmodulin-sensitive phosphodiesterase has suggested a model in which irradiation of a relatively inactive multimeric enzyme complex results in destruction of the inactivated subunit only, without energy transfer to other subunits and with dissociation of the remaining subunits into active monomers (3). This model represents one specific example of a more general set of possible models for inactivation of complex systems yielding non-linear activity–dose curves.

In this paper, we formulate and solve for the activity–dose relationships expected under the sets of conditions described above. Activity–dose relationships are calculated numerically, displayed, and explained in qualitative terms. Finally, an approach is given for interpreting and deducing important biophysical information from the complex activity–dose relationships obtained for oligomeric enzyme systems.

PROBLEM FORMULATION

Equilibration vs. Nonequilibration Models. Irradiation in most radiation inactivation experiments is carried out on frozen (-180°C to -50°C) or lyophilized samples to minimize or prevent solution free radical formation. Biological activity after irradiation is assayed after warming or rehydration. There may or may not be full reequilibration of biologically active species after irradiation, depending upon the time elapsed prior to enzyme assay and the intrinsic kinetics of the biologically active species. For each of these possibilities, equilibration and nonequilibration, a different set of mathematical equations applies.

Equilibrium Models. These models allow equilibrium of oligomers and monomers to be reestablished after irradiation and prior to assay of enzyme activity.

The total number of A subunits present initially, A_t^0 , is equal to the sum of monomeric and oligomeric subunits, $A^0 + nA_n^0$. Values for A^0 and A_n^0 are calculated from the conservation relation, $[A_t^0] = [A^0] + n[A_n^0]$ and $K = [A^0]^n/[A_n^0]$,

$$\begin{aligned} n[A^0]^n + K[A^0] - K[A_n^0] &= 0 \\ [A_n^0] &= ([A_t^0] - [A^0])/n. \end{aligned} \quad [2]$$

‡To whom reprint requests should be addressed.

In the absence of energy transfer, radiation produces independent destruction of subunits, regardless of whether the subunit is in oligomer or monomer form,

$$[A_i] = [A_i^0]e^{-D/D_0}, \quad [3]$$

in which $[A_i]$ is the total subunit concentration after radiation, D is the radiation dose, and D_0 is the radiation dose at which activity is diminished to 37% of initial activity (2).

The postradiation values for $[A_n]$ and $[A]$ are obtained by solving Eq. 2 with $[A_n^0]$ replaced by $[A_n]$, $[A^0]$ replaced by $[A]$, and $[A_i^0]$ replaced by $[A_i^0]e^{-D/D_0}$. The relative enzyme activity, R , is then

$$R = \frac{\alpha[A] + \beta[A_n]}{\alpha[A^0] + \beta[A_n^0]}, \quad [4]$$

in which α and β are the fractional activities of A and A_n , respectively, and $\alpha + \beta = 1$.

If energy transfer occurs, a given radiation dose destroys subunits very effectively because two or more oligomeric subunits are destroyed by a single hit. If m subunits are destroyed by a single hit in the oligomer, Eq. 3 is replaced by

$$[A_i] = [A^0]e^{-D/D_0} + n[A_n^0]e^{-mD/D_0}. \quad [5]$$

In an equilibration model, the problem of requiring multiple hits to inactivate an oligomer is not physically meaningful because equilibration restores active oligomers after irradiation.

Nonequilibrium Models. These models assume that equilibrium between monomers and oligomers is not reestablished after irradiation. Enzyme activity is determined from the number of active oligomers and monomers immediately at the completion of irradiation.

For each target subunit in an n -oligomer, the probability of hitting the subunit 1 or more times is $1 - e^{-D/D_0}$ and of missing the subunit is e^{-D/D_0} . The probability of missing all n subunits in the oligomer is e^{-nD/D_0} . The probability of hitting one subunit and only one subunit is the product of the probability of hitting one subunit and the probability of not hitting $n - 1$ subunits, multiplied by the number of ways, n , in which a single subunit can be hit, $ne^{-(n-1)D/D_0}(1 - e^{-D/D_0})$. In general, the probability of hitting i and only i subunits, P_i , is,

$$P_i = \frac{n!}{(n-i)!i!} e^{-(n-i)D/D_0}(1 - e^{-D/D_0})^i. \quad [6]$$

Eq. 6 is a binomial distribution and satisfies the normalization condition,

$$1 = \sum_{i=0}^n P_i. \quad [7]$$

If inactivation of an oligomer requires h or more subunits to be hit 1 or more times, then

$$[A_n] = [A_n^0] \left(1 - \sum_{i=h}^n \frac{n!}{(n-i)!i!} e^{-(n-i)D/D_0}(1 - e^{-D/D_0})^i \right) \quad [8a]$$

$$[A] = [A^0]e^{-D/D_0}. \quad [8b]$$

Eqs. 4, 8a, and 8b give the activity-dose relationship for a system requiring h hits to inactivate an oligomer where there is no equilibration between A_n and A after radiation. Eq. 8a shows correct limiting cases: $[A_n] = [A_n^0]$ when $D = 0$, $A_n = 0$ when $h = 0$, $[A_n] = [A_n^0]e^{-D/D_0}$ when $n = h = 1$, and $[A_n] =$

$[A_n^0](1 - (1 - e^{-D/D_0})^n)$ for $h = n$. The latter limiting case is the well-known expression for the single-hit, multi-target model in which all targets must be hit for inactivation.

If the oligomer dissociates into monomers after h or more hits, as suggested by Kincaid *et al.* (3) for calmodulin-activated cyclic nucleotide phosphodiesterase, the number of surviving oligomers is given by Eq. 8a and surviving and newly formed monomers becomes

$$[A] = [A^0]e^{-D/D_0} + [A_n^0] \sum_{i=h}^n \frac{(n-i)n! e^{-nD/D_0} (1 - e^{-D/D_0})^i}{(n-i)!i!}. \quad [9]$$

The addition of the $(n-i)$ term in Eq. 9 arises because $n-i$ active monomers are formed when the oligomer is hit i times.

When m subunits are destroyed by a single hit with energy transfer between subunits, the h term in Eq. 8a is replaced by $\text{int}(h/m)$, in which $\text{int}(x)$ is the smallest integer greater than or equal to x .

RESULTS

In most examples a tetrameric oligomer was used to compute predicted activity-dose relationships. The effect of oligomer size on activity-dose relations was also examined.

Fig. 1 shows the activity-dose curve for tetramer inactivation in an equilibrium model in which monomer possesses enzyme activity. At $K = \infty$, the straight line represents the exponential inactivation of monomer, $R = e^{-D/D_0}$. The shoulder appearing at low doses results from a shift in equilibrium from oligomer to active monomer that occurs as subunits are destroyed. The shoulder is greatest when the initial equilibrium favors the oligomer, which serves as a buffer to supply active monomer. At high radiation doses, however, the curves become parallel, with slope equal to $-1/D_0$, as in the case of monomer inactivation when $K = \infty$.

The same system is shown in Fig. 2 in a nonequilibrium model. Destruction of a tetramer by a single radiation hit gives three active monomers. At low radiation doses there is apparent enzyme activation because the number of active monomers formed from tetramer destruction exceeds the number of inactivated monomers (see Eq. 9). Activation is more pronounced at small K , when initial equilibrium favors the tetramer.

Although arbitrary values for $[A_i]$ and D_0 were chosen to display the activity-dose relationships in Figs. 1 and 2, the detailed shapes of the curves are valid for any values of $[A_i]$ and D_0 . When D_0 is replaced by D'_0 , each of the curves displayed is scaled by a factor of D'_0/D_0 in the x direction. Replacement of $[A_i] = 1$ by $[A_i]'$ would change the interpretation of the K values, and the new K values would depend

FIG. 1. Tetramer inactivation in equilibration model. Complete equilibration of $A_4 \rightleftharpoons 4A$ is allowed after radiation. Only monomer is active. Curves obtained from Eqs. 2-4 were generated with $[A_i] = 1$, $D_0 = 2$, $n = 4$, $\alpha = 1$, and $\beta = 0$ with K values as shown. Curves are normalized to unit activity at zero radiation dose. $[A_i]$, D_0 , and K are taken as dimensionless parameters throughout as discussed in the text.

FIG. 2. Tetramer inactivation in nonequilibrium model. Inactive tetramers are destroyed by a single radiation hit and dissociate into active monomers as described by Eqs. 4, 8a, and 9. Conditions: $[A_t] = 1$, $D_o = 2$, $n = 4$, $\alpha = 1$, $\beta = 0$, and K values as shown.

upon $[A_t]/A$ and n , the number of subunits making up the oligomer. It is thus possible to use these curves to interpret experimentally derived nonlinear activity-dose relationships for a wide variety of enzyme systems.

Fig. 3 shows the effect of allowing the oligomer, as well as the monomer, to have enzyme activity in equilibration and nonequilibrium models. In Fig. 3 *Left* there is relatively little change in the shape of the concave downward inactivation curve until monomer-to-oligomer activity, α/β , becomes less than 0.5. When $\alpha/\beta = 0.25$ the inactivation curve is linear; for $\alpha/\beta < 0.25$ the inactivation curves becomes concave upward.

If either oligomer or monomer activity strongly dominates, there is minimal effect of the presence of the less active species; conversely, differences among the theoretical curves are resolved sufficiently to decide whether the oligomer or monomer possesses most activity. A similar conclusion holds for the nonequilibrium model shown in Fig. 3 *Right*. At high α/β , there is enzyme activation at low radiation dose, whereas at $\alpha/\beta < 0.25$, the inactivation curve becomes concave upwards. In the limit $\alpha/\beta \rightarrow 0$, full oligomer destruction by a single radiation hit represents a specific example of energy transfer and will be discussed shortly.

Fig. 4 illustrates the effect of oligomer size, n , on activity-dose curves for both equilibration and nonequilibrium models. In the equilibration model, the initial plateau becomes flatter and shorter as n increases. Similarly, in the nonequilibrium model, the activation hump at low dose extends less far along the dose axis. If inactivation data are sufficiently well resolved, it should be possible to estimate oligomer size from the shape of the inactivation curve at low dose.

In the equilibrium model, energy transfer increases the observed inactivation at low radiation doses, when the oligomer-to-monomer ratio is greatest. Fig. 5 demonstrates that as the number of subunits destroyed by a single hit increases

FIG. 3. Effect of tetramer activity in equilibration and nonequilibrium models. Data from Figs. 1 and 2 are plotted for $K = 0.1$ at several values of monomer-to-oligomer activity, α/β , defined in Eq. 4. Curves are normalized to unit activity at zero radiation dose. (*Left*) Equilibration model; (*Right*) nonequilibrium model.

FIG. 4. Effect of oligomer size in equilibration and nonequilibrium models. (*Left*) Data calculated from equilibration model described by Eqs. 2-4. Conditions: $[A_t] = 1$, $D_o = 2$, $K = 0.01$, $\alpha = 1$, $\beta = 0$, and n values as shown. (*Right*) Data calculated from nonequilibrium model in Eqs. 4, 8a, and 9. Conditions: $[A_t] = 1$, $D_o = 2$, $K = 0.1$, $\alpha = 1$, $\beta = 0$, and n values as shown.

to approach the total number of oligomer subunits, n , the activity-dose relationship changes from a concave-downward curve to a straight line. It is interesting that at high doses the curves for $m \geq 2$ overlap yet remain distinct from the inactivation curve for $m = 1$. The conceptual explanation follows from the condition that oligomer-monomer equilibration does not occur until after radiation exposure. At high doses, for $m \geq 2$, there is preferential destruction of nearly all oligomers. Therefore, separation between the $m = 1$ and $m \geq 2$ curves is a measure of the oligomer concentration before irradiation.

Thus far, concave-upward inactivation curves have been seen only for inactivation at low α/β in Fig. 3. A concave upward curve is generally taken to mean independent inactivation of two targets, A_1 and A_2 , with activities γ_1 and γ_2 and D_o s of D_1 and D_2 ,

$$R = \frac{\gamma_1 A_1 e^{-D/D_1} + \gamma_2 A_2 e^{-D/D_2}}{\gamma_1 A_1 + \gamma_2 A_2} \quad [10]$$

In terms of an oligomeric enzyme system, Eq. 10 applies to the case when both monomer and oligomer have activity and there is no equilibrium or dissociation of oligomers after radiation inactivation. Fig. 6 illustrates tetramer/monomer inactivation under these conditions for single-hit inactivation. Concave-upward activity-dose relationships do not occur for situations in which oligomers and monomers interact with each other except for the case of high oligomer-to-monomer activity ratios shown in Fig. 3. Therefore, the observation of such a concave-upward activity-dose relationship generally implies the presence of independent targets of various functional sizes that are not in equilibrium with each other.

While more precise definition of accurate target sizes for oligomer and monomer may not always be possible from ex-

FIG. 5. Effect of energy transfer on tetramer inactivation in the equilibration model. Curves were obtained from Eqs. 2, 4, and 5, with $[A_t] = 1$, $D_o = 2$, $K = 0.1$, $n = 4$, $\alpha = 1$, $\beta = 0$, and m values as shown.

FIG. 6. Independent destruction of tetramers and monomers without equilibration or dissociation. Curves were obtained from Eq. 10 with monomer $D_1 = 1$ and tetramer $D_2 = 4D_1 = 4$ for values of $\gamma_1 A_1 / \gamma_2 A_2$ as shown.

perimental data, it is evident that the shapes of activity-dose curves allow distinction among differing models of oligomer-monomer interaction.

The requirement of multiple hits for inactivation gives concave-downward inactivation curves similar to those in Figs. 1–4. This can occur by the single-hit multitarget model given by Eqs. 6 and 8a or by the multihit single-target model described by a Poisson distribution (4).

$$R = e^{-D/D_0} \sum_{i=0}^{h'-1} \frac{(D/D_0)^i}{i!}, \quad [11]$$

in which h' is the number of required hits for inactivation. Eq. 11 predicts concave-downward inactivation curves even for inactivation of monomers alone. A proposed example of the multihit single-target model is the target analysis of erythrocyte hypertonic cryohemolysis (5). It was postulated that multiple hits are required for inactivation of the very large spectrin-actin molecular complex, resulting in concave-downward radiation inactivation curves with an extended initial shoulder at low radiation dose.

Fig. 7 shows the results of a multitarget single-hit inactivation model for an active tetramer under conditions in which monomers are not present. The shoulder, most pronounced when the number of hits required for inactivation is large, represents enzyme protection at low doses, when the probability of obtaining the required number of hits is small. The presence of monomers in a nonequilibrium model would introduce additional complexity into the curves but would not alter the presence of the shoulder at low doses. A multitarget single-hit model is not defined when equilibration occurs, because oligomer-monomer shift after radiation gives no preference to single vs. multiple subunit inactivations in an oligomer.

FIG. 7. Multitarget single-hit tetramer inactivation. Curves were drawn from Eq. 8a for a system containing only tetramers, with $D_0 = 2$, $n = 4$, and h values as shown.

DISCUSSION

Radiation inactivation measurements are used to obtain information about the size and functional structure of soluble or membrane-bound enzymes as a complementary tool to classical biochemical techniques. Useful information can be obtained without enzyme purification, which may disrupt functionally important subunit assembly. The effect of various activators or inhibitors on subunit association may be examined. However, it is clear from the previous section that a given activity-dose curve may be obtained from a large number of different permutations of model parameters— K , α , β , n , m , h , and h' —and physical conditions, including extent of equilibration after radiation exposure and the presence or absence of energy transfer between oligomeric subunits. It is important to establish a systematic approach to the use of radiation inactivation as a probe for enzyme structure and function and to understand the practical limitations of the method.

The basic shape of the activity-dose curve restricts the class of models to be considered. A straight line has been encountered for the vast majority of enzyme systems and is interpreted as single-hit inactivation of a homogeneous enzyme system. Target theory gives the relationship between slope of a single exponential activity-dose curve, $1/D_0$, and the enzyme molecular weight, M_r ,

$$M_r = \frac{\sigma}{D_0} 6.4 \times 10^{11}. \quad [12]$$

There is a temperature-dependent correction factor, σ , which is 2.0–2.6 at -150°C (2). The molecular weight for a number of enzyme systems determined by radiation inactivation has been in good agreement with the molecular weight determined by biochemical methods.

It has been proposed that the degree of coupling between enzyme subunits determines whether radiation inactivation selects the molecular weight for the whole enzyme or for a single active subunit (6). In a number of studies energy transfer among noncovalently linked proteins that are enzyme subunits was not apparent (3, 6, 7). Experimentally it may be possible to rule out energy transfer among oligomer subunits if enzyme activity can be assayed in monomer form, as, for example, by addition of an agent that causes oligomer dissociation. It may be concluded that energy transfer does not occur when inactivation data do not depend upon whether the dissociating agent is added before or after radiation exposure. For example, radiation inactivation data show similar linear activity-dose relationships when vasopressin is added either before or after radiation exposure to a pig kidney adenylate cyclase preparation, whereas the activity-dose curve is concave downward in the absence of vasopressin (unpublished observations).

It is important to distinguish between equilibration and nonequilibrium models. Enzyme activation cannot occur in an equilibration model. Since $[A_i]$ decreases exponentially (Eq. 3), the concentration of every reactant in chemical equilibrium with A_i must decrease with increasing radiation dose. A nonequilibrium model can result in enzyme activation as shown in Fig. 2. If radiation causes dissociation of an oligomer into active monomers, activation must occur at low radiation doses. This has been observed by Kincaid *et al.* (3) for basal cyclic nucleotide phosphodiesterase activity. Destruction of an inhibitor can also give apparent activation in a nonequilibrium model, as proposed by Harmon *et al.* (8) for the insulin receptor. The molecular weight for an inhibitor in such cases would be substantially larger than the molecular weight of the receptor or enzyme itself.

As demonstrated in *Results*, concave-downward curves are predicted from oligomer inactivation for cases of equili-

bration (Fig. 1), nonequilibrium (Fig. 2), energy transfer (Fig. 5), and multihit requirements (Fig. 7). In contrast, concave-upward inactivation curves represent the independent destruction of one or more active enzymes of different molecular weights or oligomeric sizes (Fig. 6). Schlegel *et al.* (9) obtained concave-upward activity-dose curves for inactivation of adenylate cyclase in hepatic membranes. They proposed that the enzyme could exist in both oligomeric and monomeric forms and they estimated the oligomer size from Eq. 12, in which $-1/D_0$ is the slope of the initial, steep part of the inactivation curve, where single-hit oligomer inactivation dominates. As already discussed, there are few other models that predict concave-upward inactivation curves. If the oligomer has much greater activity than monomer ($\alpha/\beta < 0.25$ in Fig. 3), the inactivation curves will be concave upward. However, it should be possible to distinguish independent destruction of more than one enzyme from oligomer-monomer equilibration at high oligomer-to-monomer activity ratios by performing experiments under conditions in which most of the enzyme is in monomer form ($[A^0] \gg [A^0_2]$). If interaction is present, the initial rate of inactivation at low dose will increase as $[A^0_2]/[A^0]$ increases, whereas, if two independent inactivations exist, data will show two constant inactivation rates with amplitude ratios $\gamma_1 A_1/\gamma_2 A_2$ (Eq. 10) depending on the oligomer-to-monomer ratio.

It is important to evaluate whether the presence of concave-downward inactivation curves proves the presence of an oligomeric enzyme structure. Multihit single-target requirements generally imply the presence of a very large structural protein as proposed for the spectrin-actin molecular complex in erythrocytes (5). It should be possible to distinguish experimental multihit single-target inactivation curves described by Eq. 11 from oligomeric inactivation curves as in Fig. 1. The multihit single-target curve shape gives a plateau of zero slope at low radiation dose with a shape similar to curves in Fig. 7, whereas oligomer inactivation gives an initial nonzero slope at low radiation dose. Furthermore, the limiting slope at high radiation dose should be very large for the multihit case, implying inactivation of a high molecular weight protein [$M_r = 16 \times 10^6$ (ref. 5)]. There are no proven cases of multihit requirements for monomeric enzyme systems. As Simon *et al.* (10) point out, however, the presence of multiple equilibria among species at several molecular weights can theoretically produce complex inactivation curves. Concave-downward inactivation curves may

also arise from the interaction of several species all required for enzyme activity or its modulation.

In summary, it is evident that an explicit statement of the assumptions used to derive a model for radiation inactivation is required for meaningful interpretation of experimental data as well as systematic exploration of alternative plausible models. The presence of an oligomeric structure can be strongly supported by quantitative agreement of experimental inactivation data with a theoretical model.

Experimental approaches have been suggested to evaluate the presence of equilibration and energy transfer. It is stressed that only when a framework for interpretation of complex inactivation curves is firmly established does it become possible to use the radiation inactivation method as a limited tool to probe the structure and function of complex biological systems. Within such a framework, it should be possible to propose mechanisms of biological importance that account for transitions in radiation inactivation curves produced by selected experimental interventions such as addition of hormone or enzyme activator.

We gratefully acknowledge the critical suggestions provided by Drs. Chan Jung and Robert Spangler, who kindly reviewed the manuscript; Dr. A. K. Solomon, in whose laboratory the computer analysis was performed; and Ms. Beth King for her expert editorial and secretarial assistance. K.S. and D.A.A. are supported by U.S. Public Health Service Grants AM 30410 and AM19406, respectively.

1. Lea, D. E. (1955) *Action of Radiation on Living Cells* (Cambridge Univ. Press, Cambridge, England), 2nd Ed.
2. Kempner, E. S. & Schlegel, W. (1979) *Anal. Biochem.* **92**, 2-10.
3. Kincaid, R. L., Kempner, E. S., Manganiello, V. C., Osbourne, J. C. & Vaughan, M. (1981) *J. Biol. Chem.* **256**, 11351-11355.
4. Oliver, R. & Shepstone, B. J. (1964) *Phys. Med. Biol.* **9**, 167-175.
5. Green, F. A., Jung, C. Y., Cuppoletti, J. & Owens, N. (1981) *Biochim. Biophys. Acta* **648**, 225-230.
6. Kempner, E. S., Miller, J. H., Schlegel, W. & Hearon, J. Z. (1980) *J. Biol. Chem.* **255**, 6826-6831.
7. Blum, E. & Alper, T. (1971) *Biochem. J.* **122**, 677-680.
8. Harmon, J. T., Kahn, C. R., Kempner, E. S. & Schlegel, W. (1980) *J. Biol. Chem.* **255**, 3412-3419.
9. Schlegel, W., Kempner, E. D. & Rodbell, M. (1979) *J. Biol. Chem.* **254**, 5168-5176.
10. Simon, P., Swillens, S. & Dumont, T. E. (1982) *Biochem. J.* **205**, 477-483.