The substructure of phosphodiesterase as established by radiation inactivation

A reinterpretation of results

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Received 9 September 1982

A model for the activation of phosphodiesterase by calmoduling based on a conversion of inactive dimers to active monomers, derived from radiation inactivation studies J. Biol. Chem. (1981) 256, 11351–11355 has been re-examined using a simple probability argument. We conclude that the original model is not supported by the radiation inactivation studies, since our analysis of this model would predict that the rate of radiation inactivation of calmodulin-dependent phosphodiesterase activity be exactly twice that for the decay in total activity in marked contrast with the results obtained.

Phosphodiesterase *Calmodulin* *Radiation inactivation* *Dimerisation*

1. INTRODUCTION

Radiation inactivation represents an empirical technique for investigating the molecular size and possible subunit structure of biologically relevant proteins. Regardless of some practical difficulties and some unproven theoretical assumptions, the technique is often successful. An empirical equation relating dose to molecular size, derived by Kepner and Macey in 1968 [1] is almost universally employed (although it has been modified for low temperature studies [2]): it has, as an obvious condition, that the logarithm of the activity remaining after irradiation be linearly related to the radiation dose. The equation can be written as:

$$X_r = X_o e^{-kr}$$

where X_o and X_r are the concentrations of the target being investigated before and after receiving a radiation dose r, and k is a constant proportional to the molecular mass of the target.

Recently, the technique has been applied to more complex systems to investigate such problems as the reaction of hormone receptors with adenylate kinase [3,4] and the activation of phosphodiesterase by calmodulin [2]. Radiation inactivation analysis has suggested the operation of models requiring selective or partial inactivation of some component(s) of these multimeric systems.

In view of the interest generated by such models, we feel it necessary to point to some of the difficulties involved in the interpretation of a model developed for the activation of phosphodiesterase by calmodulin [2].

According to this model, phosphodiesterase exists as a non-equilibrium mixture of active monomers and inactive dimers. Treatment with calmodulin converts each dimer into two active monomers, and all monomers, whatever their origin, have identical enzymic activity.

An interesting result of irradiating phosphodiesterase preparations with high energy electrons is an apparent increase in activity [2]. This is interpreted as the activation of dimers which have had one of their component monomers destroyed by radiation. This model is illustrated in fig. 1.

Published by Elsevier Biomedical Press
00145793/82/0000—0000/$2.75

According to the above assumptions, the *basal* phosphodiesterase activity of a non-irradiated preparation will be proportional to the concentration of monomers present. After irradiation, the basal phosphodiesterase activity will depend upon the concentration of surviving monomers plus the concentration of monomers formed from hits on dimers. Since calmodulin is proposed to activate all dimers present, the calmodulin-dependent phosphodiesterase activity will be proportional to the concentration of dimers present. Since all monomers, whatever their origin, are assumed to have the same phosphodiesterase activity, the enzyme activity assayed in the presence of calmodulin (i.e., total activity) will be proportional to the total number of monomers in the preparation, regardless of their origin. Table 1 lists the mathematical relationships corresponding to the above three activities.

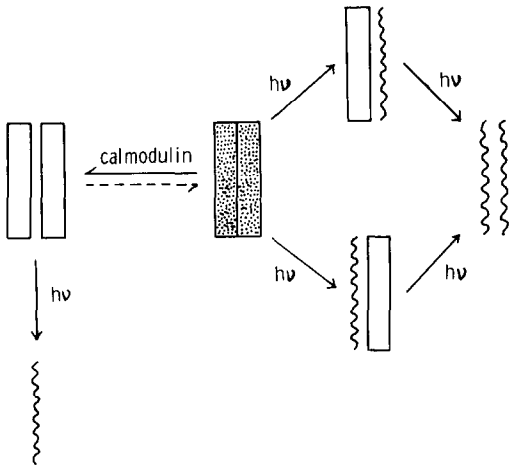

Fig. 1. Diagrammatic representation of the model proposed in [2,5] to explain the activation of phosphodiesterase by calmodulin and by low doses of radiation ($h\nu$): (□) active units; (▨) inactive units; (⦚) radiation-activated units.

2. PROBABILITY ARGUMENT

In the irradiated state there are 4 possibilities:

(i) Unhit dimer;
(ii) Dimer hit at least once on one subunit and not on the other;
(iii) Dimer hit at least once on the other subunit; i.e., the same as (ii) in reverse;
(iv) Dimer hit at least once on each subunit.

After irradiation, the sum of the 4 probabilities must equal unity.

$$p(\mathrm{a}) = \mathrm{e}^{-k}D^{\mathrm{r}} = \mathrm{e}^{-2k}M^{\mathrm{r}}$$

$$p(\mathrm{b}) = \mathrm{e}^{-k}M^{\mathrm{r}}(1 - \mathrm{e}^{-k}M^{\mathrm{r}})$$

Table 1

	Phosphodiesterase activity after		Fractional phosphodiesterase activity remaining after
	0 rads	r rads	r rads
1: Basal activity	$\propto M^{\mathrm{o}}$	$\propto M^{\mathrm{o}}\mathrm{e}^{-kr} + 2D^{\mathrm{o}}(1 - \mathrm{e}^{-kr})\mathrm{e}^{-kr}$	$A^{\mathrm{r}}/A^{\mathrm{o}} = \left(1 + 2\frac{D^{\mathrm{o}}}{M^{\mathrm{o}}}\right)\mathrm{e}^{-kr} - 2\frac{D^{\mathrm{o}}}{M^{\mathrm{o}}}\mathrm{e}^{-2kr}$
2: Calmodulin-dependent activity	$\propto 2D^{\mathrm{o}}$	$\propto 2D^{\mathrm{o}}\mathrm{e}^{-2kr}$	$\frac{A_{\mathrm{r}}^{\mathrm{cal}}}{A_{\mathrm{o}}^{\mathrm{cal}}} = \mathrm{e}^{-2kr}$
3: Total activity	$\propto M^{\mathrm{o}} + 2D^{\mathrm{o}}$	$\propto (M^{\mathrm{o}} + 2D^{\mathrm{o}})\mathrm{e}^{-kr}$	$\frac{A_{\mathrm{r}}^{\mathrm{total}}}{A_{\mathrm{o}}^{\mathrm{total}}} = \mathrm{e}^{-kr}$

M^{o} and D^{o} = monomer and dimer concentrations before irradiation

M^{r} and D^{r} = monomer and dimer concentrations after irradiation with r rads

k = an inactivation constant proportional to the radiation target size (i.e., monomer molecular mass)

A, A^{cal}, A^{total} = basal, calmodulin-dependent and total enzyme activities; the subscripts o and r refer to activities of the enzyme before and after irradiation with a dose of r rads

$$p(c) = e^{-k}M^r(1 - e^{-k}M^r)$$
$$= 2e^{-k}M^r - 2e^{-2k}M^r$$
$$p(d) = (1 - e^{-k}M^r)(1 - e^{-k}M^r)$$
$$= 1 - 2e^{-k}M^r + e^{-2k}M^r$$

and

$$p(a) + p(b) + p(c) + p(d) = 1$$

(This would not hold if the '2' derived from the sum of $p(b)$ and $p(c)$ was not present)

Then, since M^r (the number of active monomers) is the same as the number of radiation damaged dimers, we would multiply the sum of (b) + (c) by D_0 the number of dimers initially present, to obtain the phosphodiesterase activity. This produces our eq. (1).

3. DISCUSSION

The present simple final equations are in marked contrast with those in [2]. Apart from an unfortunate orthographic error which makes it difficult to follow the derivation of their equations, these authors fail to take into account the probability factor involved in producing an active monomer by hitting either half of the original dimer (see fig. 1). A further complication is that a monomer $\rightleftharpoons$ dimer equilibrium once disturbed by radiation inactivation may reequilibrate.

This analysis of the model in [2] requires that the rate of radiation inactivation of calmodulin-dependent phosphodiesterase activity be exactly twice that for the decay in total activity (lines 2 and 3, table 1). Since the final results in [2] are only presented in refined form fitted by incorrect equations, it is impossible to assess the validity of the model from their calculated molecular mass values. However, if those of their experimental results that are presented in graphical form (redrawn here in fig. 2) are representative of all the experiments made, they are far from showing a decrease in target size to one-half following calmodulin activation.

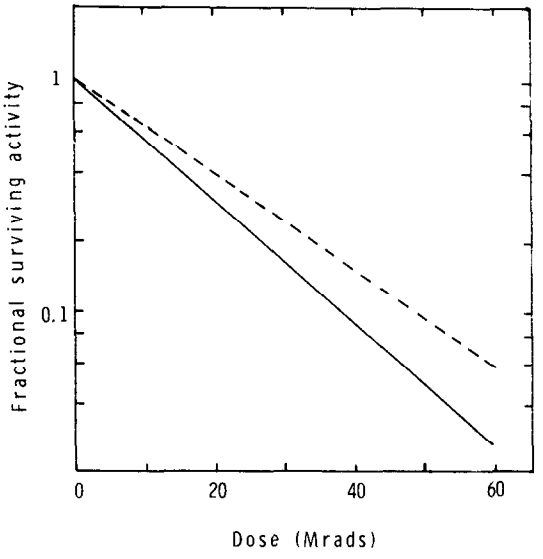

Fig. 2. Fraction of phosphodiesterase activity surviving irradiation. Redrawn from fig. 2 and 3 in [2]: (——) calmodulin-dependent phosphodiesterase activity; (– – –) Total phosphodiesterase activity.

We must therefore conclude that the model for the calmodulin activation of dimeric phosphodiesterase developed in [2,5] is not supported by their radiation inactivation data.

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