A PITFALL IN THE INTERPRETATION OF DATA ON ADENYLATE CYCLASE INACTIVATION BY IRRADIATION

Stéphane SWILLENS and Jacques E. DUMONT
Institut de Recherche Interdisciplinaire, School of Medicine, Free University of Brussels, 1000 Brussels, Belgium

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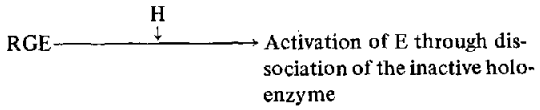
1. Introduction

Enzyme inactivation by irradiation has been recognized as a valuable technique for the determination of enzyme size (review [1]). This approach has been used by some groups for elucidating the dynamic structure of hormone-responsive adenylate cyclase [2–5]. These authors described the organization of the known components of the system (hormone receptor (R), guanine nucleotide binding protein (G), adenylate cyclase enzyme (E)) prior to and subsequent to activation of the system by different effectors as hormones, guanine nucleotides and ions. For example, on the basis of target size analysis, it was proposed [5] that the ground state of the turkey erythrocyte adenylate cyclase system consists of a tightly bound ternary complex RGE which dissociates upon isoproterenol action and releases an active E unit (precoupled model). This description is in complete disagreement with the model derived from kinetic experiments (review [6]) which suggests that hormone binding to the uncoupled receptor leads to the formation of a transient ternary complex HRG necessary for the activation of the G-unit which in turn activates the adenylate cyclase E. The latter model (preuncoupled model) is conceptually compatible with the cyclic model in [7], the collision coupling model [8], the ternary complex model [9]. In our opinion, it is impossible to reconcile these contradictory concepts as defined in fig.1.

Here, we show that the target size analysis applied to the adenylate cyclase system is misleading as this method cannot discriminate between the 2 models at least on the basis of the data reported so far.

Precoupled Model



Preuncoupled Model

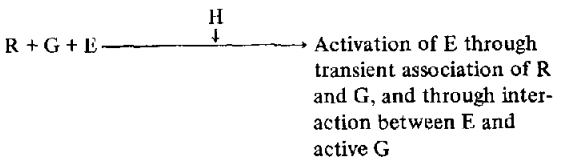


Fig.1. Definitions of two hypothetical models describing adenylate cyclase activation by hormone: R, hormone receptor; G, guanine nucleotide binding protein; E, adenylate cyclase.

2. Rationale

The rationale of the target size analysis was previously described (review [1]). Briefly, irradiation of a sample leads to the deposition of ionization energy. If ionization occurs inside an enzyme molecule, the enzymatic activity is completely lost. As the ionization occurs randomly, the larger the enzyme size, the more likely it will be destroyed. The concentration of survival active enzyme [E] obeys a simple exponential law:

$$[E] = [E]_0 \, e^{-\mu_E D} \qquad (1)$$

where D is the radiation dose (rads), $[E]_0$ is the enzyme concentration prior to irradiation and μ_E is a

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factor proportional to the relative molecular mass ($M_{r,E}$) of the enzyme [10]:

$$\mu_E = \frac{M_{r,E}}{6.4 \times 10^{11}} \quad (2)$$

Thus

$$\log ([E]/[E]_0) = \log (\nu/\nu_0) = -\mu_E D \quad (3)$$

where ν and ν_0 are the enzymatic activities corresponding to the concentrations [E] and $[E]_0$, respectively. The measure of activity decay due to increasing doses of irradiation allows the determination of μ_E as the slope of the straight line in the plot $\log (\nu/\nu_0)$ vs D.

The key eq. (3) permits one to predict how the enzymatic activity is related to the amount of radiation exposure. For one type of enzyme, eq. (3) leads to the determination of μ_E. If the same activity resides independently in two types of enzyme E_1 and E_2, then:

$$\nu \sim [E_1] + [E_2]$$

$$\sim [E_1]_0 e^{-\mu_{E_1} D} + [E_2]_0 e^{-\mu_{E_2} D}$$

Thus the plot $\log (\nu/\nu_0)$ vs D should exhibit the combination of two linear components from which both M_r-values and the relative proportion of the two enzymes can be evaluated.

The application of the target size analysis to the adenylate cyclase is more complex as the enzymatic activity is elicited by only one of the components, i.e., the E unit, whereas the two other components, R and G, are required for transmitting the hormone stimulus. Thus the adenylate cyclase activity as measured in the presence of hormone, is the consequence of the interactions between the 3 components and is thus dependent on the concentration of the three components all of them sensitive to the radiation inactivation depending on their respective M_r-value.

For the turkey erythrocyte ground state, it was reported that the $\log (\nu/\nu_0)$ vs D plot is linear and that the target size is equivalent to the sum of the sizes of the 3 components [5]. This observation led these authors to conclude that the 3 components are associated prior to activation (precoupled model). We would like to present a line of evidence that this experimental observation can be accounted for by the preuncoupled model as well and that the observed target size does not depend on the state of coupling of the different components.

(1) For the precoupled model, the hit of any component of the complex induces the loss of the associated adenylate cyclase activity in response to hormonal stimulus, either by the transfer of the ionization energy to the 2 other components or by the simple destruction of the whole function since all the 3 components are required for the adenylate cyclase activation by the hormone. Thus, it can be expected that if the activity is proportional to the holoenzyme concentration, then the survival activity in response to hormone addition is equal to:

$$\nu = \nu_0 e^{-(\mu_R + \mu_G + \mu_E) D}$$

where μ_R, μ_G and μ_E are proportional to the M_r-values of R, G and E, respectively [eq. (2)]. Thus the observed size of the target is equal to the size of the holoenzyme.

(2) For the preuncoupled model, the regulatory components R and G are also required for the adenylate cyclase activation by hormone. The activation could be described as a trimolecular process and in this case the activity is proportional to the product of the concentration of the 3 components:

$$\nu \sim [R]\,[G]\,[E]$$

and thus

$$\nu \sim [R]_0 e^{-\mu_R D}\,[G]_0 e^{-\mu_G D}\,[E]_0 e^{-\mu_E D}$$

or also

$$\nu = \nu_0 e^{-(\mu_R + \mu_G + \mu_E) D}$$

The plot $\log (\nu/\nu_0)$ vs D exhibits only one component which is characterized by a slope proportional to the sum of the sizes of the 3 units. Thus in the case of this model, target size analysis observations will be similar to those predicted for the precoupled model.

Such a result is in fact not astonishing since the function of the system, i.e., the hormonal stimulation of adenylate cyclase, is lost if only one component is destroyed. The probability to hit any component does not depend on the state of coupling of these

components and for each model the size of the global entity responsible for the biochemical function consists of the sum of the sizes of the 3 components. Since the structure of the system prior to activation does not play any role in the sensitivity of the system to the radiation inactivation, both models should exhibit similar decay curves.

3. Discussion and conclusion

It was argued [5] that for the preuncoupled model the ground state target should reveal a non-linear exponential decay curve indicative of more than one target, one of which represents the independent R-unit. As shown here, the preuncoupled model is drastically different from a heterogenous population of 3 enzymes exhibiting the same function. In this latter case, the activity is the sum of the activities of the 3 independent components and is thus proportional to the sum of the concentrations of the 3 enzymes. The destruction of one enzyme does not affect the function of the 2 other enzymes. On the contrary, for the preuncoupled model, as well as for the precoupled model, the destruction of any component leads to the loss of the function. For both models, the exponential decay of the hormone sensitive adenylate cyclase activity is linear, at least in the considered cases, and reveals an apparent target size equivalent to the sum of the sizes of the 3 components.

The precoupled model was proposed on the basis of target size analysis whereas the preuncoupled model was presented as incompatible with these data [5]. In fact, target size analysis is unable to discriminate between the 2 models, at least on the basis of the current data. A special version of the preuncoupled model was previously analyzed. It was shown that such a model can account for many observations on the adenylate cyclase activation by hormones [6]. On the contrary, at least in the case of the turkey erythrocyte system, the precoupled model can be rejected on the basis of kinetic studies [8].

Here, we have presented only one case where the activity is proportional to the concentration of the components. In fact a more detailed study (in preparation) can show that the apparent target size might depend on the kinetics of the interactions between the components but both preuncoupled and precoupled models can exhibit similar decay curves.

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References

[1] Kempner, E. S. and Schlegel, W. (1979) Anal. Biochem. 92, 2–10.
[2] Houslay, M. D., Ellory, J. C., Smith, G. A., Hesketh, T. R., Stein, J. M., Warren, G. B. and Metcalfe, J. C. (1977) Biochim. Biophys. Acta 467, 208–219.
[3] Martin, R., Stein, J. M., Kennedy, E. L., Doberska, C. A. and Metcalfe, J. C. (1979) Biochem. J. 184, 253–260.
[4] Schlegel, W., Kempner, E. S. and Rodbell, M. (1979) J. Biol. Chem. 254, 5168–5176.
[5] Nielsen, T. B., Lad, P. M., Preston, M. S., Kempner, E., Schlegel, W. and Rodbell, M. (1981) Proc. Natl. Acad. Sci. USA 78, 722–726.
[6] Swillens, S. and Dumont, J. E. (1980) Life Sci. 27, 1013–1028.
[7] Cassel, D. and Selinger, Z. (1978) Proc. Natl. Acad. Sci. USA 75, 4155–4159.
[8] Tolkovsky, A. M. and Levitzki, A. (1978) Biochemistry 17, 3795–3810.
[9] De Lean, A., Stadel, J. M. and Lefkowitz, R. J. (1980) J. Biol. Chem. 255, 7108–7117.
[10] Kepner, G. R. and Macey, R. I. (1963) Biochim. Biophys. Acta 163, 188–203.