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ORIGINAL PAPER

A general model for non-autocatalytic zymogen activation in the presence of two different and mutually exclusive inhibitors. I. Kinetic analysis

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Abstract In this paper, a general mechanism of zymogen activation and the simultaneous action of two different, mutually exclusive, two-step inhibitors acting on both the activating and the activated enzymes is proposed and kinetically analyzed. This generalization offers the advantage of being applicable to a high number of real cases, since most mechanisms of zymogen activation involving reversible or irreversible, one or two step, equal or different inhibitors are particular cases of the general model here studied. Analogously, from the equations corresponding to the general model or from any of their particular cases, it is possible to obtain the equations corresponding to cases in which one or more of the reversible steps are in rapid equilibrium. The number and type of the particular cases arising from the general model are obtained in a systematic way.

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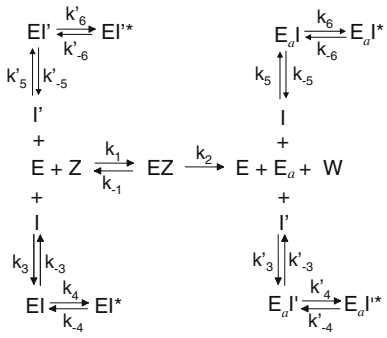
1 Introduction

Proteolytic enzymes are normally synthesized and secreted as inactive precursors, termed proenzymes or zymogens, with the aim of protecting the cells which produce them. These zymogens must undergo an activation process at a suitable time and place, usually consisting of a limited proteolysis involving selective cleavage of a peptide bond, to attain their catalytic activity. This is a very important phenomenon in many fundamental physiological processes, such as digestion, metabolism, immunity, blood coagulation, fibrinolysis, cell apoptosis, tumor growth and metastasis [1]. In recent times, work on proteolytic enzymes has accelerated greatly, fuelled by numerous practical applications in biotechnology and the realization that these enzymes are major therapeutic targets. Notably, the success of retropepsin inhibitors in the treatment of AIDS has provided an unambiguous validation of the principle that protease inhibitors can be successful drugs [2,3].

Limited proteolysis reactions are irreversible because, in common with many other hydrolytic reactions, proteolysis is an exergonic reaction under normal physiological conditions and there are no simple biological mechanism to repair a broken peptide bond. Accordingly, it is easy to see that misregulated proteolysis can lead to pathological conditions. For this reason, living organisms have developed a series of mechanisms to regulate the activity of proteolytic enzymes, including the control of enzyme synthesis and secretion and the presence of natural protease inhibitors in cells and body fluids [4,5]. Limited proteolytic reactions are also highly specific, which makes it possible to inhibit proteases when they are involved in pathological processes. Hence, protease inhibitors have considerable potential for therapeutic intervention in a variety of disease states. A good knowledge of the kinetics of protease inhibitors is obviously usefultool.

There are few contributions on the kinetic behaviour of zymogen activation processes subjected to the simultaneous action of inhibitors. Previously, we studied the transient phase of a zymogen activation process involving an irreversible inhibitor of both the activating and the active enzyme [6]. Other authors [7] have performed a kinetic analysis of zymogen autoactivation in the presence of a reversible inhibitor. More recently, Valero et al. [8] reported the kinetic analysis of a model of zymogen activation in the presence of two competitive inhibitors, one of the activating enzyme and the other one of the activated enzyme. The aim of this paper was to consider a wider reaction scheme (Scheme 1), covering the inhibition of both activating and activated enzymes by two different, mutually exclusive, inhibitors. This situation has been studied for enzymatic systems not involving zymogen activation [9–13], but not for zymogen activation processes. Figure 1 illustrates the current status of this subject. The importance of the model proposed here is that it includes, as particular cases, thousands of zymogen activation mechanisms, very few of which have been studied as contributions about the effect on zymogen activation processes in the presence of one [14] or two inhibitors [8].

Scheme 1



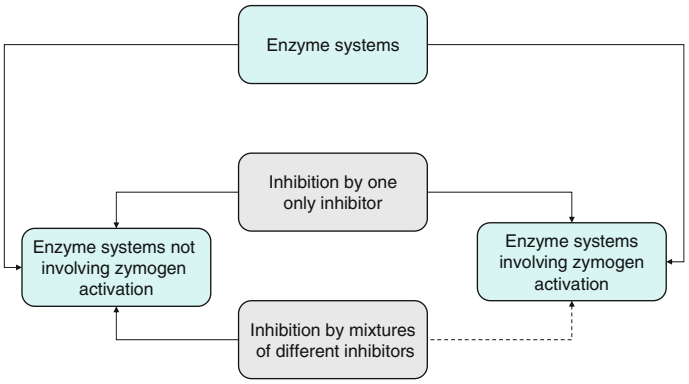


Fig. 1 Schematic representation of the current status of studies on inhibition enzymatic systems

2 Methods

Approximated analytical solutions from the set of differential equations corresponding to Scheme 1, once linearized, were obtained by using the well-known mathematical method of Laplace transformation [15].

Numerical solutions for the set of differential equations corresponding to Scheme 1 were obtained by means of the classical fourth-order Runge-Kutta formula, without resort to its linearization, but applying an adaptative stepsize control originally invented by Fehlberg [16], using a computer program implemented in Visual C++ 6.0 [17]. The above program was run on a PC compatible computer based on a Pentium IV/2 GHz processor with 512 Mbytes of RAM.

Finally, data obtained from analytical and numerical solutions were plotted using the SigmaPlot Scientific Graphing System for Windows version 8.02.

3 Theory

3.1 Notation

The following notation will be used in the present paper:

[E], [Z], [I], [I′], [EZ], [E$_a$], [EI], [EI′], [EI*], [EI′*], [E$_a$I], [E$_a$I′], [E$_a$I*], [E$_a$I′*]: Instantaneous concentrations of the species indicated.

[E]$_0$, [Z]$_0$, [I]$_0$, [I′]$_0$: Initial concentrations of the species E, Z, I and I′, respectively.

rt: reaction time.

K_m: Michaelis-Menten constant for the zymogen in relation with the activating protease, i.e.:

$$K_m = (k_{-1} + k_2)/k_1 \tag{1}$$

$K_j (j = 1, 3, 4, 5, 6)$: Equilibrium constants defined as:

$$K_j = k_{-j}/k_j \tag{2}$$

K'_j $(j = 3, 4, 5, 6)$: Equilibrium constants defined as:

$$K'_j = k'_{-j}/k'_j \tag{3}$$

$[\Sigma, E_a]$: Sum of the concentrations of all of the enzymatic species involved in Scheme 1 containing E$_a$, i.e.:

$$[\Sigma, E_a] = [E_a] + [E_a I'] + [E_a I'^*] + [E_a I] + [E_a I^*] \tag{4}$$

3.2 Kinetic behavior of the enzymatic system

The differential equation system describing the kinetic behavior of the enzymatic system shown in Scheme 1 is shown in the Appendix, Eqs. A.1–A.15. It is nonlinear and so it does not admit any analytical solution. However, under certain reasonable assumptions that are easy to implement experimentally, it is possible to linealize it to obtain approximate analytical solutions. To do this, we shall assume that the only species present at the onset of the reaction are E, Z, I and I'. In addition, we will assume the following initial conditions:

$$[Z]_0,\ [I]_0,\ [I']_0 >> [E]_0 \tag{5}$$

$$[I],\ [I']_0 >> [Z]_0 \tag{6}$$

We shall consider a reaction time, *rt*, during which $[E_a]$ is much smaller than $[Z]_0$, i.e.

$$[E_a] << [Z]_0 \text{ during time } rt \tag{7}$$

In these conditions, at any reaction time, it is observed that $[Z] \approx [Z]_0$, $[I] \approx [I]_0$ and $[I'] \approx [I']_0$, and thus the nonlinear set of differential Eqs. A.1–A.15 in the

Appendix becomes the following linear one:

$$\frac{d[E]}{dt} = -k_1[E][Z]_0 - k_3[E][I]_0 - k'_5[E]\left[I'\right]_0 + (k_{-1}+k_2)[EZ] + k_{-3}[EI] + k'_{-5}\left[EI'\right] \quad (8)$$

$$\frac{d[EZ]}{dt} = k_1[E][Z]_0 - (k_{-1}+k_2)[EZ] \quad (9)$$

$$\frac{d[E_a]}{dt} = k_2[EZ] - k'_3[E_a]\left[I'\right]_0 + k'_{-3}\left[E_aI'\right] - k_5[E_a][I]_0 + k_{-5}[E_aI] \quad (10)$$

$$\frac{d[EI]}{dt} = k_3[E][I]_0 - (k_{-3}+k_4)[EI] + k_{-4}[EI^*] \quad (11)$$

$$\frac{d[EI^*]}{dt} = k_4[EI] - k_{-4}\left[EI^*\right] \quad (12)$$

$$\frac{d[E_aI']}{dt} = k'_3[E_a]\left[I'\right]_0 - \left(k'_{-3}+k'_4\right)\left[E_aI'\right]_0 + k'_{-4}\left[E_aI'^*\right] \quad (13)$$

$$\frac{d[E_aI'^*]}{dt} = k'_4\left[E_aI'\right] - k'_{-4}\left[E_aI'^*\right] \quad (14)$$

$$\frac{d[EI']}{dt} = k'_5[E]\left[I'\right]_0 - \left(k'_{-5}+k'_6\right)\left[EI'\right] + k'_{-6}\left[EI'^*\right] \quad (15)$$

$$\frac{d[EI'^*]}{dt} = k'_6\left[EI'\right] - k'_{-6}\left[EI'^*\right] \quad (16)$$

$$\frac{d[E_aI]}{dt} = k_5[E_a][I]_0 - (k_{-5}+k_6)[E_aI] + k_{-6}\left[E_aI^*\right] \quad (17)$$

$$\frac{d[E_aI^*]}{dt} = k_6[E_aI] - k_{-6}\left[E_aI^*\right] \quad (18)$$

The differential Eqs. 8–18 can be analytically solved by any integration method, e.g. the Laplace transformation method [15]. Some of the results obtained are shown below.

3.3 Time course of the activated enzyme concentration

The equation obtained for the species E_a is the following (the detailed derivation is available for interested readers upon request):

$$[E_a] = \beta + \alpha t + \sum_{h=1}^{9} \gamma_h e^{\lambda_h t} \quad (19)$$

where λ_1, λ_2, λ_3, λ_4 and λ_5 are the roots of the equation:

$$\lambda^5 + F_1\lambda^4 + F_2\lambda^3 + F_3\lambda^2 + F_4\lambda + F_5 = 0 \quad (20)$$

and λ_6, λ_7, λ_8 and λ_9 are the roots of the equation:

$$\lambda^4 + F'_1\lambda^3 + F'_2\lambda^2 + F'_3\lambda + F'_4 = 0 \quad (21)$$

Any of the λ_h (h = 1,2,…,9) are real and negative or complex with a negative real part.

The expressions for F_i $(i = 1, 2, 3, 4, 5)$ and F_i' $(i = 1, 2, 3, 4)$ are indicated in the Appendix, Eqs. A.16–A.24. The expressions for the coefficients α, β and $\gamma_h (h = 1, 2, \ldots, 9)$ in Eq. 19 are the following:

$$\alpha = \frac{k_1 k_2 f_8 [Z]_0 [E]_0}{F_5 F_4'} \tag{22}$$

$$\gamma_h = \frac{k_1 k_2 \left(\lambda_h^8 + f_1 \lambda_h^7 + f_2 \lambda_h^6 + f_3 \lambda_h^5 + f_4 \lambda_h^4 + f_5 \lambda_h^3 + f_6 \lambda_h^2 + f_7 \lambda_h + f_8\right) [Z]_0 [E]_0}{\lambda_h^2 \prod_{\substack{p=1 \\ p \neq h}}^{9} \left(\lambda_p - \lambda_h\right)}$$

$$(\mathrm{h} = 1, 2, \ldots, 9) \tag{23}$$

$$\beta = -(\gamma_1 + \gamma_2 + \ldots + \gamma_9) \tag{24}$$

The expressions for f_q (q = 1,2,3,4,5,6,7,8) are indicated in the Appendix, Eqs. A.25–A.40. At relatively high rt-values, i.e., in the steady-state, exponential terms in Eq. 19 can be neglected and so the equation is simplified to:

$$[E_a]_{ss} = \beta + \alpha t \tag{25}$$

where $[E_a]_{ss}$ denotes the concentration of E_a in the steady-state.

3.4 Time course equation of $[\sum, E_a]$

Analogously, the equation corresponding to the concentration of any of the other nine enzymatic species involved in the mechanism shown in Scheme 1 can be obtained. For example, and because it will be used later, the equation describing the time-dependence of the concentration of the species $[EZ]$ is the following:

$$[EZ] = B_{6,0} + \sum_{h=1}^{5} A_{6,h} e^{\lambda_h t} \tag{26}$$

where:

$$B_{6,0} = \frac{k_1 g_4 [Z]_0 [E]_0}{F_5} \tag{27}$$

$$A_{6,h} = \frac{k_1 \left(\lambda_h^4 + g_1 \lambda_h^3 + g_2 \lambda_h^2 + g_3 \lambda_h + g_4\right) [Z]_0 [E]_0}{\lambda_h \prod_{\substack{p=1 \\ p \neq h}}^{5} \left(\lambda_p - \lambda_h\right)} \tag{28}$$

The coefficients g_1, g_2, g_3 and g_4 are functions of the rate constants and initial concentrations of inhibitors. Their expressions are given by Eqs. A.41–A.44 in the Appendix.

Deriving Eq. 4 with respect to time, gives:

$$\frac{d\,[\Sigma,\, E_a]}{dt} = k_2[EZ] \tag{29}$$

Inserting Eq. 26 into Eq. 29 and integrating, taking into account that $[\Sigma,\, E_a] = 0$ at $t = 0$:

$$[\Sigma,\, E_a] = C_0 + C_1 t + \sum_{h=1}^{5} \delta_h e^{\lambda_h t} \tag{30}$$

where C_0, C_1 and δ_h are given by:

$$C_0 = C_1 \left(\frac{g_3}{g_4} - \frac{F_4}{F_5} \right) \tag{31}$$

$$C_1 = \frac{k_1 k_2 g_4 [Z]_0 [E]_0}{F_5} \tag{32}$$

$$\delta_h = \frac{k_1 k_2 \left(\lambda_h^4 + g_1 \lambda_h^3 + g_2 \lambda_h^2 + g_3 \lambda_h + g_4 \right) [Z]_0 [E]_0}{\lambda_h^2 \prod_{\substack{p=1 \\ p \neq h}}^{5} (\lambda_p - \lambda_h)} (\mathrm{h} = 1, 2, \ldots, 10) \tag{33}$$

In the steady-state, i.e. when $t \to \infty$, exponential terms in Eq. 30 can be neglected, and so the equation can be simplified to:

$$[\Sigma,\, E_a]_{ss} = C_0 + C_1 t \tag{34}$$

4 Results and discussion

A complete kinetic analysis of the general model shown in Scheme 1 has been performed in the present paper, providing approximate analytical solutions for all of the enzymatic species. These equations are valid during the transient phase and the steady-state, provided that the conditions assumed are fulfilled.

The dependence of the activated enzyme concentration on time is described by Eq. 19, which consists of a first-order polynomial part and a sum of nine exponential terms. Bearing in mind that the arguments of the exponential terms are real and negative, or complex with the real part negative, these exponential terms can be neglected in the steady-state and so Eq. 25 is obtained.

The activity of the activated enzyme is described by Eq. 30, which consists of a first-order polynomial part and five exponential terms. In the same way as above, this equation is simplified to Eq. 34 in the steady-state.

4.1 Comparison between data obtained and simulated progress curves

The quality of the approximate analytical solutions here obtained can be assessed by comparing them with the corresponding particular numerical solutions obtained from the system of differential Eqs. A.1–A.15. This has been done for the species $[E_a]$ and $[\Sigma, E_a]$ for an arbitrary set of rate constants and initial conditions (Fig. 2). As can be seen, there is good agreement between the analytical and numerical solutions for approximately the first 30 s. Obviously, the deviation of theoretical data from the analytical solutions with respect to the numerical solutions will be greater as the time considered increases.

4.2 Particular cases of the general model

In this contribution we have made a kinetic analysis of a general model of proenzyme activation in the presence of two different, mutually exclusive, inhibitors (Scheme 1). Besides its physiological interest, this analysis will address, systematically, all the particular cases of the same. Indeed, one of the most important applications of the present paper is that the results obtained for the general model are applicable, without much mathematical effort, to any of its many particular cases (16,724 as we shall see

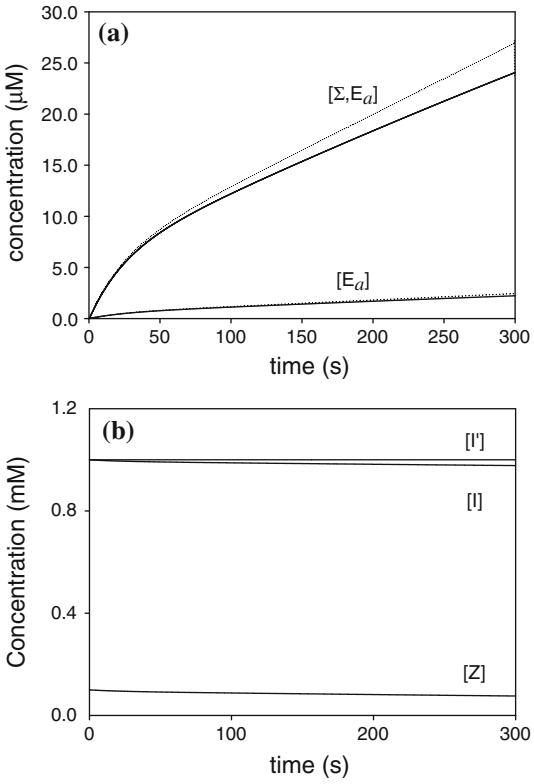


Fig. 2 **a** Progress curves of E_a and $[\Sigma, E_a]$ obtained by plotting Eq. 19 and 30 (*broken lines*) and by numerical integration of the system of differential Eqs. A.1–A.15 (*solid lines*). **b** Progress curves of *Z*, *I* and *I*′ consumption obtained by numerical integration. The following values were used for the rate constants and initial conditions: $k_1 = 10^4\ \mathrm{M^{-1}s^{-1}}$, $k_{-1} = 0.1\mathrm{s^{-1}}$, $k_2 = 1\mathrm{s^{-1}}$, $k_3 = 10^5\mathrm{M^{-1}s^{-1}}$, $k_{-3} = 10^3\mathrm{s^{-1}}$, $k_4 = 10^{-2}\mathrm{s^{-1}}$, $k_{-4} = 100\mathrm{s^{-1}}$, $k'_3 = 10^5\mathrm{M^{-1}s^{-1}}$, $k'_{-3} = 10\mathrm{s^{-1}}$, $k'_4 = 10^{-2}\mathrm{s^{-1}}$, $k'_{-4} = 10\mathrm{s^{-1}}$, $k'_5 = 10^3\mathrm{M^{-1}s^{-1}}$, $k'_{-5} = 1\mathrm{s^{-1}}$, $k'_6 = 0.1\mathrm{s^{-1}}$, $k'_{-6} = 0.01\mathrm{s^{-1}}$, $k_5 = 10^3\mathrm{M^{-1}s^{-1}}$, $k_{-5} = 50\,\mathrm{s^{-1}}$, $k_6 = 0.01\,\mathrm{s^{-1}}$, $k_{-6} = 0.1\,\mathrm{s^{-1}}$, $[E]_0 = 1\mu\mathrm{M}$, $[I]_0 = [I']_0 = 1\mathrm{mM}$ and $[Z]_0 = 0.1\,\mathrm{mM}$

later). Therefore, for the first time this study offers researchers on this topic a method based on general solutions that only need to be particularized to a specific problem of zymogen activation.

A large number of enzyme mechanisms can be considered particular cases (or derived) of another mechanism, called the primitive mechanism and more complex, after making certain changes [18]. These changes will be the same as those needed to be performed in the equations of the primitive mechanism to obtain the ones corresponding to the derived mechanism.

The most common changes to be made in the primitive mechanism to transform it into any of its particular cases, and which have been made in the present study, are the following: (A) make certain rate constants zero, (B) make one or more of the rate constants much greater than the others, i.e. let them tend to infinity and (C) match two different ligand species (in our case this change will be $I \equiv I'$ when they do not act on the same enzymatic species). Of course combinations of changes AB, AC, BC and ABC are also possible. For example, an AB change type means that certain constants are made zero in the primitive mechanism and, moreover, others tend to infinity.

4.2.1 Systematic retrieval of particular cases

In this section we shall systematically establish those mechanisms which can be considered particular cases of the mechanism shown in Scheme 1. For this, we shall distinguish four inhibition routes (two for the activating enzyme and two for the activated one) and one route of zymogen activation (Fig. 3a). Figure 3b shows the number of possibilities for each route. The following six situations, shown in Fig. 4, will be distinguished: (a) Particular cases where the inhibition routes of I' on E and of I on E_a are missing. (b) Particular cases where none of the inhibition routes is missing. (c) Particular cases where the inhibition route of I on E_a is missing. (d) Particular cases where the inhibition route of I' on E is missing. (e) Particular cases where both inhibition routes on E_a are missing. (f) Particular cases where both inhibition routes on E are missing.

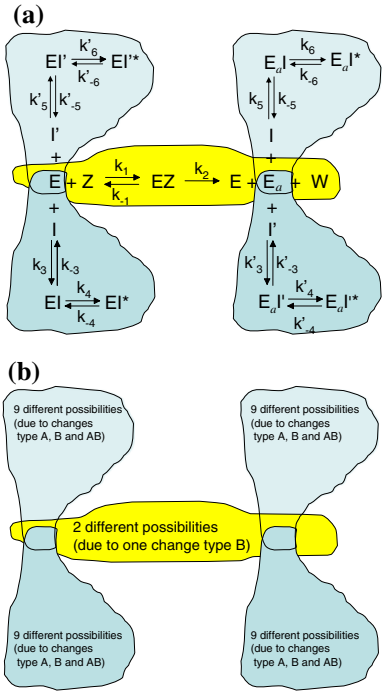
(a) Particular cases where the inhibition routes of I' on E and of I on E_a are missing.

This case involves all the particular cases corresponding to the simplified mechanism shown in Scheme 2. It is possible to distinguish three routes in this scheme. In the activation route, which is a particular type A case ($k_3 = k'_3 = 0$), only a type B change is possible, yielding the two following possibilities:

$$E + Z \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} EZ \xrightarrow{k_2} E + E_a + W \qquad \text{(a)}$$

$$E + Z \overset{K_1}{\rightleftarrows} EZ \xrightarrow{k_2} E + E_a + W \qquad \text{(b)}$$

In turn, in each of the two inhibition routes there are nine possibilities corresponding to A, B and AB changes. Scheme 3 shows this in the case of the inhibition of the activating enzyme. The nine possibilities for E_a can be established in a similar way.



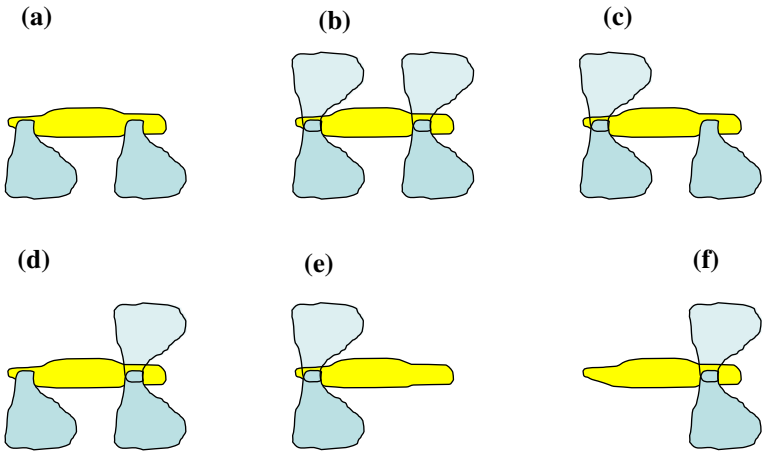
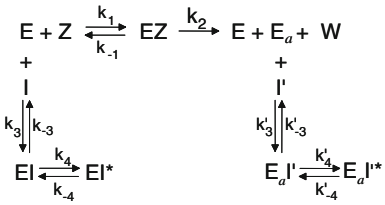


Fig. 3 **a** Indication of the four inhibition routes (two routes for the activating enzyme and two for the activated one) and the activation route for the zymogen. **b**. Indication of the number of possibilities in each route

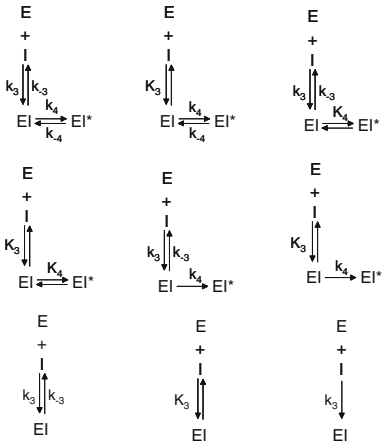
Fig. 4 Different possibilities of each route involved in Scheme 1

We could have included more possibilities in each of the inhibition routes, for example $E + I \rightarrow EI \rightarrow EI^*$, but these steps are non-viable. Nevertheless, the inclusion of more possibilities, whether they make sense or not, does not change the analysis being carried out, although it does influence the number of particular cases, which would be greater. We have preferred to limit the possibilities to those which have an accepted meaning in the literature.

Scheme 2



Scheme 3



We shall now to determine all the particular cases from Scheme 2. For this, four situations will be distinguished: (a1) Particular cases where none of the inhibition routes is missing; (a2) particular cases where the inhibition route on E is missing; (a3) particular cases where the inhibition route on E_a is missing and a4) particular cases where both inhibition routes are missing.

(a1) Particular cases where none of the inhibition routes is missing

The number of particular type A, B or AB cases, which will be denoted as $N_{I,I'}$, is the product of the number of possibilities of each one of the three routes (two inhibition and one activation), i.e. $N_{I,I'} = 9 \times 9 \times 2 = 162$, including the starting mechanism (Scheme 2). It is also possible that both inhibitors will match in each one of these mechanisms, so that the total number of particular cases with type C changes, which will be denoted as $N_{I,I}$, will also be 162, which indicates that the total number of cases is $N_{I,I'} + N_{I,I} = 162 + 162 = 324$.

(a2) Particular cases where the inhibition route on E is missing

The number of particular type A, B or AB cases, which will be denoted as $N_{I'}$, is the product of the number of possibilities of both the inhibition and the activation routes, i.e. $N_{I'} = 9 \times 2 = 18$. In this case it is not possible to make changes type C because there is only one inhibitor.

(a3) Particular cases where the inhibition route on E_a is missing

The number of particular type A, B or AB cases, which will be denoted as N_I, is the product of the number of possibilities of both the inhibition and the activation routes,

i.e. $N_I = 9 \times 2 = 18$. In this case it is not possible to make type C changes because there is only one inhibitor.

(a4) Particular cases where both inhibition routes are missing

In this case, there are only two possibilities and so two particular cases, one type A and the other one type AB. This number will be denoted as $N_0 (N_0 = 2)$. No particular type C case is possible because there is no inhibitor.

Therefore, the total number of particular cases which can be derived from Scheme 2, including itself is: $N_{I,I'} + N_{I,I} + N_{I'} + N_I + N_0 = 324 + 18 + 18 + 2 = 362$, of which $N_{I,I'} + N_{I'} + N_I + N_0 = 162 + 18 + 18 + 2 = 200$ correspond to type A, B and AB changes, and the other 162 ($N_{I,I}$) correspond to particular cases involving a type C change, i.e. C, AC, BC and ABC changes.

(b) Particular cases from Scheme 1 where none of the inhibition routes is missing

In agreement with the above, the number of particular cases in this situation will be $N_{I,I'} \times 9 \times 9 = 162 \times 9 \times 9 = 13,122$. A result obtained by combining the datum obtained in point a1) with the 9 possibilities of each inhibition route.

(c) Particular cases where the inhibition route of *I* on E_a is missing

Analogously, the number of particular cases is $N_{I,I'} \times 9 = 162 \times 9 = 1,458$.

(d) Particular cases where the inhibition route of I' on *E* is missing

The number of particular cases is $N_{I,I'} \times 9 = 162 \times 9 = 1,458$.

(e) Particular cases where both inhibition routes on E_a are missing

The number of particular cases is $N_I \times 9 = 18 \times 9 = 162$.

(f) Particular cases where both inhibition routes on *E* are missing

The number of particular cases is $N_{I'} \times 9 = 18 \times 9 = 162$.

Therefore, the number of particular cases derived from the general model shown in Scheme 1, including itself, is: 362 + 13,122 + 1,458 + 1,458 + 162 + 162 = 16,724. In this way, this study offers, for the first time, to the scientific community working on limited proteolysis regulation, a method based on general solutions which only needs to be particularized to the specific problem of zymogen activation.

4.2.2 Obtaining the kinetic equations for the particular cases

Each one of the particular cases derived from a primitive mechanism can be analyzed, in an individualized way, from its corresponding system of differential equations, taking into account the initial conditions and the reaction time allowing their linearization. However, this procedure would lose the power provided by having kinetic equations available for a model that includes the mechanism under study as a particular case, which constitutes a considerable saving of time and effort. In turn, in general, any of the particular cases of a primitive mechanism can be analyzed from the results of another particular case of the primitive mechanism if the mechanism under study is a particular case of the derived one.

In the changes that consist of, besides other possibilities, cancelling one or more of the rate constants in order to obtain equations of the particular case from those of the general model or any of their particular cases, type 0/0 uncertainties may arise, that in general, can be solved by making these constants equal to ε and then making $\varepsilon \to 0$. When, by annulling all the rate constants that transform a primitive mechanism

into the mechanism under study, mathematical problems, irresolvable uncertainties or absurd result arise, the following steps should be followed: (1) In the equations of the primitive mechanism, cancel the minimum set of rate constants that, under the initial conditions used, convert it *de facto* (though not really) into an equivalent mechanism to the derived one and then (2) cancel, in the resulting equations, all other rate constants appearing in them but not in the mechanism under study.

Example 1 Mechanism shown in Scheme 2

Kinetic equations for Scheme 2 can be obtained from those of Scheme 1 by setting:

$$k_5 = k_{-5} = k'_5 = k'_{-5} = k_6 = k_{-6} = k'_6 = k'_{-6} \quad = \quad 0 \tag{35}$$

The result is (details of the derivation are available to interested readers upon request):

$$[E_a] = \beta + \alpha t + \sum_{h=1}^{5} \gamma_h e^{\lambda_h t} \tag{36}$$

The parameters α and β are given by the following Eq.:

$$\alpha = \frac{k_1 k_2 k_{-3} k_{-4} k'_{-3} k'_{-4} [Z]_0 [E]_0}{F_3 F'_2}$$

$$\beta = \alpha \left(\frac{k_{-3} + k_4 + k_{-4}}{k_{-3} k_{-4}} + \frac{k'_{-3} + k'_4 + k'_{-4}}{k'_{-3} k'_{-4}} - \frac{F_2}{F_3} - \frac{F'_1}{F'_2} \right) \tag{37}$$

and γ_h is:

$$\gamma_h = \frac{k_1 k_2 \left(\lambda_h^4 + f_1 \lambda_h^3 + f_2 \lambda_h^2 + f_3 \lambda_h^+ f_4 \right) [Z]_0 [E]_0}{\lambda_h^2 \prod\limits_{\substack{p=1 \\ p \neq h}}^{5} (\lambda_p - \lambda_h)} \text{ (h = 1, 2, 3, 4, 5)} \tag{38}$$

λ_1, λ_2 and λ_3 are the roots of the polynomial $\lambda^3 + F_1 \lambda^2 + F_2 \lambda + F_3 = 0$ and λ_4 and λ_5 those of the polynomial $\lambda^2 + F'_1 \lambda + F'_2 = 0$, with:

$$F_1 = k_1 [Z]_0 + k_3 [I]_0 + k_{-1} + k_2 + k_{-3} + k_4 + k_{-4} \tag{39}$$

$$F_2 = k_1 [Z]_0 (k_{-3} + k_4 + k_{-4}) + k_3 [I]_0 (k_{-1} + k_2 + k_4 + k_{-4}) + (k_{-1} + k_2)(k_{-3} + k_4 + k_{-4}) + k_{-3} k_{-4} \tag{40}$$

$$F_3 = k_1 [Z]_0 k_{-3} k_{-4} + k_3 [I]_0 (k_{-1} + k_2)(k_4 + k_{-4}) + k_{-3} k_{-4} (k_{-1} + k_2) \tag{41}$$

$$F'_1 = k'_3 [I']_0 + k'_{-3} + k'_4 + k'_{-4} \tag{42}$$

$$F'_2 = k'_3 [I']_0 (k'_4 + k'_{-4}) + k'_{-3} k'_{-4} \tag{43}$$

In turn, the expressions of f_i (i = 1,2,3,4) involved in Eq. 38 are:

$$f_1 = \mathrm{k}_{-3} + \mathrm{k}_4 + \mathrm{k}_{-4} + \mathrm{k}'_{-3} + \mathrm{k}'_4 + \mathrm{k}'_{-4} \tag{44}$$

$$f_2 = (\mathrm{k}_{-3} + \mathrm{k}_4 + \mathrm{k}_{-4})(\mathrm{k}'_{-3} + \mathrm{k}'_4 + \mathrm{k}'_{-4}) + \mathrm{k}_{-3}\mathrm{k}_{-4} + \mathrm{k}'_{-3}\mathrm{k}'_{-4} \tag{45}$$

$$f_3 = (\mathrm{k}_{-3} + \mathrm{k}_4 + \mathrm{k}_{-4})\mathrm{k}'_{-3}\mathrm{k}'_{-4} + (\mathrm{k}'_{-3} + \mathrm{k}'_4 + \mathrm{k}'_{-4})\mathrm{k}_{-3}\mathrm{k}_{-4} \tag{46}$$

$$f_4 = \mathrm{k}_{-3}\mathrm{k}_{-4}\mathrm{k}'_{-3}\mathrm{k}'_{-4} \tag{47}$$

These results coincide, as they should, with those obtained for Scheme 2 from the corresponding set of differential equations [8], but in a much more laborious way in the latter case.

4.3 Final Remarks

Once the equations corresponding to a given mechanism have been obtained, it is possible to make $[I]_0 = 0$ and/or $[I']_0 = 0$ to analyze the behavior of the enzymatic system under study in the absence of one or both inhibitors. Finally, knowlwdge of the kinetic behaviour of any of the particular cases will help in experimental designs and in the analysis of kinetic data to estimate all or part of the kinetic parameters involved in the system, i.e., to characterize it.

The kinetic analysis described here in has introduced new kinetic parameters that provide information on the relative weight of the activation and inhibition routes, total inhibition efficiency, the absolute and relative contributions of both inhibition pathways to total inhibition in the overall model and, like kinetic equations, they can be particularized to any of their particular cases. The introduction and analysis of these parameters is the subject of the paper II of this series [19].

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Appendix

Set of differential equations describing the kinetic behavior of enzymatic systems that fit the model shown in Scheme 1

$$\frac{d[E]}{dt} = -k_1[E][Z] - k_3[E][I] - k'_5[E]\left[I'\right] + (k_{-1} + k_2)[EZ] + k_{-3}[EI] + k'_{-5}\left[EI'\right] \tag{A.1}$$

$$\frac{d[EZ]}{dt} = k_1[E][Z] - (k_{-1} + k_2)[EZ] \tag{A.2}$$

$$\frac{d[E_a]}{dt} = k_2\,[EZ] - k'_3\,[E_a]\left[I'\right] + k'_{-3}\left[E_aI'\right] - k_5\,[E_a]\,[I] + k_{-5}\,[E_aI] \quad \text{(A.3)}$$

$$\frac{d[EI]}{dt} = k_3\,[E]\,[I] - (k_{-3} + k_4)\,[EI] + k_{-4}[EI^*] \quad \text{(A.4)}$$

$$\frac{d[EI^*]}{dt} = k_4\,[EI] - k_{-4}\left[EI^*\right] \quad \text{(A.5)}$$

$$\frac{d[E_aI']}{dt} = k'_3\,[E_a]\left[I'\right] - \left(k'_{-3} + k'_4\right)\left[E_aI'\right] + k'_{-4}\left[E_aI'^*\right] \quad \text{(A.6)}$$

$$\frac{d[E_aI'^*]}{dt} = k'_4\left[E_aI'\right] - k'_{-4}\left[E_aI'^*\right] \quad \text{(A.7)}$$

$$\frac{d[EI']}{dt} = k'_5\,[E]\left[I'\right] - \left(k'_{-5} + k'_6\right)\left[EI'\right] + k'_{-6}\left[EI'^*\right] \quad \text{(A.8)}$$

$$\frac{d[EI'^*]}{dt} = k'_6\left[EI'\right] - k'_{-6}\left[EI'^*\right] \quad \text{(A.9)}$$

$$\frac{d[E_aI]}{dt} = k_5\,[E_a]\,[I] - (k_{-5} + k_6)\,[E_aI] + k_{-6}\left[E_aI^*\right] \quad \text{(A.10)}$$

$$\frac{d[E_aI^*]}{dt} = k_6\,[E_aI] - k_{-6}\left[E_aI^*\right] \quad \text{(A.11)}$$

$$\frac{d[Z]}{dt} = -k_1\,[E]\,[Z] + k_{-1}\,[EZ] \quad \text{(A.12)}$$

$$\frac{d[I]}{dt} = -k_3\,[E]\,[I] + k_{-3}\,[EI] - k_5\,[E_a]\,[I] + k'_{-5}\,[E_aI] \quad \text{(A.13)}$$

$$\frac{d[I']}{dt} = -k'_3[E_a][I'] + k'_{-3}[E_aI'] - k'_5[E][I'] + k'_{-5}[EI'] \quad \text{(A.14)}$$

$$\frac{d[W]}{dt} = k_2[EZ] \quad \text{(A.15)}$$

Expressions of the coefficients $F_i (i = 1,2,3,4,5)$ and F'_j $(j = 1,2,3,4)$ from Eqs. 20 and 21

$$\begin{aligned} F_1 = {} & \mathrm{k}_{-1} + \mathrm{k}_2 + \mathrm{k}_{-3} + \mathrm{k}_4 + \mathrm{k}_{-4} + \mathrm{k}'_{-5} + \mathrm{k}'_6 + \mathrm{k}'_{-6} + \mathrm{k}_1\,[\mathrm{Z}]_0 + \mathrm{k}_3\,[\mathrm{I}]_0 \\ & + \mathrm{k}'_5\left[\mathrm{I}'\right]_0 \end{aligned} \quad \text{(A.16)}$$

$$\begin{aligned} F_2 = {} & \mathrm{k}_{-3}\mathrm{k}_{-4} + \mathrm{k}_{-3}\left(\mathrm{k}'_{-5} + \mathrm{k}'_6 + \mathrm{k}'_{-6} + \mathrm{k}_{-1} + \mathrm{k}_2\right) \\ & + \mathrm{k}_4\left(\mathrm{k}'_{-5} + \mathrm{k}'_6 + \mathrm{k}'_{-6} + \mathrm{k}_{-1} + \mathrm{k}_2\right) + \mathrm{k}_{-4}\left(\mathrm{k}'_{-5} + \mathrm{k}'_6 + \mathrm{k}'_{-6} + \mathrm{k}_{-1} + \mathrm{k}_2\right) \\ & + \mathrm{k}'_{-5}\left(\mathrm{k}'_{-6} + \mathrm{k}_{-1} + \mathrm{k}_2\right) + \left(\mathrm{k}'_6 + \mathrm{k}'_{-6}\right)\left(\mathrm{k}_{-1} + \mathrm{k}_2\right) \\ & + \mathrm{k}_1\left(\mathrm{k}_{-3} + \mathrm{k}_4 + \mathrm{k}_{-4} + \mathrm{k}'_{-5} + \mathrm{k}'_6 + \mathrm{k}'_{-6}\right)[\mathrm{Z}]_0 \\ & + \mathrm{k}_3\left(\mathrm{k}_4 + \mathrm{k}_{-4} + \mathrm{k}'_{-5} + \mathrm{k}'_6 + \mathrm{k}'_{-6} + \mathrm{k}_{-1} + \mathrm{k}_2\right)[\mathrm{I}]_0 \\ & + \mathrm{k}'_5\left(\mathrm{k}_{-1} + \mathrm{k}_2 + \mathrm{k}_{-3} + \mathrm{k}_4 + \mathrm{k}_{-4} + \mathrm{k}'_6 + \mathrm{k}'_{-6}\right)\left[\mathrm{I}'\right]_0 \end{aligned} \quad \text{(A.17)}$$

$$
\begin{aligned}
F_3 = {} & \mathrm{k}_{-3}\mathrm{k}_{-4}(\mathrm{k}'_{-5}+\mathrm{k}'_{6}+\mathrm{k}'_{-6}+\mathrm{k}_{-1}+\mathrm{k}_{2}) \\
& +\mathrm{k}_{-3}\mathrm{k}'_{-5}(\mathrm{k}'_{-6}+\mathrm{k}_{-1}+\mathrm{k}_{2})+(\mathrm{k}_{-3}+\mathrm{k}_{4})(\mathrm{k}_{-1}+\mathrm{k}_{2})(\mathrm{k}'_{6}+\mathrm{k}'_{-6}) \\
& +\mathrm{k}'_{-5}(\mathrm{k}'_{-6}+\mathrm{k}_{-1}+\mathrm{k}_{2})(\mathrm{k}_{4}+\mathrm{k}_{-4})+(\mathrm{k}_{-1}+\mathrm{k}_{2})(\mathrm{k}_{-4}\mathrm{k}'_{6}+\mathrm{k}_{-4}\mathrm{k}'_{-6}+\mathrm{k}'_{-5}\mathrm{k}'_{-6}) \\
& +\mathrm{k}_{1}\{\mathrm{k}_{-3}(\mathrm{k}_{-4}+\mathrm{k}'_{-5}+\mathrm{k}'_{6}+\mathrm{k}'_{-6})+(\mathrm{k}'_{-5}+\mathrm{k}'_{6}+\mathrm{k}'_{-6})(\mathrm{k}_{4}+\mathrm{k}_{-4}) \\
& +\mathrm{k}'_{-5}\mathrm{k}'_{-6}\}[Z]_0+\mathrm{k}_{3}\{\mathrm{k}_{4}(\mathrm{k}'_{-5}+\mathrm{k}'_{6}+\mathrm{k}'_{-6}+\mathrm{k}_{-1}+\mathrm{k}_{2}) \\
& +\mathrm{k}_{-4}(\mathrm{k}'_{-5}+\mathrm{k}'_{6}+\mathrm{k}'_{-6}+\mathrm{k}_{-1}+\mathrm{k}_{2})+\mathrm{k}'_{-5}\mathrm{k}'_{-6}+(\mathrm{k}_{-1}+\mathrm{k}_{2})(\mathrm{k}'_{-5}+\mathrm{k}'_{6}+\mathrm{k}'_{-6})\} \\
& [\mathrm{I}]_0+\mathrm{k}'_{5}\{\mathrm{k}_{-3}(\mathrm{k}_{-4}+\mathrm{k}'_{6}+\mathrm{k}'_{-6}+\mathrm{k}_{-1}+\mathrm{k}_{2}) \\
& +\mathrm{k}_{4}(\mathrm{k}'_{6}+\mathrm{k}'_{-6}+\mathrm{k}_{-1}+\mathrm{k}_{2})+\mathrm{k}_{-4}(\mathrm{k}'_{6}+\mathrm{k}'_{-6}+\mathrm{k}_{-1}+\mathrm{k}_{2}) \\
& +(\mathrm{k}_{-1}+\mathrm{k}_{2})(\mathrm{k}'_{6}+\mathrm{k}'_{-6})\}[\mathrm{I}']_0 \qquad \text{(A.18)}
\end{aligned}
$$

$$
\begin{aligned}
F_4 = {} & \mathrm{k}_{-3}\mathrm{k}_{-4}\mathrm{k}'_{-5}(\mathrm{k}'_{-6}+\mathrm{k}_{-1}+\mathrm{k}_{2})+(\mathrm{k}_{-1}+\mathrm{k}_{2})\left\{\mathrm{k}_{-3}\mathrm{k}_{-4}(\mathrm{k}'_{6}+\mathrm{k}'_{-6})\right. \\
& \left.+\mathrm{k}'_{-5}\mathrm{k}'_{-6}(\mathrm{k}_{-3}+\mathrm{k}_{4}+\mathrm{k}_{-4})\right\}+\mathrm{k}_{1}\left\{\mathrm{k}_{-3}\left(\mathrm{k}_{-4}\mathrm{k}'_{-5}+\mathrm{k}_{-4}\mathrm{k}'_{6}+\mathrm{k}_{-4}\mathrm{k}'_{-6}\right.\right. \\
& \left.\left.+\mathrm{k}'_{-5}\mathrm{k}'_{-6}\right)+\mathrm{k}'_{-5}\mathrm{k}'_{-6}(\mathrm{k}_{4}+\mathrm{k}_{-4})\right\}[\mathrm{Z}]_0 \\
& +\mathrm{k}_{3}\left\{\mathrm{k}_{4}\mathrm{k}'_{-5}(\mathrm{k}'_{-6}+\mathrm{k}_{-1}+\mathrm{k}_{2})+\mathrm{k}_{4}(\mathrm{k}_{-1}+\mathrm{k}_{2})(\mathrm{k}'_{6}+\mathrm{k}'_{-6})+\mathrm{k}_{-4}\mathrm{k}'_{-5}\mathrm{k}'_{-6}\right. \\
& \left.+(\mathrm{k}_{-1}+\mathrm{k}_{2})(\mathrm{k}_{-4}\mathrm{k}'_{-5}+\mathrm{k}_{-4}\mathrm{k}'_{6}+\mathrm{k}_{-4}\mathrm{k}'_{-6}+\mathrm{k}'_{-5}\mathrm{k}'_{-6})\right\}[\mathrm{I}]_0 \\
& +\mathrm{k}'_{5}\{\mathrm{k}_{-3}\mathrm{k}_{-4}(\mathrm{k}'_{6}+\mathrm{k}'_{-6})+(\mathrm{k}_{-1}+\mathrm{k}_{2})(\mathrm{k}_{-3}\mathrm{k}_{-4}+\mathrm{k}_{-3}\mathrm{k}'_{6} \\
& +\mathrm{k}_{-3}\mathrm{k}'_{-6}+\mathrm{k}_{4}\mathrm{k}'_{6}+\mathrm{k}_{4}\mathrm{k}'_{-6}+\mathrm{k}_{-4}\mathrm{k}'_{6}+\mathrm{k}_{-4}\mathrm{k}'_{-6})\}[\mathrm{I}']_0 \qquad \text{(A.19)}
\end{aligned}
$$

$$
\begin{aligned}
F_5 = {} & \mathrm{k}_{-3}\mathrm{k}_{-4}\mathrm{k}'_{-5}\mathrm{k}'_{-6}(\mathrm{k}_{-1}+\mathrm{k}_{2})+\mathrm{k}_{1}\mathrm{k}_{-3}\mathrm{k}_{-4}\mathrm{k}'_{-5}\mathrm{k}'_{-6}[\mathrm{Z}]_0 \\
& +\mathrm{k}_{3}\mathrm{k}'_{-5}\mathrm{k}'_{-6}(\mathrm{k}_{-1}+\mathrm{k}_{2})(\mathrm{k}_{4}+\mathrm{k}_{-4})[\mathrm{I}]_0+\mathrm{k}'_{5}\mathrm{k}_{-3}\mathrm{k}_{-4}(\mathrm{k}_{-1}+\mathrm{k}_{2})(\mathrm{k}'_{6}+\mathrm{k}'_{-6})[\mathrm{I}']_0 \\
& \qquad \text{(A.20)}
\end{aligned}
$$

$$
F'_1 = \mathrm{k}'_{-3}+\mathrm{k}'_{4}+\mathrm{k}'_{-4}+\mathrm{k}_{-5}+\mathrm{k}_{6}+\mathrm{k}_{-6}+\mathrm{k}_{5}[\mathrm{I}]_0+\mathrm{k}'_{3}[\mathrm{I}']_0 \qquad \text{(A.21)}
$$

$$
\begin{aligned}
F'_2 = {} & \mathrm{k}'_{-3}(\mathrm{k}'_{-4}+\mathrm{k}_{-5}+\mathrm{k}_{6}+\mathrm{k}_{-6})+\mathrm{k}'_{4}(\mathrm{k}_{-5}+\mathrm{k}_{6}+\mathrm{k}_{-6})+\mathrm{k}'_{-4}(\mathrm{k}_{-5}+\mathrm{k}_{6}+\mathrm{k}_{-6}) \\
& +\mathrm{k}_{-5}\mathrm{k}_{-6}+\mathrm{k}_{5}(\mathrm{k}'_{-3}+\mathrm{k}'_{4}+\mathrm{k}'_{-4}+\mathrm{k}_{6}+\mathrm{k}_{-6})[\mathrm{I}]_0 \\
& +\mathrm{k}'_{3}(\mathrm{k}'_{4}+\mathrm{k}'_{-4}+\mathrm{k}_{-5}+\mathrm{k}_{6}+\mathrm{k}_{-6})[\mathrm{I}']_0 \qquad \text{(A.22)}
\end{aligned}
$$

$$
\begin{aligned}
F'_3 = {} & \mathrm{k}'_{-3}(\mathrm{k}'_{-4}\mathrm{k}_{-5}+\mathrm{k}'_{-4}\mathrm{k}_{6}+\mathrm{k}'_{-4}\mathrm{k}_{-6}+\mathrm{k}_{-5}\mathrm{k}_{-6})+\mathrm{k}_{-5}\mathrm{k}_{-6}(\mathrm{k}'_{4}+\mathrm{k}'_{-4}) \\
& +\mathrm{k}_{5}\{\mathrm{k}'_{-3}(\mathrm{k}'_{-4}+\mathrm{k}_{6}+\mathrm{k}_{-6})+(\mathrm{k}'_{4}+\mathrm{k}'_{-4})(\mathrm{k}_{6}+\mathrm{k}_{-6})\}[\mathrm{I}]_0+\mathrm{k}'_{3}\{(\mathrm{k}'_{4}+\mathrm{k}'_{-4}) \\
& \times(\mathrm{k}_{-5}+\mathrm{k}_{6}+\mathrm{k}_{-6})+\mathrm{k}_{-5}\mathrm{k}_{-6}\}[\mathrm{I}']_0 \qquad \text{(A.23)}
\end{aligned}
$$

$$
F'_4 = \mathrm{k}'_{-3}\mathrm{k}'_{-4}\mathrm{k}_{-5}\mathrm{k}_{-6}+\mathrm{k}_{5}\mathrm{k}'_{-3}\mathrm{k}'_{-4}(\mathrm{k}_{6}+\mathrm{k}_{-6})[\mathrm{I}]_0+\mathrm{k}'_{3}\mathrm{k}_{-5}\mathrm{k}_{-6}(\mathrm{k}'_{4}+\mathrm{k}'_{-4})[\mathrm{I}']_0 \qquad \text{(A.24)}
$$

Expressions of the coefficients *fq* (q = 1,2,…,8) involved in Eq. 23

$$
f_1 = a_1+a_2+a_3+a_4 \qquad \text{(A.25)}
$$

$$
f_2 = a_1a_2+a_3a_4+(a_1+a_2)(a_3+a_4)+b_1+b_2+b_3+b_4 \qquad \text{(A.26)}
$$

$$
\begin{aligned}
f_3 = {} & (a_1+a_2)(a_3a_4+b_3+b_4)+(a_3+a_4)(a_1a_2+b_1+b_2) \\
& +a_1b_2+a_2b_1+a_3b_4+a_4b_3 \qquad \text{(A.27)}
\end{aligned}
$$

$$f_4 = (a_1 + a_2)(a_3b_4 + a_4b_3) + (a_3 + a_4)(a_1b_2 + a_2b_1) + (a_1a_2 + b_1 + b_2)(a_3a_4 + b_3 + b_4) + b_1b_2 + b_3b_4 \quad \text{(A.28)}$$

$$f_5 = (a_1 + a_2)b_3b_4 + (a_3 + a_4)b_1b_2 + (a_1a_2 + b_1 + b_2)(a_3b_4 + a_4b_3) + (a_1b_2 + a_2b_1)(a_3a_4 + b_3 + b_4) \quad \text{(A.29)}$$

$$f_6 = b_1b_2(a_3a_4 + b_3 + b_4) + b_3b_4(a_1a_2 + b_1 + b_2) + (a_1b_2 + a_2b_1)(a_3b_4 + a_4b_3) \quad \text{(A.30)}$$

$$f_7 = b_1b_2(a_3b_4 + a_4b_3) + b_3b_4(a_1b_2 + a_2b_1) \quad \text{(A.31)}$$

$$f_8 = b_1b_2b_3b_4 \quad \text{(A.32)}$$

where:

$$a_1 = \mathrm{k}_{-3} + \mathrm{k}_4 + \mathrm{k}_{-4} \quad \text{(A.33)}$$

$$b_1 = \mathrm{k}_{-3}\mathrm{k}_{-4} \quad \text{(A.34)}$$

$$a_2 = \mathrm{k}'_{-5} + \mathrm{k}'_6 + \mathrm{k}'_{-6} \quad \text{(A.35)}$$

$$b_2 = \mathrm{k}'_{-5}\mathrm{k}'_{-6} \quad \text{(A.36)}$$

$$a_3 = \mathrm{k}'_{-3} + \mathrm{k}'_4 + \mathrm{k}'_{-4} \quad \text{(A.37)}$$

$$b_3 = \mathrm{k}'_{-3}\mathrm{k}'_{-4} \quad \text{(A.38)}$$

$$a_4 = \mathrm{k}_{-5} + \mathrm{k}_6 + \mathrm{k}_{-6} \quad \text{(A.39)}$$

$$b_4 = \mathrm{k}_{-5}\mathrm{k}_{-6} \quad \text{(A.40)}$$

Expressions of g_1, g_2, g_3 and g_4 involved in Eq. 28

$$g_1 = \mathrm{k}_{-3} + \mathrm{k}_4 + \mathrm{k}_{-4} + \mathrm{k}'_{-5} + \mathrm{k}'_6 + \mathrm{k}'_{-6} \quad \text{(A.41)}$$

$$g_2 = \mathrm{k}_{-3}\mathrm{k}_{-4} + (\mathrm{k}_{-3} + \mathrm{k}_4 + \mathrm{k}_{-4})(\mathrm{k}\prime_{-5} + \mathrm{k}\prime_6 + \mathrm{k}\prime_{-6}) + \mathrm{k}'_{-5}\mathrm{k}'_{-6} \quad \text{(A.42)}$$

$$g_3 = (\mathrm{k}_{-3} + \mathrm{k}_4 + \mathrm{k}_{-4})\mathrm{k}'_{-5}\mathrm{k}'_{-6} + (\mathrm{k}'_{-5} + \mathrm{k}'_6 + \mathrm{k}\prime_{-6})\mathrm{k}_{-3}\mathrm{k}_{-4} \quad \text{(A.43)}$$

$$g_4 = \mathrm{k}_{-3}\mathrm{k}_{-4}\mathrm{k}'_{-5}\mathrm{k}'_{-6} \quad \text{(A.44)}$$

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