anism is operative for protein kinases.

Registry No. CaM kinase, 9026-43-1; 5′-ATP, 56-65-5; 5′-ADP, 58-64-0; syntide, 108334-68-5.

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APPENDIX: VARIOUS CASES OF RAPID-EQUILIBRIUM ORDERED BIREACTANT MECHANISMS—THEIR BASES AND DIFFERENTIATION

In the rapid-equilibrium ordered (REO) mechanism for an enzyme-catalyzed reaction, the two substrates, A and B, bind to the enzyme in an ordered manner and the binding processes attain equilibrium rapidly:

$$E + A \overset{K_{ia}}{\rightleftharpoons} EA$$

$$EA + B \overset{K_b}{\rightleftharpoons} EAB \xrightarrow{k_3} E + P + Q$$

This mechanism has been treated by Segal et al. (1952) in which A is either an activator or the first substrate. The reciprocal initial-rate equation is

$$E_0/v = (1/k_3)[1 + (K_b/B) + (K_{ia}K_b/AB)] \quad (5)$$

Equation 5 illustrates the distinctive features observed with the REO mechanism: (1) In the $1/v$ versus $1/B$ plot, the lines obtained at different levels of A will intersect on the ordinate. (2) In the $1/v$ versus $1/A$ plot, the interaction point will be to the left of the ordinate, but the slopes of the lines, $K_{ia}K_b/k_3B$, when plotted against $1/B$, will generate a line that passes through the origin.

I want to point out that there are a number of conditions that can give rise to kinetic patterns typical of the REO mechanism. First, only the addition of the first substrate to enzyme needs to achieve equilibrium rapidly, as shown in Here the rapid-equilibrium segment is enclosed by dashed lines. The resultant rate equation from Scheme III

$$E_0/v = (1/k_3)[1 + ((k_{-2} + k_3)/k_2B) + (K_{ia}(k_{-2} + k_3)/k_2AB)] \quad (6)$$

is identical in form with eq 5 except that K_b is expressed as $(k_{-2} + k_3)/k_2$. This condition, however, does not alter any kinetic patterns predicted by the usual REO mechanism. Consequently, one needs only to focus on the apparent REO cases which can occur in various ordered and random reactions. The sequential mechanisms can be described by the general rate equation

$$E_0/v = (1/k_f)[1 + (K_a/A) + (K_b/B) + (K_{ia}K_b/AB)] \quad (7a)$$

which, in parallel fashion as has been shown in eq 1 and 2, reduces to the typical REO forms

$$E_0/v = (1/k_f)[1 + (K_b/B) + (K_{ia}K_b/AB)] \quad \text{if } K_{ib} \gg B \quad (7b)$$

and

$$E_0/v = (1/k_f)[1 + (K_a/A) + (K_{ia}K_b/AB)] \quad \text{if } K_{ia} \gg A \quad (7c)$$

Note that eq 7c describes a peculiar situation (similar to that of eq 4b) since a $1/v$ vs $1/A$ plot will yield lines converging on the ordinate, thereby making A, the first substrate in an ordered mechanism, look like the second substrate. Thus, caution should be exercised in using such a plot to identify the

substrate's binding order.

For further discussion let us return to Scheme I and use the ordered Bi Bi mechanism, where apparent REO binding occurs in the reverse direction, as an example to examine the basis for observing REO kinetic patterns. To reduce eq 1 to eq 2 requires not only $K_{ip} \gg P$ but also $K_p \simeq P$ to retain the main characteristics of the REO mechanism. This means that $K_{ip} \gg K_p$, i.e., $k_4(k_{-2} + k_3)/k_{-2}k_{-3} \gg k_{-1}(k_{-2} + k_3)/k_{-3}(k_{-1} + k_{-2})$; upon simplification, $k_4 \gg k_r$ or $k_4 \gg k_{-1}$ or k_{-2}. Likewise, examination of eq 3a shows that to observe uncompetitive inhibition of P versus both A and B the condition $K_{ip} \gg K_p'$ must be satisfied:

$$k_4(k_{-2} + k_3)/k_{-2}k_{-3} \gg (k_3 + k_4)/k_{-3} \text{ or } k_4 \gg k_{-2}$$

Thus, observation of apparent REO kinetic patterns expected from both eq 2 and 3b requires that the off-rate constant for the first substrate Q (in the reverse reaction), k_4, be large relative to the constant of the rate-limiting step. This requirement can be shown to be also true for other ordered cases such as Theorell–Chance, iso-Theorell–Chance, and iso-ordered mechanisms.

As has been shown previously, the condition $K_{iq} \gg Q$ also leads to the REO type of kinetic patterns. This condition further requires $K_{iq} \gg K_q$, i.e.

$$k_4/k_{-4} \gg k_{-1}k_{-2}/k_{-4}(k_{-1} + k_2) \text{ or } k_4 \gg k_r$$

Clearly, apparent REO phenomena, whether they arise from $K_{ip} \gg P$ or $K_{iq} \gg Q$, all depend on the off-rate constant for the first substrate being much larger than the catalytic rate constant. This common requirement is evident from the relationship $K_{ip}K_q = K_{iq}K_p$; if $K_{ip} \gg K_p$, then $K_{iq} \gg K_q$ must be true. However, the conditions $k_{-4}Q > k_r$ (if $K_{ip} \gg P$) and $k_{-3}P > k_r$ (if $K_{iq} \gg Q$) are necessary to retain REO kinetic patterns since, to keep the term $K_{ip}K_q/PQ$ in proper size, $K_q < Q$ is needed if $K_{ip} \gg P$ and $K_p < P$ if $K_{iq} \gg Q$. It is interesting to note that in the case of CaM kinase II, given the very slow backward reaction ($k_r = k_{-2} = 0.026\ s^{-1}$), one is almost certain to observe apparent REO kinetics because the condition $k_4 \gg k_r$ can be easily fulfilled.

For a rapid-equilibrium random mechanism the condition $K_{ip} \gg P$ means that the initial dissociation constant of P from enzyme is large relative to the concentration of P. Schimerlik and Cleland (1973) reported observing REO substrate addition in the reverse direction of creatine kinase reaction at pH 7. They considered the case to be a degenerate rapid-equilibrium random mechanism and assigned $K_{ip} = \infty$ and $K_q = 0$. In view of the fact that $K_{ip}K_q$ must equal $K_{iq}K_p$ and that $\infty \cdot 0$ is indeterminate, it is preferred to view the apparent REO cases in terms of the relative sizes of Michaelis or dissociation constants for a substrate to its concentration.

Table II summarizes the product inhibition patterns for five sequential mechanisms displaying apparent REO features in the reverse reaction. Predicted inhibition patterns in the forward and reverse direction of the reaction are listed to facilitate the differentiation of mechanisms, including those containing "true" REO mechanisms in the reverse reaction not listed in Table II. For example, in several cases in Table II, P or Q has no inhibitory effect, making the mechanism appear to be true REO. In some cases (see footnotes to Table II), techniques other than product inhibition are needed to make a distinction between mechanisms.

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Halocyamines: Novel Antimicrobial Tetrapeptide-like Substances Isolated from the Hemocytes of the Solitary Ascidian *Halocynthia roretzi*†

Kaoru Azumi, Hideyoshi Yokosawa,* and Shin-ichi Ishii

Department of Biochemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

Received May 1, 1989; Revised Manuscript Received July 14, 1989

ABSTRACT: Two novel antimicrobial tetrapeptide-like substances, halocyamine A and B, were isolated from the solitary ascidian *Halocynthia roretzi* by a procedure including extraction steps, chromatographies on coarse and fine HP-20 columns, and preparative reversed-phase high-performance liquid chromatography. The structures of halocyamine A and B were determined to be L-histidyl-L-6,7-dihydroxyphenylalanyl-glycyl-6-bromo-8,9-didehydrotryptamine and L-threonyl-L-6,7-dihydroxyphenylalanyl-L-histidyl-6-bromo-8,9-didehydrotryptamine, respectively, by spectral analyses and degradation studies. Besides antimicrobial activities against several kinds of bacteria and yeasts, both of them showed cytotoxic activities against neuronal cells cultured from rat fetal brain, mouse neuroblastoma N-18 cells, and human hepatoma Hep-G2 cells. They were only detected in the "morula"-like cells, which are of the most abundant cell type among the hemocytes of *H. roretzi*.

It has been proposed that antimicrobial substances function as humoral factors in defense mechanisms of invertebrates which lack humoral immunoglobulins (Boman & Hultmark, 1981). Among invertebrates, ascidians are noticeable animals from a viewpoint of the evolution of immune systems, because they are protochordata which occupy the phylogenetical position between vertebrates and true invertebrates. Several antimicrobial substances have been reported to be present in colonial ascidians and shown also to exhibit antiviral and antitumor activities (Rinehart et al., 1981, 1984; Ireland et al., 1982; Kobayashi et al., 1984, 1988; Ishibashi et al., 1987). We cannot define the tissue where they exist, however, because all of them were extracted from the whole animal bodies.

† This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

0006-2960/90/0429-159$02.50/0