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Zinc Inhibition of Renin and the Protease from Human Immunodeficiency Virus Type 1

Zhong-Yin Zhang, Ilene M. Reardon, John O. Hui, Kathy L. O'Connell, Roger A. Poorman, Alfredo G. Tomasselli, and Robert L. Heinrikson*

Biochemistry Unit, Upjohn Laboratories, Kalamazoo, Michigan 49001

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ABSTRACT: We report here for the first time that Zn^{2+} is an effective inhibitor of renin and the protease from HIV-1, two aspartyl proteinases of considerable physiological importance. Inhibition of renin is noncompetitive and is accompanied by binding of 1 mol of Zn^{2+}/mol of enzyme. Depending on the substrate, inhibition of the HIV protease by Zn^{2+} can be either competitive or noncompetitive, but in neither case is loss of activity due to disruption of the protease dimer. Inhibition of both enzymes is first order with respect to Zn^{2+} and is rapidly reversed by addition of EDTA. K_i values are strongly pH dependent and optimal in the range of 20 μM at or above pH 7. All of the data in hand suggest that the inhibitory effect of Zn^{2+} is a consequence of its binding at, or near, the active-site carboxyl groups of these aspartyl proteinases. This inhibition of the viral enzyme may help to explain some of the beneficial effects seen in AIDS patients who have received Zn^{2+} therapy.

The protease from human immunodeficiency virus (HIV) has become a popular target for development of inhibitors that might find useful application in the treatment of acquired immunodeficiency syndrome (AIDS) (Mitsuya et al., 1990). This enyzme plays an essential role in processing the *gag* and *gag/pol* viral polyproteins during the final maturation step leading to production of infectious virus. Immature, newly budded particles containing defective (Kohl et al., 1988) or inhibited (Ashorn et al., 1990) protease fail to undergo processing and assembly required for infectivity. An impressive body of information is now available regarding the structure and function of the HIV protease [see Tomasselli et al. (1991), Debouck and Metcalf (1990), Krausslich et al. (1989), Blundell et al. (1990), and Meek et al. (1990a) for reviews and further references]. Recognition that the HIV protease is a member of the well-characterized mechanistic set of aspartyl enzymes led to immediate inferences with regard to its structure and function, which were soon borne out experimentally. This wealth of structural and mechanistic information, coupled with experience gained in the design and synthesis of inhibitors of a related enzyme, renin, set the stage for rapid advances in the discovery of substrate-based, competitive inhibitors of the HIV protease that have shown antiviral activity in cell culture assays (McQuade et al., 1990; Meek et al., 1990b; Roberts et al., 1990; Erickson et al., 1990; Vacca et al., 1991). The vast majority of inhibitors described thus far are peptidomimetic compounds and, generally speaking, those that show antiviral activity have poor solubility, low oral uptake, and rapid clearance. This has hampered clinical trials of such compounds and, at the time of this writing, the concept of HIV protease as a target in AIDS therapy has yet to be proven in an infected animal.

In our analysis of fractions from the venom of the American copperhead snake (*Agkistrodon contortrix*), we discovered an inhibitor of renin and identified it to be Zn^{2+} (unpublished results). This was a surprise because, to our knowledge, metal ion inhibition of aspartyl proteases had not been reported. In the present paper we report the kinetics of inhibition of renin and the HIV protease by Zn^{2+} and discuss implications of the work relative to possible modes of metal ion binding and potential therapeutic applications.

Experimental Procedures

Zn^{2+} Inhibition of Renin and HIV Protease. Recombinant human renin was purified from Chinese hamster ovary (CHO) cell extracts and assayed with the decapeptide substrate RSP (H_2N-Pro-His-Pro-Phe-His-Leu-↓-Val-Ile-His-D-Lys-COOH) as described earlier (Poorman et al., 1986; Heinrikson &

* Corresponding author.

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Poorman, 1990). Reactions were performed at 37 °C in 50 mM 3,3-dimethylglutarate buffer, pH 7.0, containing 50 mM NaCl and 5 mM octyl β-glucoside and the course of hydrolysis in the presence of 190 nM enzyme was monitored continuously at 231 nm. The apparent k_{cat} and K_m at each concentration of Zn^{2+} were determined by a nonlinear least-squares analysis using the integrated form of the Michaelis–Menten equation. Recombinant HIV-1 protease was isolated from *Escherichia coli* inclusion bodies and assayed either against a peptide corresponding to the HIV *gag* p17/p24 region, H_2N-Val-Ser-Gln-Asn-Tyr-↓-Pro-Ile-Val-COOH (GSP; Tomasselli et al., 1990a), or with the chromogenic substrate H_2N-Lys-Ala-Arg-Val-Nle-↓-4-NO_2Phe-Glu-Ala-Nle-NH_2 (Richards et al., 1990) at 37 °C.

pH Dependence of Zn^{2+} Inhibition of Renin and HIV Protease. For determination of the pH dependence of Zn^{2+} inhibition of renin, the following buffers were used: pH 5.0–6.5, 50 mM succinate; pH 6.6–7.2, 50 mM 3,3-dimethylglutarate; pH 7.5 and above, 50 mM Tris–acetate. All buffers contained 50 mM NaCl and 5 mM octyl β-glucoside. For HIV protease, the chromogenic substrate H_2N-Lys-Ala-Arg-Val-Nle-↓-4-NO_2Phe-Glu-Ala-Nle-NH_2 was used, and the reaction was monitored continuously at 300 nm (Richards et al., 1990). All the buffers contained 50 mM sodium acetate, 50 mM MES, and 100 mM Tris; ionic strength was kept at 1.0 M by addition of NaCl. Kinetic parameters k_{cat} and K_m were obtained from nonlinear least-squares fits of the integrated Michaelis–Menten equation and, from these values, noncompetitive K_i's were calculated for renin and competitive K_i's for the HIV-1 protease. The pH dependency of K_i was analyzed according to

$$K_i = K_i^\circ\left(1 + \frac{[H^+]}{K_a}\right)$$

where K_i° is the pH-independent inhibition constant and K_a is the acid dissociation constant. This equation was employed for calculation of pK_a values.

Equilibrium Dialysis. Aliquots (65 μL) of renin (48 μM) were distributed into six dialysis chambers and allowed to equilibrate at room temperature for 24 h with 65 μL of 18, 36, 80, 180, 360, and 720 μM Zn^{2+} added to the corresponding chamber separated by a membrane with 14000 MW cutoff. Each chamber contained 13000 cpm of $^{65}Zn^{2+}$ (New England Nuclear) as a tracer. The buffer was 50 mM succinate, pH 6.5, containing 200 mM NaCl and 5 mM octyl β-glucoside. After equilibration, the samples were counted and the amount of free and bound Zn^{2+} was determined. The data were analyzed by a nonlinear least-squares algorithm using

$$[Zn]_T = [Zn]_B\left(1 + \frac{K_d}{[E]_T - [Zn]_B}\right)$$

where $[Zn]_T$ and $[Zn]_B$ refer to total and bound concentrations of zinc, respectively, and $[E]_T$ = total enzyme concentration.

Results

In Figure 1 is shown the kinetic analysis of renin inhibition of Zn^{2+} of the hydrolysis of a decapeptide substrate (Poorman et al., 1986; Heinrikson & Poorman, 1990) as assayed at pH 7.0. Experimentally determined k_{cat} values fit precisely to the equation given in the legend to Figure 1. The results demonstrate that Zn^{2+} inhibition is first order with respect to the metal ion and purely noncompetitive; i.e., Zn^{2+} does not interfere at all with binding of this substrate, but catalysis is fully suppressed. Zn^{2+} inhibition is strongly pH dependent, a proton

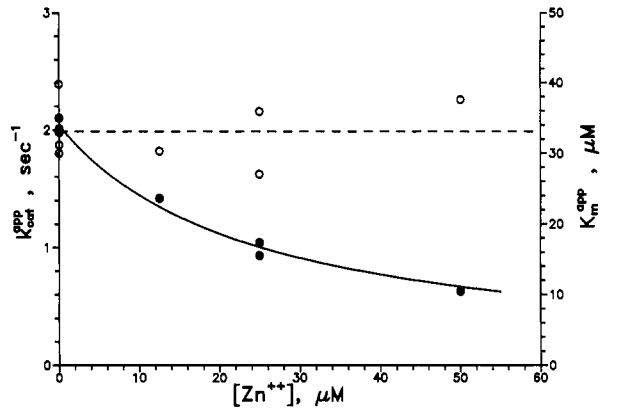

FIGURE 1: Inhibition of renin by Zn^{2+}; dependence of apparent k_{cat} (●) and K_m (○) on Zn^{2+} concentration. The dependence of k_{cat}(app) on $[Zn^{2+}]$ was then analyzed by a nonlinear least-squares algorithm using $k_{cat}(app) = k_{cat}/(1 + ([Zn^{2+}]/K_{Zn}))$. From this analysis, the theoretical curve (solid line) was generated by using $k_{cat} = 2.04\ s^{-1}$ and $K_{Zn} = 24.2\ \mu M$. The kinetic analysis revealed that Zn^{2+} inhibition of renin is noncompetitive, and this is borne out by the constancy of K_m over the entire range of $[Zn^{2+}]$.

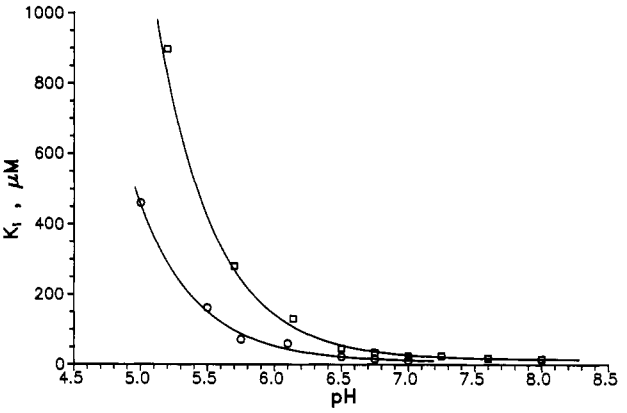

FIGURE 2: pH dependence of K_i values for inhibition of renin (□) and the HIV-1 protease (○) by Zn^{2+}. Substrates employed in these studies were as follows: for renin, the decapeptide RSP (H_2N-Pro-His-Pro-Phe-His-Leu-↓-Val-Ile-His-D-Lys-COOH); for the HIV protease, the chromogenic peptide H_2N-Lys-Ala-Arg-Val-Nle-↓-4-NO_2Phe-Glu-Ala-Nle-NH_2 (Richards et al., 1990). Conditions are described in the text.

acting as its competitor for a group with pK_a = 7.0 ± 0.1 as calculated from the equation presented under Experimental Procedures. As shown in Figure 2, K_i values range from 890 μM at pH 5.2 to 24 μM at pH 7.0 and above. Addition of excess EDTA leads to the rapid and complete reversal of inhibition, establishing that loss of activity is due to reversible binding of Zn^{2+}.

If the inhibition of renin occurs at the catalytic site of the enzyme, then one would expect that other aspartyl proteinases which share with renin all of the major features of this region should also be inhibited by Zn^{2+}. One such enzyme with a pH optimum similar to that of renin is the HIV protease, and indeed, Zn^{2+} proved to be an effective inhibitor of this enzyme. The kinetic profile obtained from Zn^{2+} inhibition of the HIV protease was more complex than that seen with renin. In this case, inhibition was found to be competitive in a continuous assay with a chromogenic substrate (Richards et al., 1990) containing a P_1' 4-nitrophenylalanine (Figure 3A) and noncompetitive toward a standard peptide substrate (GSP; Tomasselli et al., 1990a) representative of the p17/p24 junction in the viral polyprotein and having a Tyr-Pro scissile bond (Figure 3B). As was the case for renin, K_i values were found to depend upon pH, irrespective of the substrate, with optimal

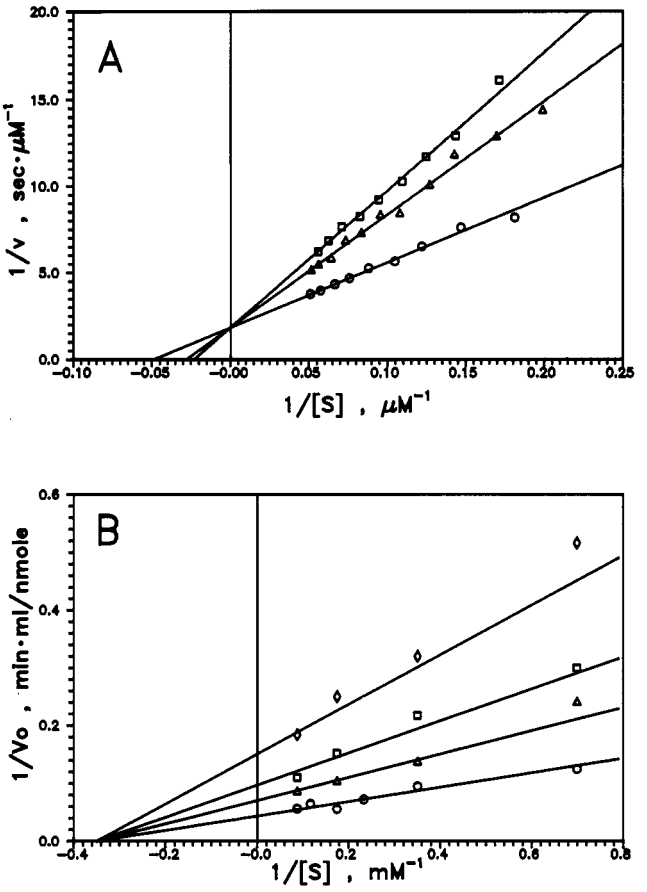

FIGURE 3: Zn^{2+} inhibition of the HIV-1 protease as monitored by hydrolysis at pH 5.5 of (A) the chromogenic substrate described (competitive inhibition; ○, △, and □, 0, 0.2, and 0.3 mM Zn^{2+}, respectively) and (B) the peptide substrate GSP corresponding to the HIV *gag* p17/p24 region, H_2N-Val-Ser-Gln-Asn-Tyr-↓-Pro-Ile-Val-COOH (noncompetitive inhibition; ○, △, □, and ◇, 0, 0.5, 1.0, and 2.0 mM Zn^{2+}, respectively).

values near 12 μM at or above pH 7 (Figure 2), and the calculated pK_a value was near neutrality (6.8 ± 0.1). Again, Zn^{2+} inhibition of hydrolysis of both substrates is abolished by addition of EDTA, thus demonstrating that there is no irreversible change in structure accompanying inhibition such as SH group oxidation. An earlier report from our laboratory established that a two-domain construct of the *Pseudomonas* exotoxin PE40 (Tomasselli et al., 1990b) is a nonviral protein that mimics the viral polyprotein as a substrate for HIV protease. Zn^{2+} inhibits hydrolysis of PE40 by the HIV protease, and the K_i at pH 5.5 is 820 μM, a value that compares to about 700 for hydrolysis of GSP at this pH. It should also be mentioned that the HIV protease has been assayed under a wide variety of conditions of ionic strength and in the presence of agents such as glycerol and ethylene glycol. We have found that neither the K_i nor the mode of inhibition of the HIV protease by Zn^{2+} is dependent on the ionic strength of the medium.

The results described above suggested that Zn^{2+} inhibition might well apply to all aspartyl proteinases, even those with low pH optimum. Indeed, Zn^{2+} was found to be a weak inhibitor of pepsin: K_i's were, once again, pH dependent and the value at pH 5.0, where pepsin still displays some activity, was about the same as those seen with HIV protease and renin. It was not possible to explore the higher range of pH where optimal Zn^{2+} inhibition is seen against the latter enzymes because pepsin activity rapidly declines above pH 5. Thus, Zn^{2+} inhibition occurs with the broad class of aspartyl proteinases, but the strong pH dependency of inhibition by the metal ion has precluded earlier detection in studies of the common members of this mechanistic set that function optimally at low pH.

Table I: Inhibitory Effects of Various Metal Ions on Renin Activity at pH 7.0

metal ion[a]	K_i (μM)	mode of inhibition
Zn^{2+}	24	noncompetitive
Cu^{2+}	729 (μM^2)[b]	competitive
Ag^{1+}	4	noncompetitive
Hg^{2+}	325	noncompetitive
Cd^{2+}	450	noncompetitive

[a] Other ions tested showed no significant inhibitory activity: calcium, magnesium, manganese, nickel, cobalt, and thallium. [b] The inhibition by Cu^{2+} is second order ($K_i = [E][Cu^{2+}]^2/[E{\cdot}2Cu^{2+}] = 729\ \mu M^2$) with respect to metal ion, whereas inhibition is first order for the other metal ions. The effective Cu^{2+} concentration for renin inhibition is very nearly the same as that of Zn^{2+}, so the K_i for Zn^{2+} should be compared with the square root of the K_i for Cu^{2+}.

We were interested to determine whether Zn^{2+} is unique among divalent metal ions in its ability to inhibit these enzymes. Among a number of metal ions tested, Zn^{2+}, Cu^{2+}, and Ag^{1+} were found to be the most effective inhibitors of renin (Table I) and the HIV protease (data not shown). Kinetic analysis revealed that inhibition of renin by Ag^{1+} is basically identical with that seen with Zn^{2+}, i.e., noncompetitive, first order with respect to the metal, and having the same pH dependence with $pK_a = 6.8 \pm 0.1$. In contrast, inhibition of renin by Cu^{2+} is competitive, i.e., k_{cat} is constant and K_m increases in the presence of this metal ion. Furthermore, the experimentally determined K_m(app) values fit exactly to the equation $K_m(app) = K_m(1 + [Cu^{2+}]^2/K_i)$ where K_m is the value in the absence of Cu^{2+}; thus, inhibition is second order with respect to Cu^{2+}. The kinetic profile of inhibition of the HIV-1 protease by Cu^{2+} was more complex. Indeed, a recent paper by Karlstrom and Levine (1991) describes Cu^{2+} inhibition of the HIV-1 protease at pH 5.5 that was reported to involve modification of the cysteine residues in the enzyme in a reaction that is not reversible by EDTA. Cu^{2+} inhibition of a mutant HIV-1 protease lacking cysteine residues was not observed by these investigators unless DTT was added to the mixture. We found that the HIV-2 protease (kindly provided by Drs. Charles Craik and Dianne DeCamp, University of California at San Francisco), an enzyme that is closely related but has no residues of cysteine or histidine, undergoes loss of activity in the presence of Cu^{2+} with no requirement for DTT. Inhibition is competitive, first order with respect to Cu^{2+}, reversed by EDTA, and shows the same pH dependency as observed with Zn^{2+} (Figure 2), optimizing in the range of 20 μM at pH 7. The HIV-2 protease is inhibited by Zn^{2+} in a manner identical with that of the HIV-1 enzyme.

Inhibition of the HIV-1 protease by Zn^{2+} is not the result of disruption of the dimeric structure of the enzyme. Gel filtration of the HIV-1 protease alone and in the presence of 5 mM Zn^{2+} gave the same molecular mass of 20 ± 2 kDa. Similarly, sedimentation equilibrium analysis of the HIV-1 protease in the presence of 5 mM concentrations of Zn^{2+} gave the same value as obtained from native enzyme, i.e., 19.5 ± 2 kDa. In all cases, the viral enzyme dimer was inactive under the conditions of exposure to this metal ion.

The first-order dependence of inactivation on Zn^{2+} concentration suggests that there is a single important site for binding of the metal ion. Nevertheless, it might be argued that Zn^{2+} could be binding at numerous sites in these aspartyl proteases. This possibility was explored by equilibrium dialysis (Tomasselli & Noda, 1979) of renin in the presence of $^{65}Zn^{2+}$ (Figure 4). Renin was chosen for this purpose because of its

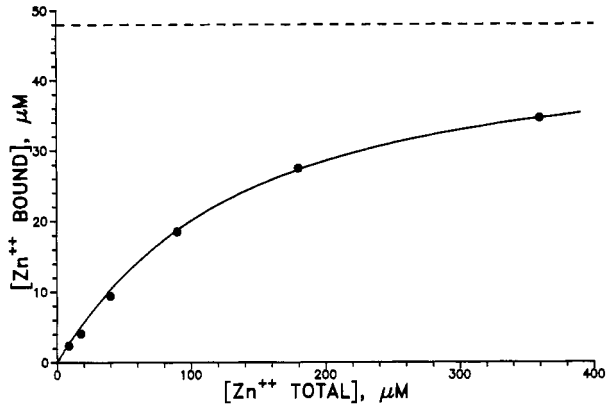

FIGURE 4: Equilibrium dialysis of renin in the presence of $^{65}Zn^{2+}$; the relation of total Zn^{2+} to Zn^{2+} bound to renin. The dotted line indicates the concentration of renin in the experiment (48 μM).

higher stability under the conditions of the experiment. Analysis of the results showed binding of 0.95 ± 0.01 mol of Zn^{2+}/mol of active renin with a K_D at pH 6.5 of 101 ± 4.7 μM. The latter value compares favorably with the K_i of 40 μM determined from the kinetic analysis of the metal ion as an inhibitor at this pH (Figure 2), considering that equilibrium dialysis was carried out at 22 °C and the kinetics at 37 °C.

Discussion

The results presented herein establish for the first time that, at neutral pH, Zn^{2+} is an effective inhibitor of aspartyl proteinases. In the case of renin and the HIV proteases, enzymes having pH optima near neutrality, inhibition by Zn^{2+} is a prominent and easily observed phenomenon. Inhibition of pepsin is more difficult to discern, but at any given pH, the K_i values are of the same order of magnitude as those obtained with enzymes having neutral pH optima. At the present time we do not have enough information to establish either the identity or the mechanistic significance of the group of pK_a near 7. Because the ionization of hydrated zinc ion occurs near pH 9, this pK_a would not appear to be related to the metal ion itself. More likely, this group of $pK_a = 7$ is the active-site carboxylate, which is catalytically active in the protonated form. Even if the metal ion does not complex directly with the two carboxylates, its binding should be facilitated by electrostatic forces, provided such binding occurs within a reasonable distance from these negatively charged moieties. If this explanation is correct, then the inhibition of pepsin should be pH independent above pH 5.5.

The two enzymes that we have studied in greatest detail are renin and the HIV-1 protease, enzymes that have pH optima near neutrality. Zn^{2+} also inhibits the HIV-2 protease, and the kinetics of inhibition of all three enzymes are nearly identical. This is true despite the fact that the only structural link among these widely disparate enzymes is found in the constellation of two aspartyl residues seen at the catalytic site. Renin contains more than 350 amino acids in a single polypeptide chain with three disulfide bonds and no free SH groups. The HIV proteases are dimers of chains having only 99 amino acids and these, in turn, may be distinguished by the fact that the HIV-1 protease has three nucleophilic amino acid side chains of Cys-67, His-69, and Cys-95 that are not present in the HIV-2 enzyme. Therefore, it seems most plausible that Zn^{2+} inhibition is a consequence of binding to or in the vicinity of the carboxyl groups of the two catalytic aspartates. Clearly, other proteins and ligands such as EDTA bind Zn^{2+} much more avidly than the neutral aspartyl proteases studied in this paper. Therefore, it seems likely that a ligand could be designed to incorporate binding both to Zn^{2+} and to the enzyme target to create a tight-binding and specific inhibitor for therapeutic evaluation. In the meantime, the question arises as to whether Zn^{2+} supplementation might present, in itself, an avenue for therapy in hypertension or in AIDS.

The importance of trace elements such as zinc in human nutrition has only been recognized since the 1960s (Prasad, 1991; Day, 1991). By now, a considerable body of information has been compiled on the use of Zn^{2+} as a therapeutic agent for a wide variety of human diseases [see Cunnane (1988) for review]. Indeed, Zn^{2+} supplementation has been reported to be beneficial in improving peripheral circulation (Henzel et al., 1972), and our work would suggest that this effect could be mediated in part by renin inhibition and consequent vasodilation. Zn^{2+} is also widely perceived to be an "antiviral" agent (Sergio, 1988). For example, it is believed to be effective against picornaviral infections (Korant et al., 1974) because it can inhibit the cysteinyl protease of the virus, which, like the HIV protease, is necessary for processing the viral polyproteins. The mechanism believed to be at work in herpes simplex virus is different; here the metal ion appears to be interfering with surface glycoprotein dependent functions of adsorption and penetration (Kumel et al., 1990).

Our finding that Zn^{2+} inhibits the HIV protease provides a rationale for the use of Zn^{2+} in AIDS therapy. In fact, independent studies have concluded that conditions of immunodeficiency such as may arise in cancer and AIDS patients are often accompanied by Zn^{2+} deficiency (Bogden, 1990; Libanore et al., 1987). Other work has questioned whether such correlations are statistically significant (Walter et al., 1990), and the subject remains controversial. Nevertheless, the idea that Zn^{2+} supplementation might improve the immune status of AIDS and cancer victims has prompted clinical trials of Zn^{2+} in humans. In one study, no significant change in total lymphocyte count nor in CD4 subsets was seen in a phase II trial of immunorestoration with zinc gluconate in immunodepressed patients with cancer and AIDS-related complex (ARC) (Mathe et al., 1986). However, other preliminary trials have shown promising effects following administration of zinc to patients with ARC (Rouveix et al., 1988) and AIDS (Caselli & Bicocchi, 1986).

Given past experience with Zn^{2+} therapy and the variable manner in which different individuals react to Zn^{2+} supplementation (Cunnane, 1988), it is not surprising that the clinical picture is complex and, seemingly, inconsistent. Any beneficial effect of zinc therapy in AIDS patients could derive from numerous interactions with the virus and with pathogens that accompany the disease, to say nothing of the general health and thymic proficiency of the host. The lack of significant improvement in other trails could be due to administration of too much or too little metal ion or to problems with absorption. Now that it is recognized that the HIV protease is a target for Zn^{2+} inhibition, clinical testing may be formulated with regard to levels needed to shut down the protease and, hence, viral maturation. Since monitoring of serum p24 protein levels is a standard measurement in the clinical setting, it should be possible to tell whether supplementation with Zn^{2+} is having an effect on polyprotein processing. If so, this would provide strong evidence that it is exerting its effect by inhibition of the HIV protease.

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References

Ashorn, P., McQuade, T. J., Thaisrivongs, S., Tomasselli, A. G., Tarpley, W. G., & Moss, B. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 7472–7476.

Blundell, T. L., Lapatto, R., Wilderspin, A. F., Hemmings, A. M., Hobart, P. M., Danley, D. E., & Whittle, P. J. (1990) *Trends Biochem. Sci. 15*, 425–430.

Bogden, J. D., Baker, H., Frank, O., Perez, G., Kemp, F., Bruening, K., & Louria, D. (1990) *Ann. N.Y. Acad. Sci. 587*, 189–195.

Caselli, M., & Bicocchi, R. (1986) *La Presse Med. 15*, 1877.

Cunnane, S. C. (1988) *Zinc: Clinical and Biochemical Significance*, CRC Press, Inc., Boca Raton, FL.

Day, H. G. (1991) *FASEB J. 5*, 2315–2316.

Debouck, C., & Metcalf, B. W. (1990) *Drug Dev. Res. 21*, 1–17.

Erickson, J., Neidhart, D. J., VanDrie, J., Kempf, D., Wang, X. C., Norbeck, D. W., Plattner, J. J., Rittenhouse, J. W., Turon, M., Wideburg, N., Kohlbrenner, W. E., Simmer, R., Helfrich, R., Paul, D. A., & Knigge, M. (1990) *Science 249*, 527–533.

Heinrikson, R. L., & Poorman, R. A. (1990) in *Hypertension: Pathophysiology, Diagnosis and Management* (Laragh, J. H., & Brenner, B. M., Eds.) pp 1179–1196, Ravel Press, Ltd., New York.

Henzel, J. H., Licht, E. L., Sheparo, W., & Paone, J. (1972) in *Clinical Applications of Zinc Metabolism* (Pories, W. J., Strain, W. H., Hsu, J. M., & Woolsey, R. L., Eds.) pp 243–259, Academic Press, New York.

Karlstrom, A. R., & Levine, R. L. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 5552–5556.

Kohl, N. E., Emini, E. A., Schleif, W. A., Davis, L. J., Heimbach, J. C., Dixon, R. A. F., Scolnick, E. M., & Sigal, I. S. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 4686–4690.

Korant, B. D., Kauer, J. C., & Butterworth, B. E. (1974) *Nature 248*, 588–590.

Krausslich, H.-G., Oroszlan, S., & Wimmer, E., Eds. (1989) *Current Communications in Molecular Biology: Viral Proteinases as Targets for Chemotherapy*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Kumel, G., Schrader, S., Zentgraf, H., Daus, H., & Brendel, M. (1990) *J. Gen. Virol. 71*, 2989.

Libanore, M., Bicocchi, R., Raise, E., Sighinolfi, L., Bedetti, A., & Montanari, P. (1987) *Minerva Med. 78*, 1805–1812.

Mathe, G., Misset, J. L., Gil-Delgado, M., Musset, M., Reizenstein, P., & Canon, C. (1986) *Biomed. Pharmacother. 40*, 383–385.

McQuade, T. J., Tomasselli, A. G., Liu, L., Karacostas, V., Moss, B., Sawyer, T. K., Heinrikson, R. L., & Tarpley, W. G. (1990) *Science 247*, 454–456.

Meek, T. D., Lambert, B. W., Metcalf, B. W., Petteway, S. R. Jr., & Dreyer, G. B. (1990a) in *Design of Anti-AIDS Drugs* (DeClercq, E., Ed.) pp 225–255, Elsevier, Amsterdam.

Meek, T. D., Lambert, D. M., Dreyer, G. B., Carr, T. J., Tomaszek, T. A., Jr., Moore, M. L., Strickler, J. E., Debouck, C., Hyland, L. J., Matthews, T. J., Metcalf, B. W., & Petteway, S. R. (1990b) *Nature 343*, 90–92.

Mitsuya, H., Yarchoan, R., & Broder, S. (1990) *Science 249*, 1533–1544.

Poorman, R. A., Palermo, D. P., Post, L. E., Murakami, K., Kinner, J. H., Smith, C. W., Reardon, I., & Heinrikson, R. L. (1986) *Proteins: Struct., Funct., Genet. 1*, 139–145.

Prasad, A. S. (1991) *Am. J. Clin. Nutr. 53*, 403–412.

Richards, A. D., Phylip, L. H., Farmerie, W. G., Scarborough, P. E., Alvarez, A., Dunn, B. M., Hirel, P.-H., Konvalinka, J., Strop, P., Pavlickova, L., Kostka, V., & Kay, J. (1990) *J. Biol. Chem. 265*, 7733–7736.

Roberts, N. A., Martin, J. A., Kinchington, D., Broadhurst, A. V., Craig, J. C., Duncan, I. B., Galpin, S. A., Handa, B. K., Kay, J., Krohn, A., Lambert, R. W., Merrett, J. H., Mills, J. S., Parkes, K. E. B., Redshaw, S., Ritchie, A. J., Taylor, D. L., Thomas, G. J., & Machin, P. J. (1990) *Science 248*, 358–361.

Rouveix, B., Zayyo, J. F., Rajagopalan, P., Lavacher, M., Girard, P. M., & Fusselier, M. (1988) *Int. J. Immunopharmacol. 10* (Suppl. 1), 36.

Sergio, W. (1988) *Med. Hypotheses 26*, 251–253.

Tomasselli, A. G., & Noda, L. H. (1979) *Eur. J. Biochem. 93*, 263–270.

Tomasselli, A. G., Olsen, M. K., Hui, J., Staples, D. J., Sawyer, T. K., Heinrikson, R. L., & Tomich, C.-S. C. (1990a) *Biochemistry 29*, 264–269.

Tomasselli, A. G., Hui, J. O., Sawyer, T. K., Staples, D. J., FitzGerald, D. J., Chaudhary, V. K., Pastan, I., & Heinrikson, R. L. (1990b) *J. Biol. Chem. 265*, 408–413.

Tomasselli, A. G., Howe, W. J., Sawyer, T. K., Wlodawer, A., & Heinrikson, R. L. (1991) *Chem. Today 9*, 6–27.

Vacca, J. P., Guare, J. P., deSolms, S. J., Sanders, W. M., Giuliani, E. A., Young, S. D., Darke, P. L., Sigal, I. S., Schleif, W. A., Quintero, J. C., Emini, E. A., Anderson, P. S., & Huff, J. R. (1991) *J. Med. Chem.* (in press).

Walter, R. M., Oster, M. H., Lee, T. J., Flynn, N., & Keen, C. L. (1990) *Life Sci. 46*, 1597–1600.