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Short communication

De novo drug design of a new copper chelate molecule acting as HIV-1 protease inhibitor

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Abstract – An original pharmacophore for the active site of the HIV-1 protease was built based on the presence of a catalytic water molecule found between the two catalytic residues Asp25 and Asp125. Using this pharmacophore, a screening of metallo-organic complexes was performed based on the fact that such metallic compounds are known to inhibit the enzyme activity. We observed that diaqua [bis-(2-pyridylcarbonyl)-amido] copper (II) nitrate dihydrate fitted in the active site of the enzyme. Experimentally, it was found to be a competitive inhibitor of the protease ($K_i = 480 \pm 120$ μM). The de novo discovery of this novel HIV-1 protease inhibitor stressed the fact that the catalytic water molecule has to be taken into consideration for the design of non-peptide inhibitors of the protease. © Elsevier, Paris

HIV-1 protease / inhibitor / drug design / copper chelates / metallo-organic compounds

1. Introduction

Human type 1 immunodeficiency virus (HIV-1) has become one of the most studied viruses due to its massive spread all over the world, and extensive work is currently going on to block its replication. As the HIV-1 protease (PR) activity is a prerequisite for viral replication [1], inhibition of the enzyme represents a promising chemotherapy of AIDS. Most of the potent inhibitors of HIV-1 PR described so far are peptidic or peptidomimetic compounds acting as transition state analogs. A great deal of work has been done attempting to synthesize low-molecular-weight inhibitors combining both antiviral potency and favorable bioavailability [2, 3]. Recently, it has been shown that simultaneous administration of several anti HIV-1 drugs, including HIV-1 PR inhibitors is effective to prevent the progression of the disease [4].

In this paper, we investigated the potency of metallo-organic compounds to act as inhibitors of the PR. Indeed, it has been previously shown that cupric chloride inhibits the PR by acting on the non-active-site cysteines 67 and 95 in both monomers [5–7]. A protease lacking these cysteines is only inhibited by $CuCl_2$ in the presence of compounds acting as metal chelators, and such complexes behave as competitive inhibitors targeting the active site [8]. These results suggest that copper chelates could be useful as inhibitors of HIV-1 PR. We built an original pharmacophore, based on the presence of the catalytic water molecule necessary for the hydrolytic process, to search for a copper chelate that could fit into the active site of the enzyme. Several compounds were found as potential inhibitors of the enzyme and we report the results obtained with one of them. This work opens the way for the development of a new family of HIV-1 PR potent inhibitors.

2. Materials and methods

2.1. General

HIV-1 protease was kindly supplied by H.J. Schramm and co-workers, Max-Planck Institut for Biochemie, Mar-

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tinsried, Germany, who expressed and isolated it using the plasmid pET9c-PR following the experimental procedure described by Billich and collaborators (1990). The chromogenic substrate K-A-R-V-L-G-F(NO_2)-E-A-Nle-G-NH_2 was designed and synthesized by B. Badet (Institut des Substances Naturelles, Gif-sur-Yvette, France; unpublished results). Kinetic measurements were performed using an Uvikon 941 spectrophotometer equipped with a thermostated cell holder.

2.2. *Biochemistry*

HIV-1 PR activity was assayed by following the continuous release of the hydrolysis product K-A-R-V-L-G-F(NO_2)-OH at 307 nm and 30 °C in 0.1 M acetate pH 5.5. The reaction was initiated by addition of the purified enzyme $[E]_0 = 0.1$mM to the chromogenic substrate $[S]_0 = 5 \times 10^{-5}$ M and $[S]_0 = 2 \times 10^{-4}$ M, incubated in the presence of various concentrations of inhibitors $[I]_0 = 1.25 - 3.75 \times 10^{-4}$ M. The inhibitor diaqua [bis-(2-pyridylcarbonyl)-amido] copper (II) nitrate dihydrate was dissolved in water [9]. The enzyme was first diluted (factor of 16) in 1 mM phosphate (pH = 8) containing bovine serum albumin (1 mg/mL). In all assays, the reaction was initiated by addition of the previously prepared enzyme solution (dilution of 20) to the reaction mixture containing the chromogenic substrate and the inhibitor. It was verified that the diaqua [bis-(2-pyridylcarbonyl)-amido] copper (II) nitrate dihydrate was stable in the buffer (0.1 M acetate) containing the same percentage of protease buffer concentration than in the reaction mixture with the inhibitor.

2.3. *Modeling*

The search for lead compounds was performed interactively using the program QUEST of the Cambridge Structural Database (CSD, version 5.9, 1995). Among the twenty compounds that emerged from the search, we identified diaqua [bis-(2-pyridylcarbonyl)-amido] copper (II) nitrate dihydrate. The inhibitor was docked manually into the active site of the enzyme. The initial PR structure was the X-ray crystal form of HIV-1 PR solved by Erickson et al. [10] and deposited in the Brookhaven Data Bank as 9HVP. The complex was gently minimized (500 steps of steepest descent and 500 steps of conjugate gradient). The molecular mechanics simulations were performed with the DISCOVER program (Biosym/Molecular Simulations Inc., San Diego, INSIGHTII package, version 95.0) using ESFF force field, on MSI Silicon Graphics Indigo 2 workstations.

3. Results and discussion

The design of the pharmacophore was based on the study of the crystallographic structure of HIV-1 PR complexed to the non-peptide inhibitor ucsf8, a haloperidol derivative [11]. Indeed, it was previously described that in contrast to peptidomimetic inhibitors, ucsf8 interacts mainly with the flaps of the enzyme in an open conformation [10, 12–14]. We found that the interaction of ucsf8 with the catalytic site of PR is mediated by a water molecule located at equal distance (3 Å) from the two catalytic aspartates Asp25/125. This water molecule can be assimilated to the catalytic water molecule that initiates the hydrolysis of the substrate during the proteolytic process and could be regarded as a structural water molecule which belongs to the protein. To the best of our knowledge, this molecule is only observed in the crystallographic complex of HIV-1 PR with ucsf8. In the presence of peptidomimetic inhibitors, this water molecule is replaced by hydroxyl groups belonging to the inhibitors and is therefore not observed in the crystallographic structures (*figure 1A*) [15, 16].

Based on this observation, we built a pharmacophore which takes into account the crucial catalytic water molecule that interacts with the catalytic residues Asp25/125 in the complex of HIV-1 PR with ucsf8 (*figure 1B*). We also incorporated a proton acceptor Y to interact directly with residues Ile50/150 of the flaps of the enzyme as was previously described by Lam and coworkers [17]. In fact, this proton acceptor Y mimicks the well described tetracoordinated water molecule bound to residues Ile50/150 and two carbonyl groups of peptidomimetic inhibitors [12–14]. It was postulated that the displacement of the tetracoordinated water molecule by an inhibitor would be favorable from an entropic point of view and increase retroviral protease specificity [17].

The model described in *figure 1B* was used for screening new HIV-1 PR inhibitors through the Cambridge Structural Database (CSD), which contains the crystallographic structure of approximatively 150 000 small molecules. The crystallographic structure of HIV-1 PR complexed to the peptidomimetic inhibitor A74704 was superimposed to HIV-1 PR/ucsf8. The distance between the proton acceptor Y and the catalytic water molecule was computed and served as geometric criteria for the screening. Twenty structures fitting into the active site of the enzyme emerged from this search. Five stable compounds, which could easily serve for the synthesis of new analogs, were selected and tested for their inhibitory activity. Among them, the compound diaqua [bis-(2-pyridylcarbonyl)-amido] copper (II) nitrate (*figure 2*) [9]

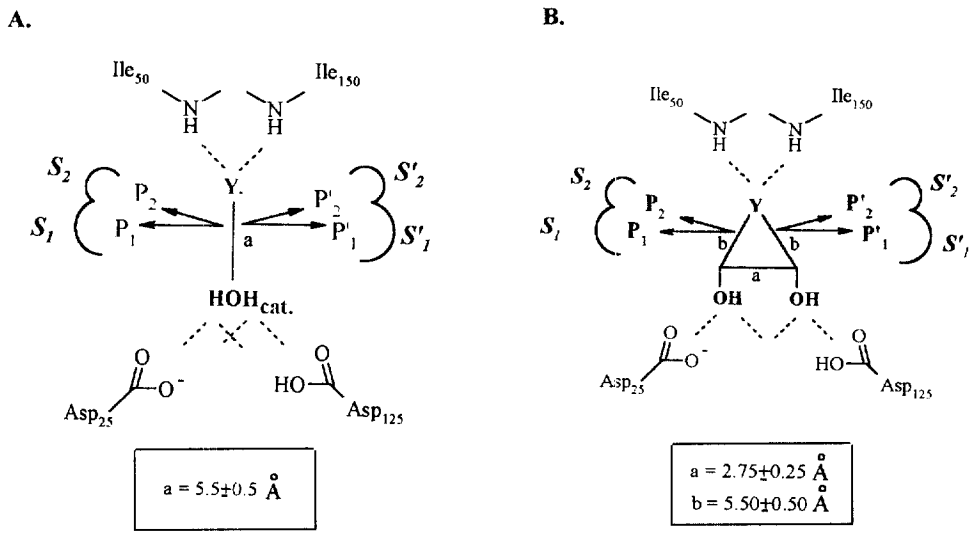

Figure 1. General pharmacophore of HIV-1 PR inhibitors. Y = proton acceptor; P1, P'1, P2 and P'2 represent hydrophobic groups; S1, S'1, S2 and S'2 represent hydrophobic pockets of the protease. A: classical pharmacophore. B: new pharmacophore including the water molecule observed near the catalytic aspartates in the complex HIV-1 PR/ucsf8.

was found to be the most active and behaved as a competitive inhibitor of HIV-1 protease (*figure 3*) with a K_i value of 480 ± 120 µM. After 1 h incubation of the copper chelate in a buffer containing EDTA, no inhibition was observed, demonstrating that the efficient molecule was the metallo-organic complex.

A similar inhibitory effect (82 and 93% inhibition respectively) was observed for 0.4 µM copper chelate and 0.4 µM $CuCl_2$ (effect of Cu^{2+} alone). However, wheras the copper chelate behaved as a competitive inhibitor (interaction within the active site), it is known that stoichiometric concentrations of copper or mercury ions inhibited HIV-1 protease by acting on the cysteine residue(s) located outside the active site [5]. Indeed, a synthetic protease lacking cysteine residues was not inhibited by exposure to copper. Cupride chloride has been shown to inhibit HIV-1 PR, as well as a mutant HIV-1 protease devoid of cysteines, only in the presence of molecules acting as metal chelators, like dithiothreitol and ascorbic acid [8]. It was postulated that the so-formed copper complexes could fit into the active site of the enzyme and inhibit its activity. It was also found that HIV-1 PR is inhibited by a boronated porphyrin Cu(II), but the interaction was not further characterized [18].

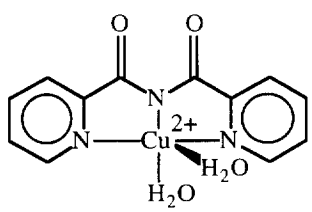

Figure 2. Diaqua [bis-(2-pyridylcarbonyl)-amido] copper (II) nitrate dihydrate.

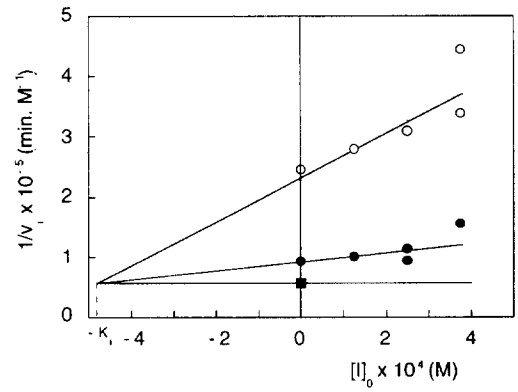

Figure 3. Dixon plot of the inhibition of HIV PR by diaqua [bis-(2-pyridylcarbonyl)-amido] copper (II) nitrate dihydrate with K-A-R-V-L-G-F(NO_2)-E-A-Nle-G-NH_2 as substrate at pH 5.5 and 30 °C. The substrate concentrations were 50 (open circles), 200 (closed circles) µM and infinite (closed squares). The enzyme concentration was 100 nM.

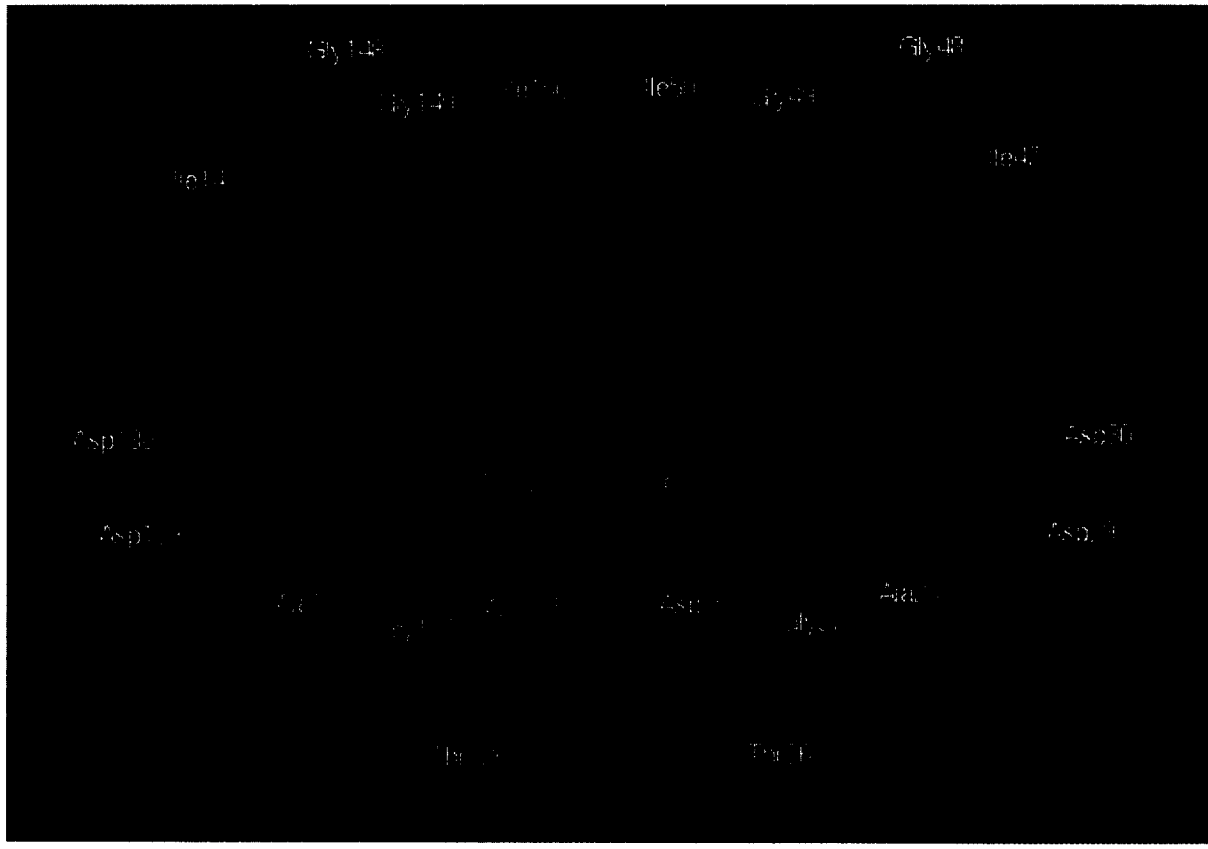

Figure 4. Interaction between HIV-1 PR and diaqua [bis-(2-pyridylcarbonyl)-amido] copper (II) nitrate dihydrate as predicted by molecular mechanics simulations and minimization. For clarity only residues 25–30 and 47–50 in both monomers are represented.

The inhibitor diaqua [bis-(2-pyridylcarbonyl)-amido] copper (II) nitrate dihydrate was modeled using the ESFF force field into the active site of the enzyme. After minimization of the potential energy of the complex, we observed an interaction of both carbonyl groups with the amino groups of the residues Ile50/150 of the flaps. As expected, a water molecule was found between the catalytic aspartates Asp25/125. These results strongly indicate that the water molecule is indeed essential for the structure of the enzyme and has to be taken into consideration in the de novo design of HIV-1 PR inhibitors which do not contain hydroxyl substitutes as it is the case of the copper chelate reported in this work. Based on the predicted interaction of the inhibitor with the enzyme, depicted in *figure 4*, the improvement of the potency of the molecule diaqua [bis-(2-pyridylcarbonyl)-amido] copper (II) nitrate dihydrate can now be undertaken.

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