Structure-based design of AIDS drugs and the development of resistance

Alexander Wlodawer

Macromolecular Crystallography Laboratory
National Cancer Institute at Frederick, Frederick, MD

ABSTRACT

AIDS is a major worldwide epidemic spread primarily through contact with infected blood during sexual activity, drug injection, birth, and, rarely now, blood transfusion. More than a dozen drugs for the treatment of AIDS have been introduced in the last 15 years and the process leading to their development offers an excellent example of the progress made in the field of rational drug design. The principal targets of the approved drugs are reverse transcriptase and protease enzymes encoded by the human immunodeficiency virus. In particular, introduction of protease inhibitors has led to a significant decrease of the mortality and morbidity associated with AIDS. My presentation will discuss methods utilized for the development of selected AIDS drugs, primarily protease inhibitors, and the emergence of drug resistance which is presently the greatest challenge in fighting this disease in developed countries.

In the last 15 years, introduction of over a dozen drugs against acquired immunodeficiency syndrome (AIDS) attests to the success of structure-assisted ("rational") drug design and discovery. This approach utilizes techniques such as protein crystallography, nuclear magnetic resonance (NMR), and computational biochemistry to guide the creation and synthesis of potential drugs (1, 2). The complementary methods of computer-aided molecular design (3) and combinatorial chemistry (4) are now routinely employed in both the lead identification and the development phases of drug design.

AIDS is caused by two variants of the human immunodeficiency virus, HIV-1 and HIV-2. The genomes of these retroviruses consist of three open reading frames (ORF), gag, pol, and env and encode only three enzymes, reverse transcriptase (RT), integrase (IN), and protease (PR). All of these enzymes have become targets for drug discovery, although they are certainly not the only possible retroviral targets. The first AIDS drugs to be identified were nucleoside inhibitors of RT, discovered and developed long before the structure of RT itself (Fig. 1) was solved (5, 6). These analogs of the polynucleotide substrates bind in the substrate-binding site and can inhibit both HIV-1 and HIV-2 RT. The inhibitory properties of nucleoside analogs against RT are due either to the lack of 2' or 3' hydroxyl groups, or to their replacement by other functional groups. So far, one nucleotide and six nucleoside analogs (NRTIs) have been approved by the U.S. Food and Drug Administration (FDA). Zidovudine (azidothymidine, AZT, Retrovir) was approved for monotherapy in 1987 as the first generally available AIDS drug, although its efficacy in that mode was shown to be only transitory (7). Another nucleoside analog AIDS drug is didanosine (dideoxyinosine, ddI, Videx), an analog of inosine, lacking both the 2'- and 3'-hydroxyl groups on its

ribose moiety. Other drugs in this category are zalcitabine (dideoxycytidine; ddC, Hivid), stavudine (d4T, 3'-deoxy-2'-thymidinene, Zerit), lamivudine (3TC, 3'-thia-2', 3'-dideoxycytidine, Epivir), and abacavir (Ziagen). Several combinations of these drugs have also been introduced. The most recently introduced drug is tenofovir (Viread), an analog of a nucleotide rather than a nucleoside.

A completely distinct class of RT-directed drugs includes compounds called non-nucleoside inhibitors (NNI's). These molecules bind in a pocket which is induced by them in the vicinity of the active site in HIV-1 RT, but which cannot be created in HIV-2 RT, thus they are potent inhibitors of HIV-1 RT, but not of HIV-2 RT. The first of these chemically very different compounds, HEPT (8) and TIBO (9), were discovered to be active in cell culture before their target was identified. A number of other members of this class, including the approved drug nevirapine (Viramune), were identified in screening programs specifically targeting HIV-1 RT (10). The two other approved drugs in this category are delaviridine (Rescriptor) and efavirenz (Sustiva). The NNI's bind in a pocket located about 10 Å from the polymerase active site (Fig. 1). When bound into that pocket in HIV-1 RT, most NNI's maintain a similar, butterfly-like shape. The mode of action of NNI's is not completely clear, although it has been suggested that they might alter the conformation of the active site due to their proximity, or else restrict the motions of the p66 thumb domain (5). The ongoing structural studies of the mode of binding of NNI's to RT should lead to the development of more and better drugs.

Figure 1.
Backbone tracing of the HIV-1 RT molecule. The enzyme consists of two domains: the larger p66 (gray) and the smaller p51 (black). Both domains are products of the same gene, with p66 containing an additional subdomain with the RNase H functionality and the sole polymerase active site. The side chains of the polymerase active site residues are shown explicitly.

Even now, only fragmentary structural data have been described for IN and no structure of the complete three-domain enzyme is available, although a number of structures of one- and two-domain constructs (Fig. 2) have been reported (11-15). Although extensive efforts in discovery of IN inhibitors have been going on for a number of years (16), only two compounds are apparently in early phase I clinical trials that have not been widely publicized.
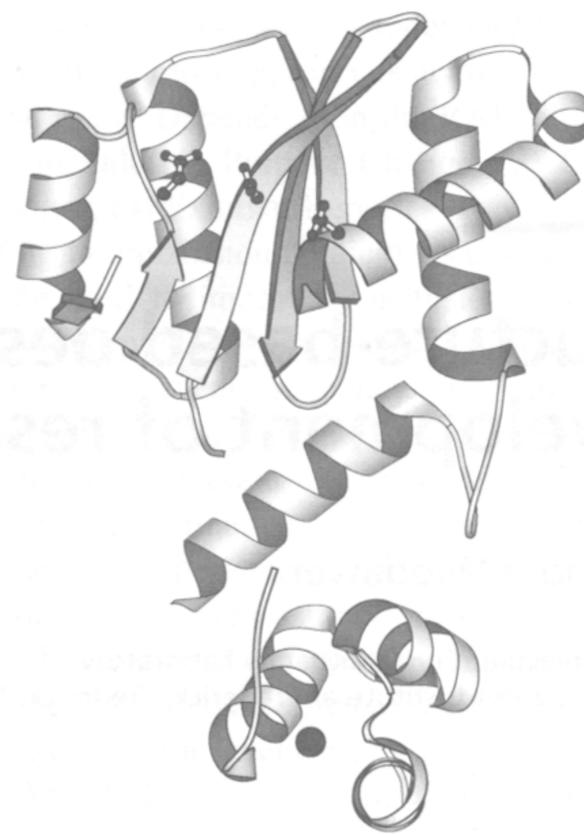

Figure 2.
The structure of a two-domain construct of HIV-1 IN (15). This fragment of the enzyme contains the N-terminal, DNA-binding domain and the catalytic domain. Three residues in the active site of the catalytic domain and the bound Zn cofactor of the N-terminal domain are shown in ball-and-stick representation.

The most successful category of AIDS drugs is the protease inhibitors. Retroviral protease was identified as a potential target very early (17, 18). Inactivation of HIV-1 PR by either mutation or chemical inhibition leads to the production of immature, non-infectious viral particles (18, 19). Thus, the function of PR was shown to be essential for proper virion assembly and maturation. For such reasons, HIV-1 PR became an important target for drug design and, since 1995, six drugs targeting this enzyme have been approved in the United States, with more certain to come.

The availability of crystal structures of HIV-1 PR was one of the important reasons for the rapid progress in drug development. The structure of the uninhibited enzyme was determined independently in several laboratories (20-22). Crystal structures of HIV-2 PR (23, 24) and simian immunodeficiency virus (SIV) PR (25, 26) also became available. Retroviral enzymes were shown to be aspartic proteases related to cellular enzymes such as pepsin (27) and consisting of two similar domains, but homodimeric rather than monomeric. Since the first structure of HIV-1 PR complexed to a peptidomimetic inhibitor was published (28), many structures of complexes of HIV-1 (Fig. 3), HIV-2, and SIV PRs with inhibitors have been reported in a number of crystal forms, some of them at a resolution as high as 1.55 Å (29). More than 230 structures of the complexes of HIV-1, HIV-2, and SIV PRs with inhibitors are publicly available in an Internet-accessible database (30). The structures of inhibitor complexes of HIV-1 PR have also been studied

using NMR (31-34). A combination of NMR with computational methods confirmed that a combination of computational models and simulations, together with NMR data, could provide a basis for further modification and design (35).

Figure 3.
Crystal structure of HIV-1 PR complexed with saquinavir (in stick representation). Selected residues in the vicinity of the active site that can mutate under drug pressure and lead to resistance are shown in ball-and-stick.

A number of inhibitors of HIV-1 PR have already been developed as anti-AIDS drugs and clinical trials of additional ones are still under way (36). The first PR inhibitor to reach clinical use was saquinavir, developed by Hoffmann-La Roche and approved by FDA in 1995. Its development provides a good example of a combination of rational drug design with traditional pharmacology. The design of saquinavir was accomplished in a rational drug design program initiated with peptide derivatives that were transition-state mimics (37). The basic design criterion relied on the observation that HIV-1 PR cleaves the sequences containing dipeptides Tyr-Pro or Phe-Pro. Mammalian aspartic proteases do not cleave peptide bonds followed by a proline, thus this target promised selectivity. Reduced amides and hydroxyethylamine isosteres most readily accommodate the imino acid moiety and, for that reason, they were chosen for further studies. A peptidic inhibitor, Ro 31-8558, was studied crystallographically in complex with HIV-1 PR and showed the expected mode of binding, as well as suggested possible future modifications (38). Replacement of a proline at P1' subsite by (S,S,S)-decahydro-isoquinoline-3-carbonyl (DIQ) significantly improved the potency of the inhibitors. A compound that included such a modification (Ro 31-8959, saquinavir) (37) was shown to be highly selective, causing only minor inhibition of human aspartic proteases. Crystallographic study of saquinavir has shown that the carbonyl of the DIQ group is able to maintain the hydrogen bond between the water molecule connecting the inhibitor with the flap regions (39). Saquinavir is available in two forms, as Invirase in hard-gel formulation, and as Fortovase in soft-gel capsules. The latter formulation increased considerably the bioavailability of the drug.

Other inhibitors of HIV-1 protease that have been approved for clinical use are ritonavir (ABT-538, Norvir), indinavir (MK-639, L-735,524, Crixivan), nelfinavir (AG-1343, Viracept), amprenavir (VX-478, 141W94, Agenerase), and Kaletra [a formulation of lopinavir (ABT-378) and ritonavir]. A number of other inhibitors are under development and in clinical trials, some of them quite advanced.

In current medical practice, monotherapy of AIDS is very rare, with the exception of its use for the prevention of mother-to-child infection, mainly in Africa. The treatment of choice is highly active antiretroviral therapy (HAART), a procedure that combines as many as 3-4 drugs given simultaneously or sequentially. Despite significant progress achieved in the treatment of AIDS with the drugs discussed above (at least in developed countries that can afford the high cost of such treatment), the development of drug resistance (that made HAART necessary in the first place) provides a new challenge in dealing with this disease. Due to the lack of an editing function in retroviral RT, transcription errors during nucleic acid replication are very common, and the viral pool contains species with all conceivable mutations. The presence of drugs provides a powerful selection pressure for virus modifications that produce lowered susceptibility to such compounds and more than 200 distinct mutations have already been described (40, 41). Development of resistance is often observed very soon after the initiation of therapy, sometimes as soon as after only a week or two.

While nucleoside inhibitors of RT have been in clinical use for almost 15 years, the mechanism of resistance to them has been elucidated only recently (42). The structure of a trapped catalytic complex of RT provided data on the exact location of the incoming deoxynucleoside triphosphate and, by extension, of the drugs. Not surprisingly, residues mutated in drug-resistant RT are located in the vicinity of the active site of the enzyme. An analysis of the steric nature of these mutations can also explain why resistance to one class of inhibitors may sensitize the enzyme to another class, forming the basis of sequential therapy. The emergence of resistance to NNIs is unusually rapid since the binding site for these compounds in not a direct part of the active site of RT (43). As a rule, mutations leading to resistance to NNIs involve residues lining the binding site of these inhibitors. However, it is now clear that resistance can be minimized, both by using NNIs in combination with other inhibitors and by starting therapy with high concentrations of the drugs.

A study of mutations resulting from the use of protease inhibitors has shown that about one third of the residues in HIV-1 PR have been found to be mutated in samples obtained after application of 27 drugs (41). While some of these mutations are in the pocket directly adjacent to the inhibitors (Fig. 3), other mutations are observed throughout the protein. The appearance of the mutations is usually sequential and remote mutations usually develop subsequently to the primary ones. The discovery of a pattern of multiple resistance mutations in patients subjected to indinavir monotherapy (44), as well as cross-resistance with

six other PR inhibitors, has raised serious questions about the possible efficacy of the drugs belonging to that category. This initial pessimism, however, has turned out to be unwarranted, since the use of sufficiently high doses of the drugs, and combination therapies in the HAART mode, have been shown to be quite successful in delaying or overcoming the appearance of resistance. The importance of the appearance of drug-resistant mutants of HIV PR is considered to be so high that resistance studies now precede any attempts of introducing such compounds into clinical practice, as was recently described for lopinavir (45). Despite all the precautions, drug-resistant strains of the virus can be detected within 18 months of initiation of therapy in almost half of the patients receiving PR inhibitors (46).

In developed countries, drug treatment is now reducing AIDS to a serious, nevertheless manageable and treatable long-term disease. However, even with all the drugs already on the market, it is clear that the long-term nature of the AIDS pandemic that has already claimed more than 20 million lives, as well as the limitations of the therapies, will make it necessary to continue the process of drug development. Until a safe, effective vaccine against HIV is found, it will be necessary to introduce new therapies and combinations of drugs to counteract the development of resistant variants. The understanding of drug-target interactions at the molecular level and extensive studies using the techniques of molecular biology are of great help in achieving rapid success in this area of drug development.

References

1. Appelt, K., Bacquet, R. J., Bartlett, C. A., Booth, C. L., Freer, S. T., Fuhry, M. A., Gehring, M. R., Herrmann, S. M., Howland, E. F., and Janson, C. A. (1991) J. Med. Chem. 34, 1925-1934
2. Klebe, G. (2000) J. Mol. Med. 78, 269-281
3. Leach, A.R. (1996) Molecular Modelling:Principles and Applications, Longman Publishers Ltd., Singapore
4. Balkenkohl, F., Von Dem Bussche-Hunnefield, C., Lansky, A., and Zechel, C. (1996) Angew. Chem. Int. 35, 2288-2337
5. Kohlstaedt, L. A., Wang, J., Friedman, J. M., Rice, P. A., and Steitz, T. A. (1992) Science 256, 1783-1790
6. Jacobo-Molina, A., Ding, J., Nanni, R. G., Clark, A. D., Jr., Lu, X., Tantillo, C., Williams, R. L., Kamer, G., Ferris, A. L., and Clark, P. (1993) Proc. Natl. Acad. Sci. USA 90, 6320-6324
7. Volberding, P. A., Lagakos, S. W., Koch, M. A., Pettinelli, C., Myers, M. W., Booth, D. K., Balfour, H. H. J., Reichman, R. C., Bartlett, J. A., and Hirsch, M. S. (1990) N. Engl. J. Med. 322, 941-949
8. Baba, M., Tanaka, H., De Clercq, E., Pauwels, R., Balzarini, J., Schols, D., Nakashima, H., Perno, C. F., Walker, R. T., and Miyasaka, T. (1989) Biochem. Biophys. Res. Commun. 165, 1375-1381
9. Pauwels, R., Andries, K., Desmyter, J., Schols, D., Kukla, M. J., Breslin, H. J., Raeymaeckers, A., Van Gelder, J., Woestenborghs, R., and Heykants, J. (1990) Nature 343, 470-474
10. Merluzzi, V. J., Hargrave, K. D., Labadia, M., Grozinger, K., Skoog, M., Wu, J. C., Shih, C. K., Eckner, K., Hattox, S., and Adams, J. (1990) Science 250, 1411-1413
11. Dyda, F., Hickman, A. B., Jenkins, T. M., Engelman, A., Craigie, R., and Davies, D. R. (1994) Science 266, 1981-1986
12. Bujacz, G., Alexandratos, J., Zhou-Liu, Q., Clement-Mella, C., and Wlodawer, A. (1996) FEBS Lett. 398, 175-178
13. Maignan, S., Guilloteau, J. P., Zhou-Liu, Q., Clement-Mella, C., and Mikol, V. (1998) J. Mol. Biol. 282, 359-368
14. Chen, J. C., Krucinski, J., Miercke, L. J., Finer-Moore, J. S., Tang, A. H., Leavitt, A. D., and Stroud, R. M. (2000) Proc. Natl. Acad. Sci. USA 97, 8233-8238
15. Wang, J. Y., Ling, H., Yang, W., and Craigie, R. (2001) EMBO J. 20, 7333-7343
16. Pommier, Y., Pilon, A. A., Bajaj, K., Mazumder, A., and Neamati, N. (1997) Antivir. Chem. Chemother. 8, 463-483
17. Katoh, I., Yasunaga, T., Ikawa, Y., and Yoshinaka, Y. (1987) Nature 329, 654-656
18. Kohl, N. E., Emini, E. A., Schleif, W. A., Davis, L. J., Heimbach, J. C., Dixon, R. A., Scolnick, E. M., and Sigal, I. S. (1988) Proc. Natl. Acad. Sci. USA 85, 4686-4690
19. Seelmeier, S., Schmidt, H., Turk, V., and von der Helm, K. (1988) Proc. Natl. Acad. Sci. USA 85, 6612-6616
20. Navia, M. A., Fitzgerald, P. M., McKeever, B. M., Leu, C. T., Heimbach, J. C., Herber, W. K., Sigal, I. S., Darke, P. L., and Springer, J. P. (1989) Nature 337, 615-620
21. Wlodawer, A., Miller, M., Jaskólski, M., Sathyanarayana, B. K., Baldwin, E., Weber, I. T., Selk, L. M., Clawson, L., Schneider, J., and Kent, S. B. H. (1989) Science 245, 616-621
22. Lapatto, R., Blundell, T., Hemmings, A., Overington, J., Wilderspin, A. F., Wood, S., Merson, J. R., Whittle, P. J., Danley, D. E., Geoghegan, K. F., Hawrylik, S. J., Lee, S. E., Scheld, K. G., and Hobart, P. M. (1989) Nature 342, 299-302
23. Tong, L., Pav, S., Pargellis, C., Do, F., Lamarre, D., and Anderson, P. C. (1993) Proc. Natl. Acad. Sci. USA 90, 8387-8391
24. Mulichak, A. M., Hui, J. O., Tomasselli, A. G., Heinrikson, R. L., Curry, K. A., Tomich, C. S., Thaisrivongs, S., Sawyer, T. K., and Watenpaugh, K. D. (1993) J. Biol. Chem. 268, 13103-13109
25. Zhao, B., Winborne, E., Minnich, M. D., Culp, J. S., Debouck, C., and Abdel-Meguid, S. S. (1993) Biochemistry 32, 13054-13060
26. Rose, R. B., Rose, J. R., Salto, R., Craik, C. S., and Stroud, R. M. (1993) Biochemistry 32, 12498-12507
27. Davies, D. R. (1990) Annu. Rev. Biophys. Biophys. Chem. 19, 189-215
28. Miller, M., Schneider, J., Sathyanarayana, B. K., Toth, M. V., Marshall, G. R., Clawson, L., Selk, L., Kent, S. B. H., and Wlodawer, A. (1989) Science 246, 1149-1152
29. Mahalingam, B., Louis, J. M., Reed, C. C., Adomat, J. M., Krouse, J., Wang, Y. F., Harrison, R. W., and Weber, I. T. (1999) Eur. J. Biochem. 263, 238-245
30. Vondrasek, J., van Buskirk, C. P., and Wlodawer, A. (1997) Nature Struct. Biol. 4, 8
31. Wonacott, A., Cooke, R., Hayes, F. R., Hann, M. M., Jhoti, H., McMeekin, P., Mistry, A., Murray-Rust, P., Singh, O. M., and Weir, M. P. (1993) J. Med. Chem. 36, 3113-3119
32. Jhoti, H., Singh, O. M., Weir, M. P., Cooke, R., Murray-Rust, P., and Wonacott, A. (1994) Biochemistry 33, 8417-8427
33. Yamazaki, T., Nicholson, L. K., Torchia, D. A., Stahl, S. J., Kaufman, J. D., Wingfield, P. T., Domaille, P. J., and Campbell-Burk, S. (1994) Eur. J. Biochem. 219, 707-712
34. Ohno, Y., Kiso, Y., and Kobayashi, Y. (1996) Bioorg. Med. Chem. 4, 1565-1572
35. Podlogar, B. L., Farr, R. A., Friedrich, D., Tarnus, C., Huber, E. W., Cregge, R. J., and Schirlin, D. (1994) J. Med. Chem. 37, 3684-3692
36. Tavel, J. A. (2000) Expert. Opin. Investig. Drugs 9, 917-928
37. 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., and Machin, P. J. (1990) Science 248, 358-361
38. Graves, B. J., Hatada, M. H., Miller, J. K., Graves, M. C., Roy, S., Cook, C. M., Krohn, A., Martin, J. A., and Roberts, N. A. (1991) Adv. Exp. Med. Biol. 306, 455-460
39. Krohn, A., Redshaw, S., Ritchie, J. C., Graves, B. J., and Hatada, M. H. (1991) J. Med. Chem. 34, 3340-3342
40. Schinazi, R. F., Larder, B. A., and Mellors, J. W. (1997) Int. Antiviral News 5, 129-142
41. Schinazi, R. F., Larder, B. A., and Mellors, J. W. (2000) Int. Antiviral News 8, 65-91
42. Huang, H., Chopra, R., Verdine, G. L., and Harrison, S. C. (1998) Science 282, 1669-1675
43. De Clercq, E. (1998) Antiviral Res. 38, 153-179
44. Condra, J. H., Schleif, W. A., Blahy, O. M., Gabryelski, L. J., Graham, D. J., Quintero, J. C., Rhodes, A., Robbins, H. L., Roth, E., Shivaprakash, M., Titus, D., Yang, T., Teppler, H., Squires, K. E., Deutsch, P. J., and Emini, E. A. (1995) Nature 374, 569-571
45. Carrillo, A., Stewart, K. D., Sham, H. L., Norbeck, D. W., Kohlbrenner, W. E., Leonard, J. M., Kempf, D. J., and Molla, A. (1998) J. Virol. 72, 7532-7541
46. Romano, L., Venturi, G., Giomi, S., Pippi, L., Valensin, P. E., and Zazzi, M. (2002) J. Med. Virol. 66, 143-150