The human immunodeficiency virus type-1 (HIV-1) transframe protein p6* is located between the structural and enzymatic domains of the Gag-Pol polyprotein, flanked by the nucleocapsid (NC) and the protease (PR) domain at its amino and carboxyl termini, respectively. Here, we report that recombinant highly purified HIV-1 p6* specifically inhibits mature HIV-1 PR activity. Kinetic analyses and cross-linking experiments revealed a competitive mechanism for PR inhibition by p6*. We further demonstrate that the four carboxyl-terminal residues of p6* are essential but not sufficient for p6*-mediated inhibition of PR activity. Based on these results, we suggest a role of the transframe protein p6* in regulating HIV-1 PR activity during viral replication.

Translation of human immunodeficiency virus type-1 (HIV-1) genetic RNA results in the production of two polypeptide precursors, Gag and Gag-Pol (reviewed in Ref. 1). The 55-kDa Gag precursor contains the structural proteins matrix (MA), capsid (CA), and nucleocapsid (NC), in addition to the p2, p1, and p6 domains (MA-CA-p2-NC-p1-p6) (2). The Gag-Pol polyprotein is generated by a −1 ribosomal frameshift at the NC-p1 junction, occurring with a frequency of about 5% of all translational events (3, 4). The 160-kDa Gag-Pol polyprotein therefore consists of the gag products MA, CA, p2, and NC, followed by the pol-encoded transframe protein p6* and the viral enzymes protease (PR), reverse transcriptase, and integrase.

Gag and Gag-Pol polyproteins are transported to the plasma membrane where assembly of type-C retroviruses and lentiviruses typically occurs (5, 6). During particle assembly, the viral PR cleaves the Gag and Gag-Pol precursors into the structural and functional proteins required for viral replication (7). Although PR activity has been observed within the cytoplasm of infected cells, the cleavages that lead to the mature proteins incorporated into virions are late events, probably occurring in the last stages of budding as the virion is being readied for release from the cell (Ref. 8; reviewed in Ref. 9).

The mature HIV-1 PR is an obligate dimer of identical 11-kDa subunits, each contributing one of the two catalytic aspartic residues. In contrast, the cell-derived members of the aspartic PR family are monomeric enzymes with two Asp-Thr-Gly-containing domains (10–13). The unique dimeric structure of retroviral PRs is mainly stabilized by an antiparallel β-sheet formed by the interdigitation of the amino- and carboxyl-terminal β-strands of each monomer (14).

The activation of HIV-1 PR, i.e. the dimerization and autocatalytic release from Gag-Pol, is a critical step in the viral life cycle. Premature processing by a PR activated too early results in separation of Gag and Gag-Pol domains from critical transport signals and prevents particle assembly and release, whereas inhibition of PR activation causes a severe defect in Gag polyprotein processing and a complete loss of viral infectivity (15–20). However, the molecular mechanisms leading to PR activation are currently unknown. The 68-amino acid transframe protein p6* encoded by the pol open reading frame directly upstream of the PR region has not been ascribed a specific function and is in a position corresponding to that of the prosegment observed in other aspartic PRs. Based on this analogy, it has been suggested that the p6* region regulates HIV-1 PR activity in a manner similar to the pepsinogen propeptide and that autocatalytic release of PR from p6* may be a triggering event in HIV polyprotein processing (21, 22).

Support for this hypothesis has primarily been drawn from the observation that deletion of the p6* region in a Gag-PR precursor led to enhanced polyprotein processing in an in vitro translation system (21).

In the present study, we have analyzed the interaction of HIV-1 PR and the viral transframe protein p6* in vitro using recombinant highly purified proteins. We report that p6* specifically inhibits mature HIV-1 PR activity. We have studied the mechanism of inhibition and mapped the region in p6* responsible for PR regulation. Based upon our results, we suggest a mechanism for PR activation different from that of zymogen conversion, in which the carboxyl-terminal residues of the transframe protein p6* block the substrate binding cleft of HIV-1 PR after amino-terminal autoprocessing of the viral enzyme.

EXPERIMENTAL PROCEDURES

Proteins and Peptides—HIV-1 PR was expressed and purified according to Ref. 23. Enzyme concentrations were determined by active-site titration (24) using inhibitory Ro 31-8599 (25) kindly provided by Roche Products Ltd. The recombinant p6, p6*M1, p6*M2, p6*M3, and p6*M4 proteins were prepared essentially as described earlier for wild-type p6* (26). Briefly, proteins were expressed in Escherichia coli M15[pREP4] as fusion proteins with glutathione S-transferase using the pGEX vector system (Amersham Pharmacia Biotech). After cell breakage the soluble part of the glutathione S-transferase fusion proteins was affinity-purified using glutathione-Sepharose matrix. Afterward p6 and p6* proteins were removed from the matrix by thrombin digestion. The isolated proteins were further purified by size exclusion chromatography and concentrated by ultrafiltration. The purification steps were monitored by SDS-polyacrylamide gel electrophoresis (27), followed by silver staining of the proteins and by Western blot analysis with polyclonal rabbit antisera raised against purified glutathione S-
transferrase fusion proteins. Protein concentrations were calculated from absorption spectra according to Gill and von Hippel (28). The chromogenic substrate Lys-Ala-Arg-Val-Nle-Phe(p-NO2)-Glu-Ala-Nle-\( \text{NH}_2 \), initially described by Richards et al. (29), and the unmodified tetrapeptide Ser-Phe-Aan-Phe-COOH were purchased from Bachem and Dr. G. Arnold (Gentzentrum München, Germany), respectively. The chemical composition and purity of synthetic oligopeptides were confirmed by mass spectrometry.

**PR Inhibition Assay**—The ability of recombinant proteins and the tetrapeptide Ser-Phe-Aan-Phe-COOH to inhibit PR activity was determined in a continuous spectrophotometric assay by monitoring the decrease in absorbance at 295 nm associated with the hydrolysis of the chromogenic substrate Lys-Ala-Arg-Val-Nle-Phe(p-NO2)-Glu-Ala-Nle-\( \text{NH}_2 \). Measurements were carried out on an UVikon 930 spectrophotometer (Kontron Instruments) equipped with a stirring device and a thermostatted cell holder maintained at 25 °C using a quartz cuvette with a 9.5-mm path length and a final volume of 1.8 ml. Assays were conducted at pH 5.0, in 0.1 M sodium acetate, 4 mM EDTA, and 5 mM dithiothreitol. Reactions were initiated by adding enzyme to a final concentration of 22 nM active dimer, and progress curves were recorded for 4 min, thereby collecting 600 data points. The initial rate was determined as the slope of the absorbance change during the linear phase of the reaction (up to 2 min) using the software package of the instrument. The protein or peptide concentration that inhibited substrate cleavage by 50% was designated the IC\(_{50}\).

**Cross-linking of HIV-1 PR**—Cross-linking reactions involved preincubating PR (100 nM total dimer) for 40 min at 25 °C with or without p6* or p6\(^*\) (100 \( \mu \text{M} \)) in 100 \( \mu \text{l} \) of buffer consisting of 20 mM sodium phosphate, pH 7.5, and 1 mM dithiothreitol. The homobifunctional cross-linker disuccinimidyl suberate (DSS, spacer arm length 11.4 Å) was obtained from Pierce and dissolved in dimethyl sulfoxide, which was added at 1% of the reaction volume, to give a final DSS concentration of 0.24 mM. Control samples received solvent only. Samples were reacted for 2 min, quenched with 40 mM Tris, and the proteins were precipitated by adding 10% trichloroacetic acid and 0.5 mg/ml sodium deoxycholate. The pellets were washed with ice-cold acetone and air-dried. The proteins were resolved on 16% SDS-polyacrylamide gels (30), electroblotted onto nitrocellulose, and probed with polyclonal antiserum ARP413 specific for HIV-1 PR obtained from the Medical Research Council AIDS Directed Program Reagent Project. The bands were visualized by enhanced chemiluminescence as recommended by the manufacturer (Amersham Pharmacia Biotech). Autoradiograms were scanned and cropped with Adobe Photoshop. Reaction products were quantitated with the Matrix program (QuantaVision, Canada) from the generated TIFF file.

**RESULTS**

**Recombinant p6\(^*\) Inhibits Mature HIV-1 PR Activity**—To investigate a possible regulatory function of the transframe protein p6\(^*\) on mature HIV-1 PR activity, both proteins were produced in bacterial expression systems and purified to homogeneity. The effect of p6\(^*\) on PR activity was then tested in a continuous enzymatic assay by monitoring the absorption change associated with the cleavage of a chromogenic substrate. The comparison of initial reaction velocities in the presence and absence of p6\(^*\) revealed that the Gag-Pol transframe protein inhibits mature HIV-1 PR in a concentration-dependent manner. The inhibition yielded a linear Dixon plot, and the IC\(_{50}\) value was determined to be 13 \( \mu \text{M} \). In contrast, the equally sized HIV-1 Gag protein p6, which was produced and purified in the same way, did not significantly alter proteolytic activity (Fig. 1).

**Determination of the Inhibition Mechanism**—Due to the obligatory dimeric structure of the HIV-1 PR, there are at least two possibilities for an inhibitor (1) to influence PR activity. It can either bind to active dimers (D), thereby competing with substrate (5) binding, or it can bind to inactive monomers (M) thereby preventing dimerization (Scheme 1). Given an earlier report that the carboxyl-terminal hexapeptide of p6\(^*\) acts as a dimerization inhibitor of HIV-1 PR (31), we questioned whether full-length p6\(^*\) protein also inhibits PR activity by interfering with dimerization. Since PR inhibition assays were started with highly concentrated dimeric PR and dimerization inhibitors bind exclusively to PR monomers, p6\(^*\) can only act as a dimerization inhibitor if significant dimer dissociation occurs during data collection. To test the dimer stability under our experimental conditions, we determined the time-dependent PR inactivation caused by dimer dissociation during preincubation in assay buffer. A plot of remaining PR activity versus preincubation time fits a first-order exponential and yields an activity decay rate of 5.84 h\(^{-1}\) (Fig. 2A). Analysis of the preincubation mixture by Western blot showed that the loss of activity after 60 min does not correlate with the appearance of truncated forms of the PR (Fig. 2A, top). Due to the slow dimer dissociation under our experimental conditions, it seems unlikely that p6\(^*\) has bound to PR monomers and prevented their dimerization.

To further support this conclusion, we next studied the effect of p6\(^*\) on the time-dependent inactivation of HIV-1 PR following dilution. For these experiments the enzyme was preincubated in the presence of either p6\(^*\) or control protein p6, and the activity as a function of time was examined. As shown in Fig. 2A, presence of p6\(^*\) decreased the rate of HIV-1 PR inactivation, while p6 had no significant effect on the kinetics of PR activity decrease. The finding that presence of p6\(^*\) obviously results in a specific stabilization of the HIV-1 PR dimer suggested that p6\(^*\) might bind to the active-site of the enzyme.

This hypothesis was further tested in cross-linking reactions of HIV-1 PR. As both subunits of retroviral PRs equally contribute to the substrate binding region an inhibitor interacting with the active site should be able to shift the PR monomer/dimer equilibrium to the dimer form. In accordance with the kinetic data the presence of p6\(^*\) in cross-linking reactions resulted in a stabilization of the PR dimer (Fig. 2B, lane 4), whereas the control protein p6 had no influence on the PR monomer/dimer equilibrium (Fig. 2B, lane 3). Other control polypeptides unrelated to HIV-1 but of similar size as the p6\(^*\) protein, like lysozyme and aprotinin, were also found to be without effect on the amount of dimer (data not shown). The additional band observed in lanes 2–4 has probably resulted from autodegradation of PR between amino acids Leu\(^5\) and Trp\(^8\) (32). Densitometric analyses of three such experiments revealed that p6\(^*\) increased the relative amount of dimer by 12%, while presence of p6 had no significant effect on the relative amount of dimer detected (Fig. 2B, top).

![Fig. 1. Dixon plot showing inhibition of mature HIV-1 PR activity by p6\(^*\).](image-url)
The type of inhibition of HIV-1 PR by p6* was further characterized by steady-state kinetic analysis. HIV-1 PR activity was measured with substrate concentrations of 10–50 μM and 0, 5, or 10 μM p6*. Fig. 2C shows double-reciprocal (Lineweaver-Burk) plots of the initial reaction rate versus concentration of substrate. The lowest line in Fig. 2C represents the results obtained in the absence of p6*. Increasing concentrations of p6* affected only the $K_m$ and not the $V_{max}$ value, leading to a single intercept of the linear regression lines on the velocity axis of the Lineweaver-Burk plot. The slopes of the lines in Fig. 2C were replotted against the concentration of p6* and the result is shown in the inset of Fig. 2C. The linearity of this replot is indicative of simple competitive inhibition.

Carboxyl-terminal Residues of p6* Are Essential for PR Inhibition—The HIV-1 transface protein p6* spans the sequence of two PR half-substrates (NC-p6* and p6*-PR) and contains an internal PR cleavage site between Phe$^8$ and Leu$^9$ (33, 34). To map the region in p6* responsible for competitive inhibition of HIV-1 PR, we analyzed the inhibitory potency of p6* deletion mutants lacking either the internal PR cleavage site spanning amino acids 5–12 (p6*M1) or the four carboxyl-terminal residues that are part of the p6*-PR cleavage site sequence (p6*M2). These experiments revealed that p6* deletions affecting the PR cleavage site between Phe$^8$ and Leu$^9$ did not significantly impair PR inhibition compared with wild-type p6*, whereas the p6* mutant missing the carboxyl-terminal cleavage site residues exhibited virtually no inhibitory potential (Fig. 3A). Thus, we conclude that p6*-induced PR inhibition is dependent on the carboxyl-terminal cleavage site residues Ser$^{65}$-Phe$^{66}$-Asn$^{67}$-Phe$^{68}$.

To examine whether the inhibitory effect of wild-type p6* is distinguishable from PR inhibition by Ser-Phe-Asn-Phe, we analyzed the influence of a synthetic oligopeptide corresponding to positions P4–P1 of the p6*-PR cleavage site (nomenclature in accordance with Ref. 35). These experiments revealed that the tetrapeptide Ser-Phe-Asn-Phe-COOH alone is a rather poor inhibitor of HIV-1 PR activity (Fig. 3A, inset). The IC$_{50}$ value of 581 μM for this inhibition is almost 45-fold higher than that for PR inhibition by full-length p6*. Thus, the four carboxyl-terminal residues of p6* are essential for blocking the PR cleavage site.

![Fig. 2. Analysis of the inhibition mechanism. A, effect of p6* on time-dependent inactivation of HIV-1 PR due to dimer dissociation during preincubation in assay buffer. An aliquot of the enzyme stock solution was diluted into the cuvette containing assay buffer without (white circle) or with 5 μM p6* (black circle) or p6 (gray circle) to yield a final concentration of 22 nM active PR dimer. After preincubation for 0 or 60 min, PR activity was measured with 10–50 μM substrate. B, effect of p6* on cross-linking of PR subunits. PR was preincubated in the presence or absence of p6 or p6*. Preincubated samples were treated with DSS or solvent only. Precipitated proteins were resolved on a 16% protein gel and transferred to a nitrocellulose membrane. PR was visualized with polyclonal antiserum ARP413, followed by enhanced chemiluminescence. The bands representing the 22-kDa PR dimer are indicated at the left, C, effect of p6* on rate of substrate hydrolysis leading to double-reciprocal (Lineweaver-Burk) plots of initial reaction rate versus substrate concentration. The linearity of results is indicative of simple competitive inhibition. The plots represent representative of two independent experiments performed in triplicates with different p6* preparations.](image-url)
boxyl-terminal residues of p6* are essential but not sufficient for HIV-1 PR inhibition.

To further confirm the role of the carboxyl-terminal residues of p6* in PR inhibition, we tested whether the inhibitory potency of p6* can be improved by replacing the natural sequence Ser-Phe-Asn-Phe by Ser-Tyr-Glu-Leu, for which molecular modeling studies predicted stronger interactions with the substrate binding cleft of the HIV-1 PR. Indeed, the p6* mutant p6*M3 containing the carboxyl-terminal sequence Ser65-Tyr66-Glu67-Leu68 was more active in PR inhibition assays than wild-type p6* (IC50 = 2.7 μM, Fig. 3B).

In contrast, inserting the carboxyl-terminal residues Ser-Phe-Asn-Phe internally into the p6* protein resulted in a p6* mutant (p6*M4) with reduced inhibitory potency compared with wild-type p6* (Fig. 3B). Apparently, the sequence Ser-Phe-Asn-Phe must be freely accessible at the very carboxyl-terminal terminus of p6* to inhibit PR activity efficiently.

**DISCUSSION**

In this report, we show for the first time that recombinant p6* protein acts as an inhibitor of mature HIV-1 PR activity, a finding that considerably extends previous observations suggesting a potential role of the p6*-encoded transframe protein in regulating PR function (21, 31, 34–40). For comparison, neither recombinant nor synthetic p6 Gag protein affects PR activity in vitro (this study and Ref. 41). This clearly demonstrates that PR inhibition is not common to all Gag and Gag-Pol cleavage products rather than specific for p6*.

The IC50 value for HIV-1 PR inhibition by wild-type p6* in vitro is 13 μM. The binding affinity of p6* is thus very similar to that reported for the well-known inhibitor of aspartic PRs pepstatin A, which has been shown to block HIV-1 replication in cell culture when added to the culture medium in a concentration of 100 μM (42, 43). Given Gag protein concentrations >5 mM in the HIV-1 virion and a ratio of approximately 1:20 for packaging of Gag-Pol and Gag precursors, the p6* concentration in viral particles is at least 250 μM. Regarding the comparably low IC50 value, we strongly suggest an inhibitory function of p6* on PR activity also during viral replication.

The possible molecular mechanism of p6*-mediated PR regulation has been controversially discussed during recent years. There is evidence from in vitro translation experiments that p6* is wrapped around the PR in a manner similar to the pepsinogen prosegment and prevents premature precursor processing by rendering the active-site and substrate binding cleft inaccessible (21). It has been also proposed that p6* affects PR activation by interfering with dimerization of PR domains in Gag-Pol precursors (37). Blockage of the release of mature PR from a Gag-PR polypeptide by introducing mutations into the amino-terminal cleavage site of the PR domain suggested another, different mechanism of p6*-induced PR regulation. Examination of the PR species generated in this experiment after autoprocessing in E. coli revealed several extended PR forms with amino termini in p6*. These 14–18-kDa p6*-PR intermediates do not appear to be hydrolyzed readily and have been proposed to occupy the substrate binding cleft of the PR precursor, thereby delaying the extent of overall processing (38).

While the molecular details of p6*-PR interaction are difficult to explore by means of cellular systems, the use of quantitative in vitro assays for study of p6* and PR as purified proteins allowed us to carefully analyze the molecular mechanism of p6*-mediated PR inhibition. Our data demonstrate that p6* is a competitive inhibitor of mature HIV-1 PR activity and that PR regulation is dependent on carboxyl-terminal residues Ser65-Phe66-Asn67-Phe68 of p6*. By contrast, Schramm and colleagues (31) have reported that synthetic hexapeptide Thr-Val-Ser-Phe-Asn-Phe, which corresponds to p6* residues 63–68, is a dimerization inhibitor of HIV-1 PR activity in vitro. In this study a rapid equilibrium assumption for PR dissocia-
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REFERENCES

1. Vaishnav, Y. N., and Wong-Staal, F. (1991) Annu. Rev. Biochem. 60, 577–630.

2. Henderson, L. E., Bowers, M. A., Sowder, R. C., Serabyn, S. A., Johnson, D. G., Bess, J. W., Jr., Arthur, L. O., Bryant, D. K., and Fenselau, C. (1992) J. Virol. 66, 1856–1865.

3. Jacks, T., Power, M. D., Masiarz, F. R., Luciw, P. A., Barr, P. J., and Varmus, H. E. (1988) Nature 331, 280–283.

4. Wilson, W., Braddock, M., Ruby, J. F., Rathjen, P. D., Kingsman, S. M. and Kingsman, A. J. (1988) Cell 55, 1159–1169.

5. Gelderblom, H. R. (1991) AIDS 5, 617–638.

6. Wills, J. W., and Craven, R. C. (1991) AIDS 5, 639–654.

7. Kobil, N. W. E., Emini, E. A., Schlenf, W. A., Davis, L. J., Heimbach, J. C., Dixon, R. A., Scnlock, E. M., and Sigal, I. S. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4686–4690.

8. Kaplan, A. H., and Swanstrom, R. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4528–4532.

9. Vogt, V. M. (1996) Curr. Top. Microbiol. Immunol. 214, 95–131.

10. Pearl, L. H., and Taylor, W. R. (1987) Nature 329, 351–354.

11. Meek, T. D., Dayton, B. D., Metcalf, B. W., Dreyer, G. B., Strickler, J. E., Gorniak, J. G., Rosenberg, M., Moore, M. L., Maagaard, V. W., and Debouck, C. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 1841–1845.

12. Toh, H., Ono, M., Saigo, K., and Miyata, T. (1985) Nature 315, 691–692.

13. Schneider, J., and Kent, S. B. H. (1988) Cell 54, 363–368.

14. Wlodawer, A., Miller, M., Jaskolski, M., Sathyanarayana, B. K., Baldwin, E., Weber, I. T., Selk, L. M., Clawson, L., Schneider, J., and Kent, S. B. H. (1989) Science 245, 615–621.

15. Krausslich, H.-G. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3213–3217.

16. Krausslich, H.-G. (1992) J. Virol. 66, 567–572.

17. Mozfarrer, K., Packer, M., and Brinkmann, V., Gelderbloom, H. R. and Krausslich, H.-G. (1992) Virology 186, 25–39.

18. Park, J., and Mower, C. D. (1991) J. Virol. 65, 5111–5117.

19. Karacostas, V., Wolfe, E., J., Nagashima, K., Gonda, M. A., and Moss, B. (1993) Virology 193, 661–671.

20. Tessmer, U., and Krausslich, H.-G. (1998) J. Virol. 72, 3459–3463.

21. Partin, K., Zyharch, G., Ehrlich, L., DeCroonbrugghe, M., Winnen, E., and Carter, C. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4776–4780.

22. Louis, J. M., Nashed, N. T., Parris, K. D., Kimmel, A. R., and Jerina, D. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7970–7974.

23. Konvalinka, J., Litterst, M. A., Welker, H., Heuser, A.-M., and Krausslich, H.-G. (1995) J. Virol. 69, 7180–7186.

24. Tomasselli, A. G., Olsen, M. K., Hui, J. O., Staples, D. J., Sawyer, T. K., Heinrikson, R. L., and Tomich, C.-S. C. (1996) Biochemistry 25, 6074–6083.

25. Roberts, N. A., Martin, J. A., Kinchington, D., Broadhurst, A. V., Craig, J. C., Heinrikson, R. L., and Tomich, C.-S. C. (1990) Biochemistry 29, 11939–11945.

26. Schechter, I., and Berger, A. (1967) Biochemistry. Bio. Rev. 27, 157–162.

27. Schagger, H., and von Jagow, G. (1987) Anal. Biochem. 166, 368–379.

28. Gill, S. C., and von Hippel, P. H. (1989) Anal. Biochem. 182, 319–326.

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

30. Laemmli, U. K. (1970) Nature 227, 680–685.

31. Schramm, H., Billic, A., Jaeger, E., Runge, K., P.-P., Arnold, G., and Schramm, W. (1993) Biochemistry. Biois. Res. Commun. 194, 595–600.

32. Rock, J. R., Salto, K., and Goe, C. K. (1980) J. Biol. Chem. 255, 11399–11405.

33. Philipp, L., Miller, J. S., Parten, B. P., Dunn, B. M., and Kay, J. (1992) FEBS Lett. 314, 449–454.

34. Almg, N., Rollet, R., Arad, G., Passi-Evin, L., Wainberg, M. A., and Koifer, M. (1990) J. Virol. 70, 7228–7233.

35. Schnechter, I., and Berger, A. (1967) Biochemistry. Biois. Res. Commun. 27, 157–162.

36. Partin, K., Krausslich, H.-G., Ehrlich, L., Winmen, E., and Carter, C. (1990) J. Virol. 64, 3839–3847.

37. Zyharch, G., and Carter, C. (1995) J. Virol. 69, 3787–3884.

38. Zyharch, G., Krausslich, H.-G., Partin, K., and Carter, C. (1994) J. Virol. 68, 240–250.

39. Louis, J. M., Dyda, F., Nashed, N. T., Kimmel, A. R., and Davies, D. R. (1998) Biochemistry 37, 2105–2110.

40. Gatch, J. Arroz, J. S., and Schatten, M. G. (1998) Virology 244, 87–96.

41. Stays, D., Blaha, L., and Strop, P. (1993) Virology. Acta 1182, 157–161.

42. Darke, P. L., Leu, C.-T., Davis, L. J., Heimbach, J. C., Diehl, R. F., Hill, W. S., Dixon, R. A. F., and Sigal, I. S. (1989) J. Biol. Chem. 264, 2307–2312.

43. von der Hel, K., Guttler, L., Eberle, J., and Deinhardt, F. (1989) FEBS Lett. 247, 349–352.

44. Darke, P. L., Jordan, D. P., Hall, D. L., Auzay, J. A., Shafer, J. A., and Kus, L. C. (1994) Biochemistry 33, 98–105.

45. Dunn, B. M., Cheynin, A., Wlodawer, A., and Kay, J. (1994) Methods Enzymol. 241, 254–278.

46. Misumi, S., Kudo, A., Aruma, R., Tomonaga, M., Furushiki, K., and Shoji, S. (1997) Biochemistry. Biois. Res. Commun. 241, 275–280.

47. Pettit, S. C., Moody, M. D., Wohlie, R. S., Kaplan, A. H., Nanterme, N. V., Klein, C. A., and Swanstrom, R. (1994) J. Virol. 68, 8017–8027.

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