The nucleocapsid protein NCp7 of the human immunodeficiency virus (HIV) type 1 is important for the annealing of HIV RNA and tRNA\(^{\text{Lys}}\), the tRNA acting as a primer during reverse transcription of HIV RNA. A wild type NCp7 and a Cys\(^{23}\) mutant having a disrupted zinc finger during reverse transcription of HIV RNA. A wild that NCp7 does not induce tRNA\(^{\text{3Lys}}\) structure after binding of NCp7, which demonstrates (HIV)\(^{1}\) type 1 consists of two identical unspliced RNA joined nealing of HIV RNA and tRNA\(^{\text{3Lys}}\).

The retroviral genome of the human immunodeficiency virus (HIV)\(^{1}\) type 1 consists of two identical unspliced RNA joined near their 5'-extremities by a dimer linkage structure (1). Numerous copies of NCp7, a basic nucleocapsid protein, are found associated to the HIV RNA (1). NCp7 binds specifically to the 5'-terminal region of the HIV RNA and facilitates viral RNA dimerization and packaging (5). The nucleoprotein complex is stable in vitro due to a large number of NCp7-HIV RNA and NCp7-NCp7 interactions and protects viral RNA against RNase degradation (6).

The 5'-terminal region of the HIV RNA contains several functional sites essential for the retroviral cycle, such as the RNA transactivation response element structure involved in the activation of provirus transcription or the primer binding site (PBS) located between RNA transactivation response element and the gag initiation codon (7). Initiation of HIV genomic reverse transcription requires the presence of a specific host cell-derived tRNA\(^{\text{3Lys}}\) primer (8, 9). tRNA\(^{\text{3Lys}}\) binds HIV RNA through its 3'-terminal 18-nucleotide region, which is complementary to the PBS site and forms a complex recognized by the reverse transcriptase (10). NCp7 activates the annealing of the primer tRNA to the PBS site in vitro (11, 12). Cross-linking experiments showed that NCp7 interacts with the anticodon domain of tRNA\(^{\text{3Lys}}\) (13). However, fluorescence experiments did not identify any high affinity binding site in tRNA and suggested a nonspecific NCp7-tRNA interaction (14).

NCp7 is a 72-amino acid protein originating from the gag 3' region and conserved among retroviruses (15). NCp7 contains two copies of a conserved Cys-X\(_2\)-Cys-X\(_2\)-His-X\(_4\)-Cys zinc finger sequence flanked with basic residues (16). Both zinc finger sequences are tetra-coordinated to a Zn\(^{2+}\) ion, although their affinity for zinc differs (17). The first zinc finger is important for RNA binding since a truncated (residues 13–64) NCp7 containing both zinc fingers binds tRNA\(^{\text{3Lys}}\) or HIV RNA, whereas a Cys\(^{23}\) (13–64) NCp7 mutant containing a disrupted first zinc finger is unable to bind RNA (18). The basic residues located outside NCp7 zinc fingers are also important for the nucleic acid binding property (12, 19).

In this study, far UV circular dichroism (CD) spectra of NCp7 and Cys\(^{23}\) NCp7 were obtained in different solvents to evaluate conformational heterogeneity. We then measured the far UV CD spectra of tRNA\(^{\text{3Lys}}\), NCp7-tRNA\(^{\text{3Lys}}\), and Cys\(^{23}\) NCp7-tRNA\(^{\text{3Lys}}\) complexes. The modification of the 260-nm band in the complexes, only due to the tRNA contribution, shows that NCp7 and Cys\(^{23}\) NCp7 bind tRNA\(^{\text{3Lys}}\). However, the A-helical character of tRNA\(^{\text{3Lys}}\) is maintained within the complexes. Our study shows that NCp7 does not induce tRNA unwinding. In addition, to evaluate conformational changes into tRNA, CD spectra were measured from 5 to 80 °C. tRNA melting was observed at 80 °C, and the resulting CD spectrum differs completely from the CD spectra of complexes. The NCp7-tRNA\(^{\text{3Lys}}\) interaction was further investigated using molecular modeling according to the NMR structure of the zinc finger-Psi RNA complex (3). This model shows that binding of the zinc finger region to the anticodon loop could position the NCp7 N-terminal region into the major groove of the tRNA anticodon stem.

**MATERIALS AND METHODS**

**Molecule Synthesis**—The chemical synthesis of NCp7 and Cys\(^{23}\) NCp7 was performed by solid phase method using a Fmoc (N-(9-fluorenyl)methoxycarbonyl) chemistry and a hydroxymethylphenoxymethyl polystyrene resin with an Applied Biosystem model 431A peptide synthe- poster, assigned previously (20). Zinc was added to the protein in a 2.5 molar ratio. Synthetic tRNA\(^{\text{3Lys}}\) was obtained from in vitro transcription of BstI-linearized pTL9 with phage T7 RNA polymerase, as described previously (8). After ethanol precipitation, tRNA\(^{\text{3Lys}}\) was lyophilized and dissolved in 10% acetic acid. A Sephadex G-25 column (Pharmacia Biotech, Upsalla, Sweden) in 10% acetic acid was used to remove salt, and then tRNA was lyophilized to remove acetic acid.

**Circular Dichroism Measurements**—CD spectra were measured in 50-μm path length cells with a Jobin-Yvon (Longjumeau, France) UV CD spectrophotometer (Mark VII). Calibration of the instrument was evaluated with (+)-10-camphorsulfonic acid. A ratio of 2.2 was found between the positive CD band at 290.5 nm and the negative band at 192.5 nm. Protein CD spectra were obtained with a sample volume of 100 μl and protein concentrations of 1 mg/ml. Complex CD spectra were obtained at 5 °C with 0.7 mg/ml of tRNA\(^{\text{3Lys}}\) mixed with 0.5 mg/ml concentrations of proteins corresponding to a molar ratio of one tRNA for three NCp7. Data was collected at 0.5 nm intervals with a scan rate of 1 nm/min. Light transmission in the far UV to 178 nm was verified with the Jobin-Yvon spectrophotometer, which is able to measure absorption and CD spectra simultaneously. Absorption spectra are reported as absorption units. CD spectra are reported as Δε/amine. Temp
temperature was measured with a Haake thermal device. Analysis of CD data to evaluate secondary structure contents was performed according to the method of Manavalan and Johnson (21) using a set of 32 reference proteins. To determine the protein concentration, amino acid analyses were performed on a model 6300 Beckman analyzer according to the method of Sanger and Thompson (22).

Molecular Modeling—Models were built with Insight II, the Homology and Discover software from MSI Technologies, Inc. (San Diego, CA) on a Silicon Graphics R5000PC workstation. NCp7 and NCp7-tRNA3Lys complex structures were optimized in terms of internal energies with the consistent valence forcefield. Molecular dynamics was performed at 300 K for 1.1 ps. 110 structures were sampled and analyzed from the 1100 structures produced in the dynamic trajectory. Energy minimization was performed at pH 7 with the steepest descent and conjugate gradient algorithms, down to a maximum derivative of 0.001 kcal/Å.

RESULTS AND DISCUSSION

CD was performed on wild type NCp7 and a Cys23 mutant, with His23 in the first zinc finger replaced by a cysteine (Fig. 1).

To determine whether this mutation could affect secondary structures, CD was measured in two different solvents. In an aqueous buffer (pH 7), NCp7 and Cys23 NCp7 show similar CD spectra, characterized by a strong negative band near 200 nm, typical of random coil structures (Fig. 2A). However, minor structural changes in the mutant are revealed by the absence of a positive band near 220 nm. Structural changes are also observed after zinc depletion (data not shown). Trifluoroethanol (TFE) is a solvent that favors α-helix structures within flexible regions. This solvent was used to check if NCp7 had flexible regions and if the Cys23 mutation had an effect on the extent of these flexible regions. In 80% TFE, NCp7 and Cys23 NCp7 display a class C CD spectra typical of α-helix structures, with a positive band at 190 nm and two negative bands near 207 and 222 nm (data not shown). The percentage of secondary structures was determined from CD spectra ac-
adopt either a non-ordered structure in aqueous solvent or an
decrease of extended structures. In TFE, the

cysteine induces an increase of non-ordered structures and a
changes observed at 260 nm are due to local modifications of the base

helical structure in TFE. The imidazol ring of His23 bound to a
Cys23/His23 replacement leads to structural changes in the first
whereas the extended structure content decreases (Table I).

In aqueous solvent, zinc depletion or substitution of His 23 by
the complexes.

tRNA3

spectra in the
cular heterogeneity is consistent with the flexibility of the
third of the NCp7 structure is solvent-dependent. This confor-
mational heterogeneity is consistent with the flexibility of the
N- and C-terminal regions proposed in a1H NMR study (24).

To understand the role of NCp7 in the HIV RNA/tRNA
annealing, two complexes were formed with tRNA\textsubscript{A\textsuperscript{5\textsuperscript{9}}}, one
involving NCp7 and the other involving Cys\textsuperscript{23} NCp7. The far UV CD spectra of these two complexes were measured from 320 to
178 nm in 20 mM phosphate buffer (pH 7) complemented with
50 mM KF and 5% glycerol at 5 °C (Fig. 3). The tRNA\textsubscript{A\textsuperscript{5\textsuperscript{9}}}, CD spectrum, measured in similar conditions, is typical of an A-
form helix, as usually observed with RNA stems (25). A ratio of one
tRNA for three NCp7 molecules was used for these com-
plexes to ensure that no free tRNA was present. The associa-
tion constant (K\textsubscript{obs}) of the NCp7-tRNA\textsubscript{A\textsuperscript{5\textsuperscript{9}}} interaction is 10\textsuperscript{-6}
M\textsuperscript{-1} (14). We used tRNA and NCp7 concentrations near 10\textsuperscript{-5} M,
higher than K\textsubscript{obs} to ensure complex formation. Precipitation of
the complexes did occur, however, when the experiments were
done at temperatures higher than 5 °C. Absorption spectra
confirmed the absence of protein precipitation when both com-
plexes were formed at 5 °C (Fig. 3). The main protein chro-
mophore has its contribution localized between 178 and 240 nm
with a maximum at 190 nm, whereas tRNA has its maximum
absorption near 190 and 260 nm (Fig. 3). Therefore, the CD
band observed near 260 nm is only due to tRNA. When NCp7 or
Cys\textsuperscript{23} NCp7 is added to tRNA, few changes occur in the CD
spectra compared with tRNA alone (Fig. 3). Native NCp7 and
Cys\textsuperscript{23} NCp7 induce a similar decrease of the 260-nm band. This
change in the 260 nm CD band shows that both native NCp7
and Cys\textsuperscript{23} NCp7 binds tRNA\textsubscript{A\textsuperscript{5\textsuperscript{9}}}, as interpreted in a former CD study, a
similar decrease of the 260-nm band was observed in the
CD\textsubscript{A\textsuperscript{5\textsuperscript{9}}} complex and interpreted as a tRNA unwinding
induced by NCp7 binding (25). We do not agree with this
interpretation. In polynucleotides, the 260-nm CD band is very
sensitive to base stacking (26), which can be modified upon
binding of a protein without affecting the general nucleic acid
structure (27). For instance, binding of an intercalating drug
induces a specific modification of the 260-nm band in the RNA
transactivation response element RNA CD spectrum (28).
Phosphorescence and optically detected magnetic resonance
studies reported modifications of tRNA base stacking induced
by NCp7 binding (29). Moreover, RNA unwinding should in-
duce more significant changes in CD spectra. The negative
band at 210 nm should completely disappear when the A-
form helix is disrupted (26, 30). The tRNA unfolding due to the
addition of organic solvents results in a dramatic decrease of the
positive CD band near 260 nm, which losses two-thirds of
its magnitude and shifts to 275 nm (31). Such modifications are
not observed when the two complexes are formed. Our results
thus suggest that there is no tRNA\textsubscript{A\textsuperscript{5\textsuperscript{9}}} unwinding upon binding
of NCp7 or Cys\textsuperscript{23} NCp7.

To observe the effect of tRNA melting on the far UV spec-
trum, we measured CD spectra of tRNA\textsubscript{A\textsuperscript{5\textsuperscript{9}}} from 5 to 80 °C (Fig.
4). The tRNA CD bands near 260 and 190 nm decreased pro-
gressively as a function of temperature. The 190-nm band
completely disappeared at 80 °C, whereas the 260-nm band
shifted to 275 nm, which was also observed in tRNA unstaking

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|l|}
\hline
Proteins & Specificity & $\alpha$ helix & Extended structure & $\beta$ turn & Other structures & Total \\
\hline
NCp7 & Aqueous\textsuperscript{a} & 0.02 (1) & 0.42 (30) & 0.33 (24) & 0.23 (17) & 0.99 (72) \\
& TFE\textsuperscript{a} & 0.39 (28) & 0.11 (8) & 0.22 (16) & 0.28 (20) & 1.00 (72) \\
& no Zn\textsuperscript{a} & 0.28 (20) & 0.30 (22) & 0.38 (27) & 1.00 (72) \\
Cys\textsuperscript{23} NCp7 & Aqueous\textsuperscript{a} & 0.46 (33) & 0.07 (5) & 0.21 (15) & 0.26 (19) & 1.00 (72) \\
& TFE\textsuperscript{a} & \textsuperscript{a} & \textsuperscript{a} & \textsuperscript{a} & \textsuperscript{a} & \textsuperscript{a} \\
\hline
\end{tabular}
\caption{CD data secondary structures analysis}
\end{table}

\textsuperscript{a} aqueous solvent with 20 mM phosphate buffer, pH 7.
\textsuperscript{b} solvent composed of 80% trifluoroethanol and 20% water.
\textsuperscript{c} aqueous solvent with 20 mM phosphate buffer, pH 7, with NCp7 without Zn\textsuperscript{2+}.
due to high salt concentration (31). Interestingly, the 210-nm bands remained almost unchanged from 5 to 55 °C, dramatically changed at 60 °C, and almost disappeared at 80 °C. If NCp7 did denature tRNA\textsuperscript{3 Lys}, the complex CD spectrum should display similarities to what was observed with tRNA alone at 80 °C or to what was observed at 60 °C if denaturation was partial (Fig. 4). Such is not the case. The 260- and 190-nm bands are sensitive to base stacking, and the intensity of these bands is the highest when bases are well superimposed (26). The intensity of the 260-nm band decreases when base stacking is altered, either by a temperature rise (Fig. 4) or by intermolecular contacts (Fig. 3). Nevertheless, base stacking can be altered in the absence of tRNA unwinding as observed from 5 to 55 °C (Fig. 4). This peculiarity made the measure of the 260-nm CD band very useful to check the binding of intercalating drugs on double-stranded RNA (28). Only the 210-nm band seems to be strictly related to the tRNA helical structure, its variation correlating clearly with wound and unwound conformational states. Fig. 4 shows that there is a partial melting of tRNA at 60 °C, which becomes complete after 80 °C. We used the variation of the 210-nm CD band to analyze the conformational stability of tRNA\textsuperscript{3 Lys}. The thermal unfolding curve shows that the equilibrium is reached at 57 °C (Fig. 4), which argues in favor of the high stability of tRNA\textsuperscript{3 Lys}.

Near 260 nm, the CD spectra of both complexes (Fig. 3) are similar to the tRNA CD spectrum at 20 °C (Fig. 4). Between 250 and 178 nm, both protein and tRNA contribute to the CD spectrum. To evaluate protein-induced changes, the tRNA\textsuperscript{3 Lys} CD spectrum was subtracted from the CD spectra of the two complexes, from 250 to 178 nm (Fig. 2B). These two difference spectra can be assigned to CD spectra of NCp7 and Cys\textsuperscript{23} NCp7 bound to tRNA\textsuperscript{3 Lys}. It is not possible to determine the percentage of secondary structure due to the tRNA contribution, but protein-specific CD bands appear, which permit evaluation of structural changes in NCp7 and Cys\textsuperscript{23} NCp7 bound to tRNA\textsuperscript{3 Lys}. Intensity of the 200-nm band, associated to random coil structures, decreases in the difference CD spectra compared with unbound NCp7. Interestingly, a negative band at 185 nm usually associated to \(\beta\)-turn structures (30) appears clearly in bound NCp7 (Fig. 2B).

To investigate possible NCp7:tRNA\textsuperscript{3 Lys} binding sites, a molecular model of the complex was realized (Fig. 5). Our NCp7 model uses the NMR structure of the first zinc finger bound to an RNA fragment analogous to the Psi region (3), available in the protein data bank (32). The second zinc finger adopts similar secondary structures with two turns and extended structures (33). In our NCp7 model, the zinc fingers and linker region were positioned according to nuclear Overhauser effects between side chain protons observed by NMR (34). No nuclear Overhauser effects are observed between side chain protons of the N- and C-terminal regions and these two regions were modeled to minimize protein-solvent interactions (Fig. 5).
model was then submitted to molecular dynamics and energy minimization in the consistent valence forcefield. The van der Waals energies reached 424 kcal/mol, which is in a range compatible with the NCp7 molecular weight. Repulsive and dispersive van der Waals energies are two of the 10 parameters in the consistent valence forcefield. Contrary to the consistent valence forcefield total energy, the van der Waals energy levels are proportional to molecular weights as they can be observed with high resolution x-ray structures. Our final NCp7 model agrees with the percentage of secondary structures calculated from the NCp7 CD spectrum in aqueous solvent (Table I). The 30 residues corresponding to the expected percentage of extended structures are found mainly in the N-terminal region and, in a lesser amount, in zinc fingers and C-terminal regions (Fig. 5). This is confirmed with dαN nuclear Overhauser effect spectroscopy connectivities observed in similar locations with NMR (34). From the six β turns that can be deduced from CD data analysis in an aqueous solvent, two are located in each zinc finger, as described in zinc finger NMR structures (3, 33). Two other β turns in our model are observed in the C-terminal region (residues 62–65 and 67–70). Conformationally restricted sequences are identified by NMR in the same region (34).

The human tRNA<sup>3Lys</sup> sequence (Fig. 1) is highly similar to that of yeast tRNA<sup>3Lys</sup>. Both are type I tRNAs with equal lengths and strictly conserved sequences in every tertiary base pair and triple. This high level of homology enabled us to use the yeast tRNA<sup>3Lys</sup> x-ray structure (35) as a template and to replace non-conserved bases (22/76 bases) by their human tRNA<sup>3Lys</sup> counterparts. This replacement did not introduce any significant changes and did not require additional refinement (Fig. 5). UV cross-linking and gel retardation assays demonstrated a tight interaction between tRNA<sup>3Lys</sup> and NCp7 involving 12 nucleotides in the anticodon domain (13). We modeled the interaction between tRNA<sup>3Lys</sup> and the first zinc finger according to the NMR structure of the zinc finger-Psi RNA complex (3). In this model, the relative orientation of the first zinc finger with the RNA was well defined from intermolecular nuclear Overhauser effects, and our first goal was to reproduce this arrangement. The tRNA anticodon loop positions 34–38 (5′-UUUAA-3′) are single-stranded (Fig. 1), exposed to solvent, and stacked in an A-helical form (Fig. 5) as the Psi analog (5′-ACGCC-3′) used in the NMR model. Consequently, this loop can be docked to the first zinc finger in an orientation very similar to that of the Psi analog in the NMR structure. The tRNA nucleotides 32–33 preceding the U turn in the anticodon loop face part of the 34–38 nucleotides and interfere with the side chain of Arg<sup>26</sup>, which can easily be moved away. The involvement of residues Lys<sup>14</sup>, Phe<sup>16</sup>, Glu<sup>21</sup>, Ile<sup>24</sup>, and Ala<sup>25</sup> in interactions with tRNA was considered as probable from the NMR model. All these residues in our model are in hydrogen bond range to anticodon loop nucleotides. The sequence differences between Psi RNA and tRNA<sup>3Lys</sup> anticodon loop did not permit specific protein-RNA contacts to reproduce, as suggested in the NMR model. It is noteworthy, however, that no other part of tRNA contains such a stretch of single-stranded
nucleotides available for a similar docking with zinc fingers (Fig. 5).

The first zinc finger is involved in tRNA binding since (13–64) NCp7 binds tRNA, whereas (13–64) Cys23 NCp7 does not (18). However, our CD data shows that full-length Cys23 NCp7 can bind tRNA59, and this result confirms that residues located outside zinc fingers are involved in the NCp7-tRNA59 interaction (12). The position of the NCp7 N-terminal region had to be changed to model the NCp7-tRNA59 interaction (Fig. 5). Table I shows that important structural changes occur in NCp7 in the function of the solvent. This conformational heterogeneity is mainly due to the N- and C-terminal regions, zinc fingers being described as rigid structures (34). The binding of the first zinc finger to tRNA brings the N-terminal region in proximity of the major groove of the anticodon stem (Fig. 5). Docking of the N-terminal region in this groove was tried and turned out to be successful, since the N-terminal region remained in the anticodon major groove after molecular dynamics and energy minimization (Fig. 5). The few arrangements that did occur during the refinement positioned the lateral chain of arginines in contact with tRNA phosphates, increasing coulombic interactions between the two molecules. These interactions involve the tRNA backbone rather than the bases, consistent with nonspecific contacts (36). A β turn appeared between the first zinc finger and the N-terminal region in the complex (Fig. 5). This is consistent with the difference CD spectra, which show an increase of β-turn structures in NCp7 bound to tRNA (Fig. 2B). Nucleotides interacting with NCp7 in our model (Figs. 1 and 5) correspond to those identified in UV cross-linking experiments (13).

CD data indicate that NCp7 does not unwind tRNA upon binding, contrary to what was proposed in a former study (25). Except for few changes in base stacking, the tRNA retains its native A-helical form. Molecular modeling suggests that the NCp7 N-terminal region and the first zinc finger could be tRNA59 binding sites. This interaction would not require a base specificity and could occur with other tRNAs as well. This is in agreement with experiments showing that NCp7 binds different types of tRNAs (14). We propose that tRNA59 unwinding is a consequence of the annealing with the PBS rather than an effect of NCp7 binding. However, NCp7 dramatically increases the efficiency of HIV RNA-tRNA59 annealing (37). The function of NCp7 in HIV RNA-tRNA59 annealing could be to facilitate the HIV RNA-tRNA59 interaction. The C-terminal region comprising the second zinc finger of NCp7 can bind HIV RNA (12). In addition, NCp7 bound to HIV RNA can interact with another NCp7 molecule (6). NCp7 could bring together the tRNA 3′ end and its complementary sequence in the PBS either via binding of NCp7 to both tRNA and HIV RNA or via a tRNA-NCp7 complex binding a HIV RNA-NCp7 complex.

Acknowledgments—We are indebted to Dr. Caroline Gabus-Darlix and Professor Bernard Roques for the generous gifts of tRNA59, NCp7, and Cys23 NCp7. We thank Nicole Zylber for technical assistance and Drs. Nathalie Jullian, Valérie Tanchou, Jean-Luc Darlix, and Claude Lazdunski for fruitful discussions.

REFERENCES
1. Darlix, J. L., Gabus, C., Nugeyre, M. T., Clavel, F. & Barré-Sinoussi, F. (1990) J. Mol. Biol. 216, 689–699
2. Sakaguchi, K., Zambrano, N., Baldwin, E. T., Shapiro, B. A., Erickson, J. W., Omiichi, J. G., Clore, G. M., Gronenborn, A. M. & Appella, E. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5219–5223
3. South, T. L. & Summers, M. F. (1993) Protein Sci. 2, 3–19
4. Dannull, J., Surovov, A., Jung, G. & Moelling, K. (1994) EMBO J. 13, 1525–1533
5. Prats, A. C., Sarith, L., Gabus, C., Litvak, S., Keith, G. & Darlix, J. L. (1988) EMBO J. 7, 1777–1783
6. Tanchou, V., Gabus, C., Rogemond, V. & Darlix, J. L. (1995) J. Mol. Biol. 252, 563–571
7. Baudin, F., Marquet, R., Isel, C., Darlix, J. L., Ehresmann, B. & Ehresmann, C. (1993) J. Mol. Biol. 228, 301–318
8. de Roquigny, H., Gabus, C., Vincent, A., Fournié-Zaluski, M. C., Roques, B. P. & Darlix, J. L. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6472–6476
9. Barat, C., Schmalzbauer, E., Dannull, J., Surovoy, A., Jung, G., & Moelling, K. (1996) Biochemistry 35, 5175–5182
10. Isel, C., Lanchy, J. M., Le Grice, S. F. J., Ehresmann, C., Ehresmann, B. & Marquet, R. (1996) Biochemistry 35, 11767–11765
11. Manavalan, P. & Johnson, W. C., Jr. (1987) Anal. Biochem. 167, 76–85
12. de Roquigny, H., Ficheux, D., Gabus, C., Fournié-Zaluski, M. C., Darlix, J. L. & Roques, B. P. (1995) Biochemistry 34, 2670–2676
13. Barat, C., Schmalzbauer, E., Dannull, J., Schmalzbauer, E., Dannull, J., Guehmann S. & Moelling, K. (1996) Biochemistry 35, 5175–5182
14. Schmalzbauer, E., Strack, B., Dannull, J., Guehmann S. & Moelling, K. (1996) Biochemistry 35, 771–777
15. de Roquigny, H., Gabus, C., Fournié-Zaluski, M. C., Darlix, J. L. & Roques, B. P. (1991) Biochem. Biophys. Res. Commun. 186, 1010–1018
16. Manavalan, P. & Johnson, W. C., Jr. (1987) Anal. Biochem. 167, 76–85
17. Jullian, N., De·méné, H., Dong, C. Z., Ottmann, M., Rouyez, M. C., Jullian, N., Morellet, N., M., Darlix, J. L., Fournié-Zaluski, M. C., Saragosti, S. & Roques, B. P. (1994) Biochemistry 33, 11677–11716
18. Schmalzbauer, E., Strack, B., Dannull, J., Guehmann S. & Moelling, K. (1996) Biochemistry 35, 771–777
19. Jullian, N., De·méné, H., Dong, C. Z., Ottmann, M., Rouyez, M. C., Jullian, N., Morellet, N., M., Darlix, J. L., Fournié-Zaluski, M. C., Saragosti, S. & Roques, B. P. (1994) Biochemistry 33, 11677–11716
20. de Roquigny, H., Ficheux, D., Gabus, C., Fournié-Zaluski, M. C., Darlix, J. L. & Roques, B. P. (1991) Biochem. Biophys. Res. Commun. 186, 1010–1018
21. Manavalan, P. & Johnson, W. C., Jr. (1987) Anal. Biochem. 167, 76–85