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Characterization of Human Immunodeficiency Virus Type 1 Dimeric RNA from Wild-Type and Protease-Defective Virions

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We have characterized the dimeric genomic RNA in particles of both wild-type and protease (PR)-deficient human immunodeficiency virus type 1 (HIV-1). We found that the dimeric RNA isolated from PR$^-$ mutant virions has a lower mobility in nondenaturing gel electrophoresis than that from wild-type virions. It also dissociates into monomers at a lower temperature than the wild-type dimer. Thus, the dimer in PR$^-$ particles is in a conformation different from that in wild-type particles. These results are quite similar to recent findings on Moloney murine leukemia virus and suggest that a postassembly, PR-dependent maturation event is a common feature in genomic RNAs of retroviruses. We also measured the thermal stability of the wild-type and PR$^-$ dimeric RNAs under different ionic conditions. Both forms of the dimer were stabilized by increasing Na^+ concentrations. However, the melting temperatures of the two forms were not significantly affected by the identity of the monovalent cation present in the incubation buffer. This observation is in contrast with recent reports on dimers formed in vitro from short segments of HIV-1 sequence: the latter dimers are specifically stabilized by K^+ ions. K^+ stabilization of dimers formed in vitro has been taken as evidence for the presence of guanine quartet structures. The results suggest that guanine quartets are not involved in the structure linking full-length, authentic genomic RNA of HIV-1 into a dimeric structure.

In retroviruses, the genome is a dimeric RNA molecule. The dimer is composed of two monomers of the same (positive-strand) polarity. While the nature of the linkage between the monomers is not understood in detail, it presumably consists of hydrogen bonds or other weak, noncovalent bonds, since the dimers can be dissociated under mild conditions (3, 10, 14). Electron microscopic studies indicate that the monomers are linked in parallel orientation near their 5′ ends (5, 6, 22, 27; reviewed in reference 12).

It has more recently been found that relatively short RNA molecules containing sense-strand sequences from near the 5′ end of the viral genome can dimerize spontaneously in vitro. A number of studies have analyzed the properties of the resulting dimers (2, 4, 7, 8, 13, 24, 29, 31, 35, 36). While these dimers of short transcripts of viral RNAs are, for a variety of reasons, much easier to study than RNAs obtained from virions, the following question remains: to what extent and in what ways are the former dimers a good model for the dimers present in virions? With this question in mind, we have characterized the RNA dimer present in human immunodeficiency virus type 1 (HIV-1) particles.

The results presented here tend to support two important conclusions. First, we have compared dimers from wild-type HIV-1 particles with those from mutant particles lacking the viral protease (PR). We found that the dimers from PR$^-$ virions have a conformation different from that of dimers from wild-type virions. These results are interpreted in light of recent findings on Moloney murine leukemia virus (Mo-MuLV). We recently showed that the dimeric RNA of Mo-MuLV undergoes a change in conformation after the virus is released from the cell (16). This change, which we termed maturation, is dependent on the action of the viral PR, and probably on the presence of a wild-type nucleocapsid (NC) protein as well. Our present results with HIV-1 are quite analogous to those with MuLV and suggest that dimeric RNA of HIV-1, like that of Mo-MuLV, undergoes a maturation event after the virus is released from the cell.

The second conclusion deals with the structure of the dimer and its resemblance to dimers formed in vitro. It was recently reported that dimers of short HIV-1 transcripts are far more stable in buffers containing K^+ than in buffers with other monovalent cations (2, 35). It was suggested, on the basis of this finding, that these dimers are stabilized by quadruple-helical structures, involving guanine base tetrads, called G quartets. In the present work, we have tested the stability of authentic viral RNA dimers, derived from either wild-type or PR$^-$ virions of HIV-1. In contrast to the results with short synthetic transcripts of viral RNA, we found that the dimers from virions are not specifically stabilized by K^+. Thus, our results suggest that G quartets do not contribute significantly to the stability of genomic RNA dimers of HIV-1, either before or after the maturation event.

MATERIALS AND METHODS

HIV-1 plasmids. HIV-1 clones used here are gifts from Ronald Swanstrom and Mari Manchester, Lineberger Cancer Research Center, University of North Carolina, Chapel Hill. The DNA clones were based on the HXB2 provirus that was cloned into the *Xba*I site of the pIBI20 vector. The plasmids are designated as follows: wild-type, pMM4-HXB2; and PR$^-$ mutant, with a GAT (aspartate) → GCT (alanine) change at amino acid 25 in the PR coding region (nucleotide 2325 in the proviral sequence [30]), pMM4-D25A.

Virus preparation and RNA isolation. HeLa cells were cultivated in 150-cm^2 flasks and maintained as described previously (18). The cells were transfected at 80% confluency with the plasmids described above either by the calcium phosphate method (19) or by using Lipofectamine reagent

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(Life Technologies, Gaithersburg, Md.). For the calcium phosphate method, 102 μg of plasmid DNA was used per 150-cm^2 flask. Transfection of cells in a 150-cm^2 flask with 16 μg of plasmid DNA and 125 μl of Lipofectamine reagent was performed as instructed by the manufacturer. Flasks were fluid changed 48 h after transfection, and two or three consecutive 24-h virus harvests were then obtained.

Virions were collected as described previously (18). Viral particles were disrupted in sterile lysis buffer consisting of 50 mM Tris (pH 7.4), 10 mM EDTA, 1% sodium dodecyl sulfate (SDS), 100 mM NaCl, 50 μg of yeast tRNA per ml, and 100 μg of proteinase K per ml. Usually, a pellet resulting from 30 ml of culture fluid was disrupted in 100 to 200 μl of lysis buffer. Resuspended pellets were incubated at room temperature for 30 min and then extracted three times with an equal volume of buffer-saturated phenol-chloroform-isoamyl alcohol (25:24:1, pH 7.9) on ice. The aqueous phase, containing the viral RNA, was precipitated in 70% ethanol, using 0.3 M sodium acetate (pH 5.2) (diethylpyrocarbonate treated).

Electrophoretic and thermal analysis of HIV-1 viral RNAs. In general, viral RNA pelleted from ethanol suspension was dissolved in a buffer containing 10 mM Tris (pH 7.5), 1 mM EDTA, 1% SDS, and 100 mM NaCl and analyzed by nondenaturing Northern (RNA) blot analysis (21) as described previously (16). In these experiments, ^{32}P-labeled HIV-1 riboprobes were generated from a subclone of the pNL4-3 clone of HIV-1 (1). The subclone extended from the *Spe*I site at nucleotide 1507 to the *Sal*I site at nucleotide 5785, inserted between the *Spe*I and *Sal*I sites of pBluescript KS+ (Stratagene, La Jolla, Calif.). The subclone was linearized with *Spe*I and transcribed with T3 polymerase as instructed by the manufacturer (Promega, Madison, Wis.).

In some experiments, a very sharp, heat-resistant band is seen near the dimer position. This band is probably residual plasmid DNA from the transient transfections used here.

In salt concentration-dependent experiments, aliquots of viral RNA from ethanol suspension were pelleted and then redissolved in buffers of the same composition as indicated above except that the concentration of NaCl was varied. Similarly, in experiments varying the identity of monovalent cations, viral RNA was pelleted out of 70% ethanol and redissolved in buffers containing 10 mM Tris (pH 7.5), 1 mM EDTA, 1% SDS, and 100 mM LiCl, NaCl, KCl, or CsCl. In experiments with Mg^{2+} ions, viral RNA from ethanol suspension was dissolved in buffers containing 5 mM $MgCl_2$, 10 mM Tris (pH 8.3), and 100 mM LiCl, NaCl, or KCl. In all melting experiments, RNA samples were incubated in parallel in a water bath for 10 min at the indicated temperatures.

Precipitates formed in buffers containing both KCl and SDS. These precipitates did not affect the melting curves, since control experiments in buffers without SDS gave the same results as those shown, for both wild-type and PR^- dimers (data not shown).

RESULTS

Comparison of dimeric RNAs in wild-type and PR^- HIV-1 virions. Previous work on Mo-MuLV shows that the dimeric RNA from PR^- mutant virions is in a more extended, less stable conformation than that from wild-type virions (16); somewhat similar data have also been presented for avian sarcoma virus (33). These results indicated that the conformation of the RNA dimers is dependent on the function of the viral protease. To investigate the effect of protease function on RNA dimers in HIV-1, we isolated genomic RNAs from both wild-type and PR^- mutant virions. The RNAs were characterized by nondenaturing Northern analysis, using a ^{32}P-labeled HIV-1 riboprobe. Figure 1 shows that there were two RNA species present in the PR^- mutant and only one in the wild-type sample before heating. The mobility of the wild-type band is close to that of dimeric Mo-MuLV RNA (data not shown). One of the two bands in the PR^- sample migrated more rapidly, and the other more slowly, than the band in the wild-type sample. After incubation at 65°C for 10 min, only one band was seen in each sample; this band migrated at the same position as the lower band in the unheated PR^- sample. The mobility of this band is also very similar to that of monomeric RNA of Mo-MuLV (data not shown). On the basis of these results, we conclude that the upper band in the unheated PR^- RNA and the band in the unheated wild-type sample are HIV-1 RNA dimers; after heating, only monomers are present. The data also indicate that PR^- dimers migrate more slowly than those from the wild type (Fig. 1, unheated samples).

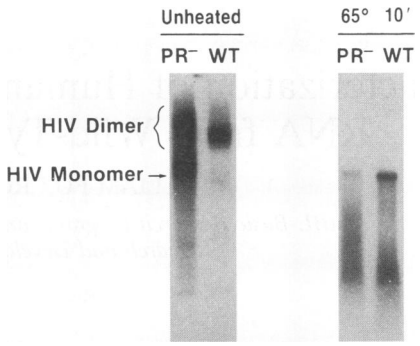

FIG. 1. Electrophoretic analysis of HIV-1 RNAs from wild-type (WT) and PR^- mutant virions. Viral RNA pellets were dissolved at room temperature in 10 mM Tris (pH 7.5)–1 mM EDTA–1% SDS–100 mM NaCl. One half of each sample was directly analyzed on a 1% agarose gel, while the second half was heated at 65°C for 10 min before analysis. RNAs were analyzed by nondenaturing Northern blotting as described in Materials and Methods.

It is interesting that the unheated PR^- sample contains a substantial fraction of monomeric RNA (Fig. 1). This is in contrast with our previous study of Mo-MuLV RNA (16). The significance of these monomers in PR^- HIV RNA will be considered in Discussion.

In further experiments, we measured the thermal stability of the dimeric RNAs in wild-type and PR^- virions by treating them at temperatures ranging from 25 to 60°C. As shown in Fig. 2, the dimeric RNA of PR^- HIV-1 was completely converted to monomeric form at 50°C, while that of wild-type HIV-1 was completely dissociated only after incubation at 55°C. Taken together, the results in Fig. 1 and 2 suggest that the HIV-1 RNA dimers from wild-type virions have a more stable, more compact conformation than those from PR^- mutant virions. Therefore, it seems likely that the RNA dimers in HIV-1, like those in Mo-MuLV, undergo a PR-dependent maturation event.

Effect of salt concentration on thermal stability of HIV-1 RNA dimers. The structure of the dimeric RNA in HIV-1 is not understood. In an effort to obtain some insight into the nature of the linkage between the monomers, we measured the thermal stability of the wild-type and PR^- dimers under a variety of ionic conditions. In one of these experiments, we determined the melting temperature (T_m) of the dimers at a number of NaCl concentrations. As shown in Fig. 3, the T_m of both wild-type and PR^- dimers increased approximately 15°C as the NaCl concentration was raised from 5 to 200 mM.

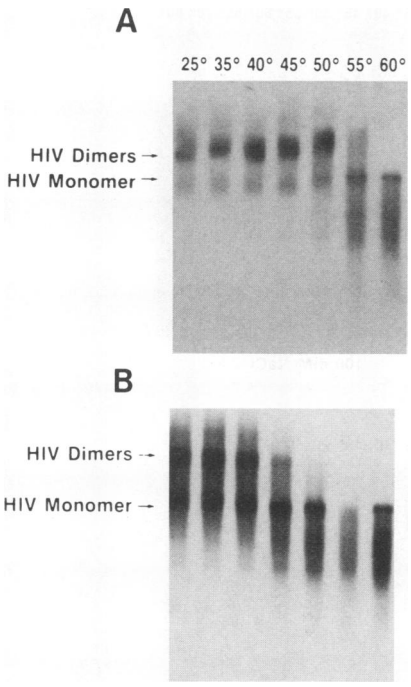

FIG. 2. Melting curves of HIV-1 dimeric RNAs from wild-type (A) and PR$^-$ mutant (B) virions. RNAs extracted from the respective virions were dissolved in a buffer containing 10 mM Tris (pH 7.5), 1 mM EDTA, 1% SDS, and 100 mM NaCl at room temperature. Aliquots of the RNAs were heated for 10 min at the indicated temperatures. The RNA samples were analyzed by nondenaturing Northern blotting as described in Materials and Methods.

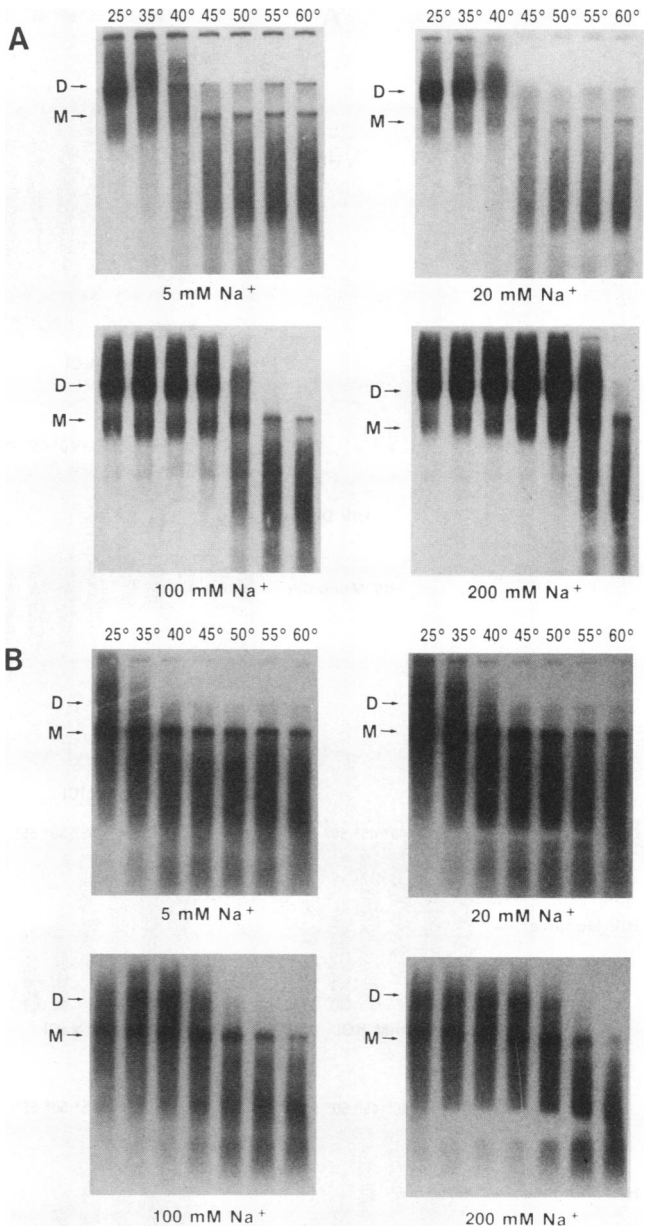

FIG. 3. Melting curves of HIV-1 dimeric RNAs from wild-type (A) and PR$^-$ mutant (B) virions in different salt concentrations. RNAs isolated from wild-type and PR$^-$ mutant virions were dissolved in a buffer containing 10 mM Tris (pH 7.5), 1 mM EDTA, 1% SDS, and either 5, 20, 100, or 200 mM NaCl as indicated. The experiments were performed as described for Fig. 2.

The results presented in Fig. 3 show that the genomic RNA dimers are stabilized by increasing salt concentrations. We also tested the effect of Mg^{2+} on the T_m of the dimers. In the presence of 100 mM NaCl, the addition of 5 mM Mg^{2+} raised the T_m of wild-type dimers from approximately 55°C (Fig. 3) to approximately 65°C (data not shown). Thus, Mg^{2+} ions apparently stabilize genomic RNA dimers far more than would be expected from a simple ionic strength effect.

Thermal stability of HIV-1 RNA dimers in different monovalent cations. As noted above, several laboratories have recently described the properties of dimeric RNAs formed in vitro from short transcripts of HIV-1 RNA sequences. Two reports have described dimers that are far more stable in the presence of potassium than in the presence of other monovalent cations (2, 35). This dramatic, specific stabilization by K^+ ions was taken as evidence that these short dimers contain G quartet structures. In an effort to determine whether the dimers of full-length genomic RNA extracted from HIV-1 particles exhibit this property, we compared the thermal stability of HIV-1 dimeric RNAs in 100 mM K^+ with that in 100 mM Na^+, Li^+, and Cs^+. The results obtained with wild-type HIV dimers are shown in Fig. 4A; it can be seen that the T_ms obtained in the four buffers are very similar to each other (all approximately 55°C).

The results shown in Fig. 4A suggest that G quartets do not contribute significantly to the linkage between the monomers in the wild-type HIV-1 dimers. However, data presented above (Fig. 1 to 3) show that dimers extracted from PR$^-$ virions are different in structure from those in wild-type virions. It therefore still seemed possible that PR$^-$ dimers are stabilized by G quartets. To test this idea, we measured the thermal stability of PR$^-$ dimers in the presence of the four monovalent cations used in Fig. 4A. As shown in Fig. 4B, the identity of the monovalent cation did not strongly affect the stability of the dimers extracted from PR$^-$ virions: in all cases, the T_m was about 50°C under these conditions.

In one of the reports describing stabilization by K^+ of short dimers formed in vitro (35), the melting experiments were performed in the presence of 5 mM Mg^{2+} and without SDS. We also tested the thermal stability of wild-type HIV-1 dimeric RNAs in the buffer used by these authors. We found that the

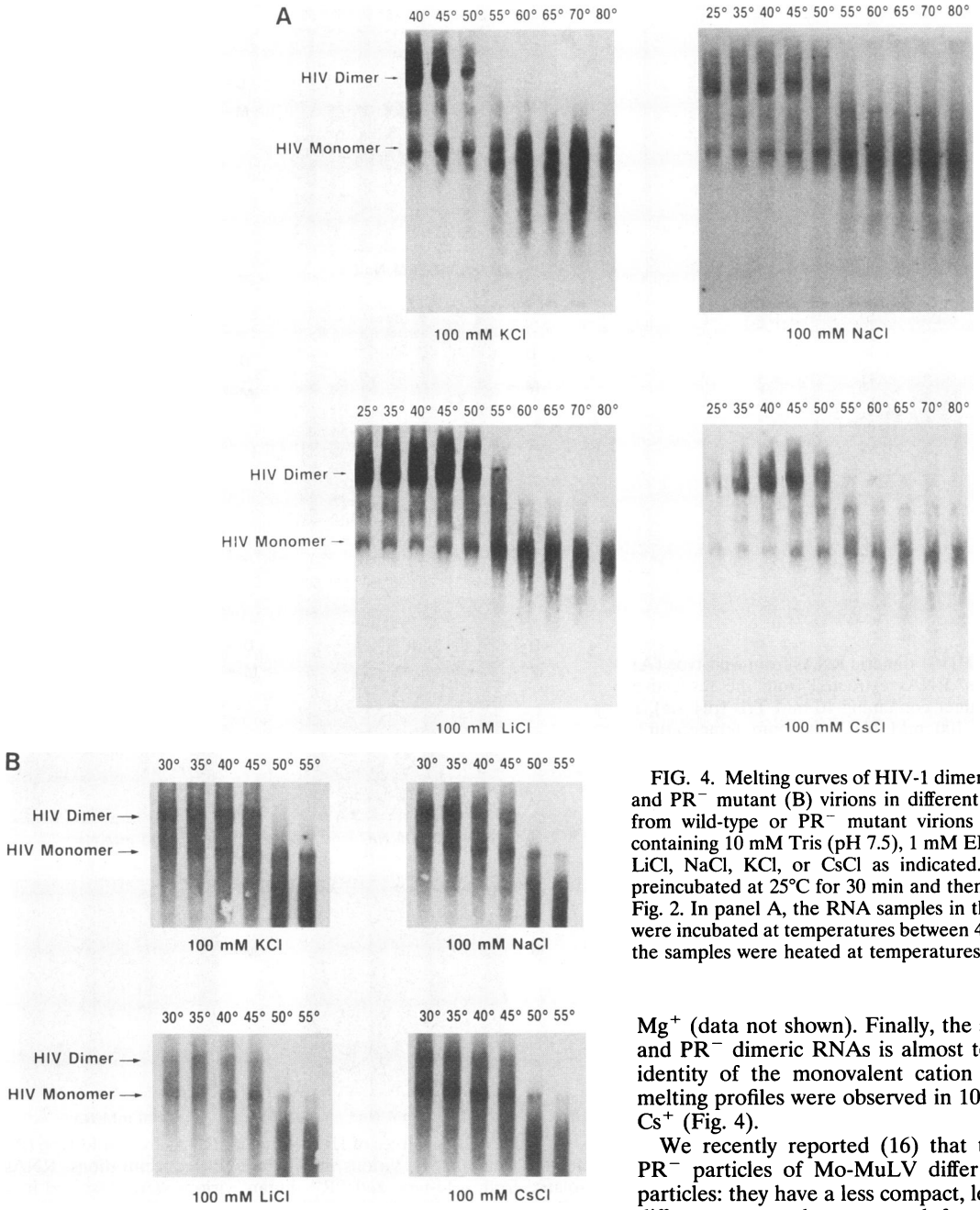

FIG. 4. Melting curves of HIV-1 dimeric RNAs from wild-type (A) and PR$^-$ mutant (B) virions in different monovalent cations. RNAs from wild-type or PR$^-$ mutant virions were dissolved in a buffer containing 10 mM Tris (pH 7.5), 1 mM EDTA, 1% SDS, and 100 mM LiCl, NaCl, KCl, or CsCl as indicated. The RNA solutions were preincubated at 25°C for 30 min and then heated and analyzed as for Fig. 2. In panel A, the RNA samples in the presence of 100 mM KCl were incubated at temperatures between 40 and 80°C, while the rest of the samples were heated at temperatures ranging from 25 to 80°C.

presence of Mg^{2+} raised the T_m of the dimers from 55 to 65°C, regardless of whether the monovalent cation was Li^+, K^+, or Na^+ (data not shown).

DISCUSSION

The results presented here can be briefly summarized as follows. First, dimeric RNA isolated from PR$^-$ particles of HIV-1 differs from that in wild-type particles. In particular, the PR$^-$ dimer migrates more slowly in nondenaturing gel electrophoresis (Fig. 1), and dissociates into monomers at a lower temperature (Fig. 2), than the wild-type dimer. Second, dimeric RNAs from either wild-type or PR$^-$ particles are stabilized by increasing concentrations of Na^+ (Fig. 3), and wild-type dimers are also stabilized by low concentrations of Mg^+ (data not shown). Finally, the stability of both wild-type and PR$^-$ dimeric RNAs is almost totally independent of the identity of the monovalent cation present: nearly identical melting profiles were observed in 100 mM Na^+, K^+, Li^+, and Cs^+ (Fig. 4).

We recently reported (16) that the dimers isolated from PR$^-$ particles of Mo-MuLV differ from those in wild-type particles: they have a less compact, less stable structure. These differences were shown to result from the lack of functional PR in the mutant particles. Similarly, dimers from newly released MuLV particles have a less compact, less stable structure than those from mature particles. Taken together, these observations suggested that wild-type particles of MuLV undergo a series of maturation events after being released from the cell. Newly released particles contain one form of dimeric RNA; after the viral proteins are cleaved by PR, the cleavage products convert the RNA to a more stable, more compact conformation. A number of observations on avian retroviruses are also all consistent with this hypothesis (11, 34). The differences described here between wild-type and PR$^-$ HIV RNAs strongly suggest that a similar phenomenon occurs in HIV as well.

In our study of MuLV dimeric RNAs, we also showed that Kirsten sarcoma virus dimers packaged by an NC mutant of

Mo-MuLV have a lower electrophoretic mobility than those packaged by wild-type Mo-MuLV (16). This observation suggested that the maturation of the RNA dimers involves the NC molecule, which is liberated from the Gag polyprotein by PR after virus assembly. It will be interesting to determine whether NC can catalyze the maturation of dimeric RNAs in vitro.

One difference between our previous study of MuLV RNA and the present results is that a substantial amount of monomeric RNA is always detected in PR^- HIV particles (e.g., Fig. 1, unheated sample). Very similar observations were also made in studies of PR^- (28, 33) and NC (9, 15, 26, 28) mutants of avian retroviruses. One conclusion which might be drawn from the presence of monomers in these RNA preparations is that HIV virions can package either monomeric or dimeric RNA. However, we also found that the dimers in the PR^- viral RNA samples are significantly less stable than wild-type dimers (Fig. 2 to 4). Thus, an alternative possibility is that only dimers are actually packaged in PR^- particles, but that some of these fragile dimeric molecules dissociate before or during RNA preparation. For the reasons discussed below, we favor the latter hypothesis.

Packaging of genomic RNAs by retroviral particles is very limited in certain experimental situations. These situations include MuLV-producing cells which have been treated with actinomycin D (23) and somewhat leaky mutants in the cysteine array of MuLV NC (17, 25). Under these conditions, a large majority of the particles contain no genomic RNA. Nevertheless, the genomic RNAs present in these virus preparations are exclusively dimeric (23, 25). This fact implies that monomers are not packaged into MuLV particles independently of each other but are probably already joined into some dimeric structure at the time of virus assembly. In other words, these observations suggest that dimerization is a prerequisite for packaging; it is possible that this is a general feature of retrovirus assembly. If this is the case, then monomers identified in PR^- or NC mutant virions would arise by breakdown of fragile dimers within the virion or during extraction.

Both wild-type and PR^- dimers were found (Fig. 3) to be stabilized by increasing Na^+ concentrations. Somewhat similar observations have previously been made for murine retroviral RNAs (3, 16a). It seems possible that a more precise analysis of the salt dependence of T_m would provide some information on the number of base pairs involved in the dimer linkage structure. A more quantitative treatment of the steepness of the melting curve could also be useful. We also found that a low concentration of Mg^{2+} has a strongly stabilizing effect on wild-type dimers, even in the presence of a much higher concentration of monovalent ion (data not shown). This effect probably reflects a significant contribution of tertiary interactions to the stability of genomic RNA dimers, by analogy with studies on other structures in RNA (20, 32).

Two recent reports have shown that dimerization in vitro of short segments of HIV-1 RNA is dependent on G-rich sequence elements and that the resulting dimers are dramatically, specifically stabilized by K^+ ions (2, 35). These observations were taken to mean that the dimers under study were stabilized by structures similar or identical to G quartets. However, the authentic, full-length genomic RNAs extracted from wild-type or PR^- virions do not seem to exhibit this specific stabilization by K^+ (Fig. 4). Thus, we find no evidence supporting the idea that G quartets are involved in the dimeric structures present in natural genomic RNAs.

Our failure to detect stabilization by K^+ could be reconciled with the presence of G quartets in RNAs from virus particles if these structures in the viral RNAs contained, and were stabilized by, sequestered, nonexchangeable K^+ ions. However, the responsiveness of the dimers to ionic conditions (Fig. 3) appears to argue against this possibility. For example, the two forms of viral RNA melt at relatively low temperatures (approximately 40 and 45°C) in 5 mM Na^+; they do not seem to be stabilized by intrinsic or nonexchangeable structures. We also attempted to determine the melting temperature of viral RNA under the same conditions as those used by Sundquist and Heaphy (35). They reported that short dimers melt at 80°C in 10 mM Tris (pH 8.3) and 100 mM KCl, with or without 5 mM $MgCl_2$. In contrast, dimers isolated from wild-type virus particles melt in this buffer at 55°C in the absence (Fig. 3) and 65°C in the presence (not shown) of $MgCl_2$. Again, these differences suggest that the structures stabilizing the short dimers studied by Sundquist and Heaphy are not involved in the linkage of dimers present in virus particles.

A report describing the properties of several dimers formed in vitro from HIV-1 synthetic RNAs appeared very recently (24a). This study compared dimers from several subregions of the 5′ end of the HIV-1 genome with dimers of the first 707 nucleotides. The results showed that dimers containing the entire 707-nucleotide sequence behaved quite differently from dimers lacking the first 311 nucleotides. Because of this observation, Marquet et al. (24a) suggested that RNAs lacking these 311 nucleotides are not good models for the dimers of the entire region. This conclusion is consistent with our finding that genomic RNA molecules isolated from virions do not appear to be stabilized by G quartet structures.

Since the original discovery by Darlix and colleagues that retroviral RNA transcripts are capable of spontaneous dimerization in vitro (8, 13, 29), a number of studies have analyzed the structures in short dimers of HIV RNA sequences (2, 4, 7, 13, 24, 35). The results presented here point up a general problem, i.e., dimers of full-length viral RNAs, formed under in vivo conditions, may not have the same structure as dimers of short synthetic RNAs, even though the sequences in these short RNAs are wholly contained in full-length viral RNA.

As noted above, we have proposed that dimerization may be a prerequisite for encapsidation of retroviral RNAs (16). We also suggested (16) that maturation of dimeric RNA may be required for infectivity. If these hypotheses are correct, then a fuller understanding of structures present in dimeric retroviral RNAs might suggest new approaches to the inhibition of virus replication. These considerations highlight the importance of structural studies on retroviral RNA dimers.

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REFERENCES

1. **Adachi, A., H. E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, and M. A. Martin.** 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. J. Virol. **59**:284–291.
2. **Awang, G., and D. Sen.** 1993. Mode of dimerization of HIV-1 genomic RNA. Biochemistry **32**:11453–11457.
3. **Bader, J. P., and T. L. Steck.** 1969. Analysis of the ribonucleic acid of murine leukemia virus. J. Virol. **4**:454–459.
4. **Baudin, F., R. Marquet, C. Isel, J. L. Darlix, B. Ehresmann, and C. Ehresmann.** 1993. Functional sites in the 5′ region of human

immunodeficiency virus type 1 RNA form defined structural domains. J. Mol. Biol. **229**:382–397.
5. **Bender, W., Y.-H. Chien, S. Chattopadhyay, P. K. Vogt, M. B. Gardner, and N. Davidson.** 1978. High-molecular-weight RNAs of AKR, NZB, and wild mouse viruses and avian reticuloendotheliosis virus all have similar dimer structures. J. Virol. **25**:888–896.
6. **Bender, W., and N. Davidson.** 1976. Mapping of poly(A) sequences in the electron microscope reveals unusual structure of type C oncornavirus RNA molecules. Cell **7**:595–607.
7. **Berkhout, B., B. B. O. Essink, and I. Schoneveld.** 1993. In vitro dimerization of HIV-2 leader RNA in the absence of PuGGAPuA motifs. FASEB J. **7**:181–187.
8. **Bieth, E., C. Gabus, and J. L. Darlix.** 1990. A study of the dimer formation of Rous sarcoma virus RNA and its effect on viral protein synthesis in vitro. Nucleic Acids Res. **18**:119–127.
9. **Bowles, N. E., P. Damay, and P.-F. Spahr.** 1993. Effect of rearrangements and duplications of the Cys-His motifs of Rous sarcoma virus nucleocapsid protein. J. Virol. **67**:623–631.
10. **Canaani, E., and P. Duesberg.** 1972. Role of subunits of 60 to 70S avian tumor virus ribonucleic acid in its template activity for the viral deoxyribonucleic acid polymerase. J. Virol. **10**:23–31.
11. **Cheung, K.-S., R. E. Smith, M. P. Stone, and W. K. Joklik.** 1972. Comparison of immature (rapid harvest) and mature Rous sarcoma virus particles. Virology **50**:851–864.
12. **Coffin, J.** 1984. Genome structure, p. 261–368. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), RNA tumor viruses. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
13. **Darlix, J. L., C. Gabus, M. T. Nugeyre, F. Clavel, and F. Barré-Sinoussi.** 1990. *cis* elements and *trans*-acting factors involved in the RNA dimerization of the human immunodeficiency virus HIV-1. J. Mol. Biol. **216**:689–699.
14. **Duesberg, P. H.** 1968. Physical properties of Rous sarcoma virus RNA. Proc. Natl. Acad. Sci. USA **60**:1511–1518.
15. **Dupraz, P., S. Oertle, C. Meric, P. Darmay, and P.-F. Spahr.** 1990. Point mutations in the proximal Cys-His box of Rous sarcoma virus nucleocapsid protein. J. Virol. **64**:4978–4987.
16. **Fu, W., and A. Rein.** 1993. Maturation of dimeric viral RNA of Moloney murine leukemia virus. J. Virol. **67**:5443–5449.
16a. **Fu, W., and A. Rein.** Unpublished data.
17. **Gorelick, R. J., L. E. Henderson, J. P. Hanser, and A. Rein.** 1988. Point mutants of Moloney murine leukemia virus that fail to package viral RNA: evidence for specific RNA recognition by a "zinc finger-like" protein sequence. Proc. Natl. Acad. Sci. USA **85**:8420–8424.
18. **Gorelick, R. J., S. M. Nigida, Jr., J. W. Bess, Jr., L. O. Arthur, L. E. Henderson, and A. Rein.** 1990. Noninfectious human immunodeficiency virus type 1 mutants deficient in genomic RNA. J. Virol. **64**:3207–3211.
19. **Graham, F. L., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–467.
20. **Holbrook, S. R., J. L. Sussman, R. W. Warrant, G. M. Church, and S.-H. Kim.** 1977. RNA-ligand interactions: (1) magnesium binding sites in yeast $tRNA^{Phe}$. Nucleic Acids Res. **4**:2811–2820.
21. **Khandjian, E. W., and C. Méric.** 1986. A procedure for Northern blot analysis of native RNA. Anal. Biochem. **159**:227–232.
22. **Kung, H.-J., S. Hu, W. Bender, J. M. Bailey, N. Davidson, M. O. Nicolson, and R. M. McAllister.** 1976. RD-114, baboon, and woolly monkey viral RNAs compared in size and structure. Cell **7**:609–620.
23. **Levin, J. G., P. M. Grimley, J. M. Ramseur, and I. K. Berezesky.** 1974. Deficiency of 60 to 70S RNA in murine leukemia virus particles assembled in cells treated with actinomycin D. J. Virol. **14**:152–161.
24. **Marquet, R., F. Baudin, C. Gabus, J. L. Darlix, M. Mougel, C. Ehresmann, and B. Ehresmann.** 1991. Dimerization of human immunodeficiency virus (type 1) RNA: stimulation by cations and possible mechanism. Nucleic Acids Res. **19**:2349–2357.
24a. **Marquet, R., J.-C. Paillart, E. Skripkin, C. Ehresmann, and B. Ehresmann.** 1994. Dimerization of human immunodeficiency virus type 1 RNA involves sequences located upstream of the splice donor site. Nucleic Acids Res. **22**:145–151.
25. **Meric, C., and S. P. Goff.** 1989. Characterization of Moloney murine leukemia virus mutants with single-amino-acid substitutions in the Cys-His box of the nucleocapsid protein. J. Virol. **63**:1558–1568.
26. **Meric, C., and P.-F. Spahr.** 1986. Rous sarcoma virus nucleic acid-binding protein p12 is necessary for viral 70S RNA dimer formation and packaging. J. Virol. **60**:450–459.
27. **Murti, K. G., M. Bondurant, and A. Tereba.** 1981. Secondary structural features in the 70S RNAs of Moloney murine leukemia and Rous sarcoma viruses as observed by electron microscopy. J. Virol. **37**:411–419.
28. **Oertle, S., and P.-F. Spahr.** 1990. Role of the *gag* polyprotein precursor in packaging and maturation of Rous sarcoma virus genomic RNA. J. Virol. **64**:5757–5763.
29. **Prats, A. C., C. Roy, P. Wang, M. Erard, V. Housset, C. Gabus, C. Paoletti, and J. L. Darlix.** 1990. *cis* elements and *trans*-acting factors involved in dimer formation of murine leukemia virus RNA. J. Virol. **64**:774–783.
30. **Ratner, L., A. Fisher, L. L. Jagodzinski, H. Mitsuya, R.-S. Liou, R. C. Gallo, and F. Wong-Staal.** 1987. Complete nucleotide sequences of functional clones of the AIDS virus. AIDS Res. Hum. Retroviruses **3**:57–69.
31. **Roy, C., N. Tounekti, M. Mougel, J. L. Darlix, C. Paoletti, C. Ehresmann, B. Ehresmann, and J. Paoletti.** 1990. An analytical study of the dimerization of *in vitro* generated RNA of Moloney murine leukemia virus MoMuLV. Nucleic Acids Res. **18**:7287–7292.
32. **Stein, A., and D. M. Crothers.** 1976. Conformational changes of transfer RNA. The role of magnesium(II). Biochemistry **15**:160–167.
33. **Stewart, L., G. Schatz, and V. M. Vogt.** 1990. Properties of avian retrovirus particles defective in viral protease. J. Virol. **64**:5076–5092.
34. **Stoltzfus, F. M., and P. N. Snyder.** 1975. Structure of B77 sarcoma virus RNA: stabilization of RNA after packaging. J. Virol. **16**: 1161–1170.
35. **Sundquist, W. I., and S. Heaphy.** 1993. Evidence for interstrand quadruplex formation in the dimerization of human immunodeficiency virus 1 genomic RNA. Proc. Natl. Acad. Sci. USA **90**:3393–3397.
36. **Weiss, S., G. Häusl, M. Famulok, and B. König.** 1993. The multimerization state of retroviral RNA is modulated by ammonium ions and affects HIV-1 full-length cDNA synthesis in vitro. Nucleic Acids Res. **21**:4879–4885.