0273-2289/00/87/0103/$13.25

Effect of *Escherichia coli* Chaperonin GroELS on Heterologously Expressed Human Immunodeficiency Virus Type 1 Reverse Transcriptase In Vivo and In Vitro

Gottfried Maier, Elena Manakova, and Hermann Heumann*

Max-Planck-Institut für Biochemie, Am Klopferspitz 18a, D-82152 Martinsried, Germany, E-mail: heumann@biochem.mpg.de

Received July 6, 1999; Revised December 30, 1999; Accepted December 30, 1999

Abstract

The two subunits of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (HIV-1 RT), p66 and p51, were coexpressed in *Escherichia coli* along with the *E. coli* chaperonin system GroEL/GroES. Coexpression increases the yield of heterodimeric HIV-1 RT by a factor of 4 to 5 and improves the nucleic acid binding affinity of HIV-1 RT by a factor of 1.6. We have analyzed the reasons for the improvements. The total increase in yield of HIV-1 RT can be attributed to an accumulation of RT subunits in the cells (factor of about 2.8) and an increased growth of the *E. coli* cells (factor of about 1.4). One reason for the accumulation in the cells is an improved stability of HIV-1 RT subunits toward bacterial proteases. In vitro studies showed that the nucleic acid binding affinity of HIV-1 RT purified from cells that did not coexpress GroELS was stimulated by adding purified GroELS (approx 1.5-fold), whereas HIV-1 RT stemming from cells coexpressing GroELS was stimulated only marginally (approx 1.1-fold). The in vivo as well as the in vitro studies suggest that the chaperonin interacts with HIV-1 RT and therefore affects the folding process of HIV-1 RT.

Index Entries: GroE; chaperonin; human immunodeficiency virus type 1; reverse transcriptase; coexpression.

Introduction

Molecular chaperones can assist folding of proteins into the active state (for reviews, *see* refs. *1* and *2*). Chaperones that can participate in

*Author to whom all correspondence and reprint requests should be addressed.

Escherichia coli in the folding of proteins after ribosomal synthesis are the chaperone hsp70 system (DnaK system) and the chaperone hsp60 system (GroE system). Both systems can subsequently interact with the protein *in statu nascendi*, thereby improving folding of the protein. This property of chaperons has been exploited to improve folding of heterologously expressed protein. It has been shown that coexpression of GroELS improves folding and assembly of Rubisco *(3)* as well as the yield of recombinant proteins *(4,5)*. It has been reported that overproduction of chaperones prevents inclusion body formation and increases the solubility of recombinant proteins *(6–9)*. Despite these examples, the molecular basis of the improvement by coexpression of GroELS is not clear.

In this study, we investigated the effect of overproduced GroELS on the yield and quality of recombinant reverse transcriptase from human immunodeficiency virus type 1 (HIV-1 RT). We also investigated the in vitro effect of purified GroELS on the specific activity of HIV-1 RT. Our aim was to determine the processes responsible for the improved expression. HIV-1 RT was coexpressed with the chaperonin system GroELS, which consists of two components, the main component GroEL and the cochaperonin GroES. Both components were overexpressed in *E. coli*.

HIV-1 RT is a DNA polymerase also carrying an RNase H activity. It catalyzes transcription of viral single-stranded RNA into proviral double-stranded DNA (dsDNA) (for reviews, *see* refs. *10* and *11*). HIV-1 RT is a heterodimer consisting of the subunits p51 and p66. Both subunits are derived from the same gene product by viral protease cleavage and have in common the same N-terminal part *(12)*. Because of its key function in viral replication, HIV-1 RT is one of the most important targets in suppressing HIV amplification. Nucleosidic as well as nonnucleosidic inhibitors are used as drugs to inhibit HIV-replication. To study the mechanism of inhibition as well as of resistance formation in vitro, recombinant HIV-1 RT is required. Expression of HIV-1 RT in *E. coli* (for an overview see, e.g., ref. *13*) is complicated owing to partial degradation of p66 by bacterial proteases that can lead to p51 derivatives *(14)*. This side reaction is especially detrimental, when (as we intend to do) subunit-specific mutagenesis is performed with the aim of obtaining information about the function of HIV-1 RT subunits. Mixed reconstitution of HIV-1 RT from, e.g., wild-type p66 and mutated p51 using subunit-specific tags is one possibility of minimizing unwanted wild-type p66/p51 reconstitutes arising from cleavage of p66 by bacterial proteases yielding p51 derivatives *(15)*. Because dimerization of p51 and p66 enhances the stability toward bacterial proteases, coexpression of both subunits is one possible way of reducing degradation by *E. coli* proteases *(15–17)*. Coexpression of both HIV-1 RT subunits along with the chaperonin system GroELS should improve folding and, as a consequence, heterodimer formation and stability. We show herein that GroELS enhances cell growth, facilitates protein accumulation, and increases protease resistance and nucleic acid binding affinity of HIV-1 RT.

Materials and Methods

Plasmids, Strains, and Nucleic Acids

Plasmids for expression of p66 containing a C-terminal His-tag and of p51 carrying glutathione-*S*-transferase (GST) at the N-terminus have been described previously *(15)*. Plasmid pOF39 was a gift from A. Plückthun. *E. coli* strain M15 was purchased from Qiagen, and strain BL21 from Novagen. The nucleic acid sequence encoding the sequence of HIV-1 RT (isolate designated D148; *[18]*) is deposited at the European Molecular Biology Laboratory (EMBL) database (accession no. AJ011836). DNA oligonucleotides (high-performance liquid chromatography purified) were purchased from Interactiva (Ulm, Germany).

Oligonucleotides used in RT activity or band-shift assays were purified on a 12% polyacrylamide-7 *M* urea gel containing 50 m*M* Tris-borate, pH 8.0, and 0.1 m*M* EDTA. The concentration of nucleic acids was determined spectrophotometrically. Oligonucleotides were radioactively labeled at the 5' end with [γ-^{32}P] adenosine triphosphate (ATP) using T4 polynucleotide kinase (Boehringer Mannheim) according to the manufacturer's recommendation. The labeled oligonucleotides were again electrophoretically purified.

Electrophoretic Methods

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli *(19)* with a modified loading buffer (100 m*M* Tris-HCl, pH 6.8; 10% [v/v] glycerol; 4% SDS; 0.01% bromophenol blue; 5% 2-mercaptoethanol; 0.1 *M* $MgCl_2$). $MgCl_2$ was added to remove chromosomal DNA *(20)*. Proteins were stained in gels by combined use of Coomassie brilliant blue R-250 and Bismarck brown R following the protocol of Choi et al. *(21)*. Agarose gels were prepared according to Sambrook et al. *(22)*. To circumvent the problem of UV damaging, 1 m*M* guanosine was added to the electrophoresis buffer as described by Gründemann and Schömig *(23)*. DNA purification from agarose gels was carried out as described by Boyle and Lew *(24)*.

Native polyacrylamide gels (4%, 20 cm long) for band-shift assays were run at room temperature for 2 h (80 V). Buffer conditions were 20 m*M* Tris-HCl, pH 8; 5 m*M* NaCl; and 0.1 m*M* EDTA. For gel densitometric analysis, Bio-Rad Imaging Densitometer GS-690 with the software Molecular Analyst® was used. PhosphorImaging was used for viewing band shifts. Binding was quantified by means of MacBAS software.

Cloning

Plasmid p66H/Gro (Fig. 1) is a derivative of p66His *(15)* that carries a copy of *groEL* and *groES* under the control of the wild-type promoter. An *Eco*RI-*Hin*dIII fragment excised from pOF39 *(25)* was inserted into *Xba*I-cleaved p66His after blunting with Klenow fragment. Both orientations of the insert showed essentially the same coexpression efficiency.

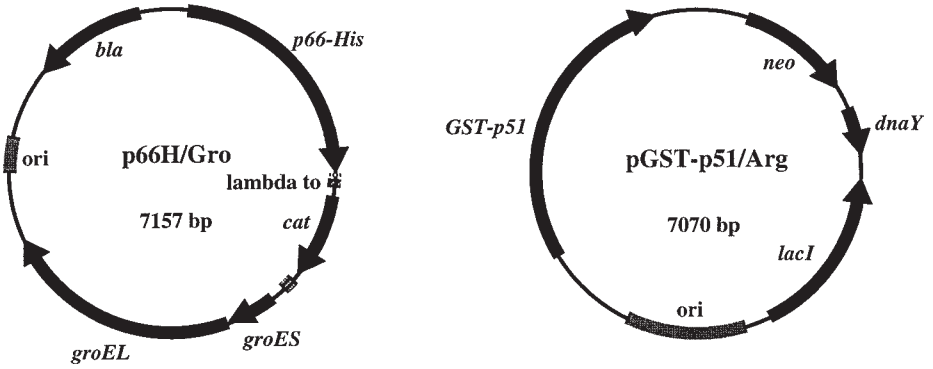

Fig. 1. Plasmids of the HIV-1 RT/GroELS coexpression system. p66H/Gro, plasmid for expression of p66 with a $(His)_6$-tag at its C-terminus (p66-His), isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible promoter, GroELS wild-type promoter; pGST-p51/Arg, plasmid for expression of p51 N-terminally fused to glutathione S-transferase (GST-p51), IPTG-inducible promoter, concomitant expression of $tRNA^{Arg4}$ (*dnaY*) and lac repressor (*lac*I); *ori ColE1*, origin of replication from pBR322; *ori pUB110*, replicon derived from pUB110 *(30)*; *bla*, β-lactamase gene; *cat*, chloramphenicol acetyltransferase gene; lambda to, transcriptional terminator to from phage lambda; T1/rrnB *E. c.*, t1 transcriptional terminator from the rrnB operon of *E. coli*; *lac*I, lac repressor gene; *neo*, aminoglycoside 3'-phosphotransferase gene, conferring resistance to kanamycin.

Expression and Purification of Protein

Expression and purification of HIV-1 RT heterodimer coexpressed with GroELS was performed in the same way as recently described for expression of HIV-1 RT alone *(15)*. Homodimer p66-His was expressed in the *E. coli* strain BL21 carrying plasmid p66H/Gro (Fig. 1) and plasmid p66His *(15)*, respectively. Purification was started with chromatography on an Ni-NTA column using conditions described in ref. *15*. The concentration of HIV-1 RT was determined by A_{280} measurements using an extinction coefficient of ε_{280nm} = 1.4 mg/(mL·cm). Preparation of GroEL/GroES and determination of concentrations was carried out as described previously *(26)*.

Preparation of Primer/Template

DNA primer/template was prehybridized before incubation with HIV-1 RT. A mixture containing the template strand (1.2 μ*M*) and ^{32}P-labeled primer (1 μ*M*) in a buffer containing 50 m*M* Tris-HCl, pH 7.8, and 50 m*M* NaCl was heated to 95°C for 2 min followed by incubation at 72°C for 10 min and cooling for 20 min to room temperature. Complete hybridization was verified by native PAGE.

Band-Shift Analysis of RT Binding to Primer/Template in Absence and Presence of GroELS

Annealed primer/template substrate (100 n*M*) was incubated for 5 min at 37°C with HIV-1 RT (10–200 n*M*) in the absence or presence, respectively,

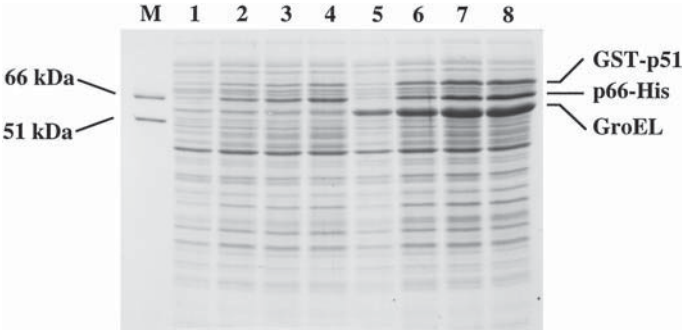

Fig. 2. Time dependence of the yield of HIV-1 RT expressed with and without overexpressed GroELS. (**Left**) (lanes 1–4) displays the protein expression without GroELS and **(right)** (lanes 5–8) with GroELS. Lanes 1 and 5 show the protein pattern directly after induction, lanes 2 and 6 after 2 h, lanes 3 and 7 after 4 h, lanes 4 and 8 after 6 h. The position of the subunits p66-His and GST-p51 and the GroEL subunits are indicated. M, the position of the subunits p66 and p51 for comparison (fifty microliters of the cell culture was loaded on each lane of the SDS-PAGE).

of GroEL, GroES, and/or bovine serum albumin (BSA) in a buffer containing 50 m*M* Tris-HCl, pH 7.8; 5 m*M* NaCl; 6 m*M* $MgCl_2$; and 30 m*M* KCl. ATP, if present, was at 10 m*M*. The first nucleotide was given as ddATP (0.5 m*M*) to stabilize the complex. The primer was 5'-CTTTCAGGTCCC TGTTCGGGCGCCA-3', and the template was 5'-GCCCGGAACCCT TCTGGTTCCCCTTTGGCGCCCGAACAGGGACCTGAAAGCGAAAG-3'. For band-shift analysis, 0.5 μL of loading buffer was added per 10 μL of sample, and 5 μL was applied on a non-denaturing gel (*see* Electrophoretic Methods). Loading buffer contained 20 m*M* Tris-HCl, pH 8.0; 0.02% bromophenol blue; and 40% sucrose.

Results

Coexpression of RT/GroELS

We have recently published an article on an expression system permitting coexpression of both HIV-1 RT subunits, p66-His and GST-p51 *(15)*. By inserting the genes *groES* and *groEL* controlled by their wild-type promoter into the p66-His subunit–expressing plasmid, as shown in Fig. 1, we analyzed the influence of the chaperonin GroELS overexpression on RT expression. The time-dependent effect of GroELS synthesis was analyzed on the SDS gel of the lysates shown in Fig. 2. Equal amounts of cell culture medium were loaded on each lane. Analysis of the protein expression without (lanes 1–4 in Fig. 2) and with (lanes 6–8 in Fig. 2) concomitant overproduction of GroELS shows that not only the RT subunits were synthesized with higher yield but that also the total amount of protein increased. This is owing to an increase in the growth rate, as the curves in Fig. 3 show. Cells expressing RT and GroELS reach a 1.4-fold higher optical density than those without GroELS overproduction 6 h after induction.

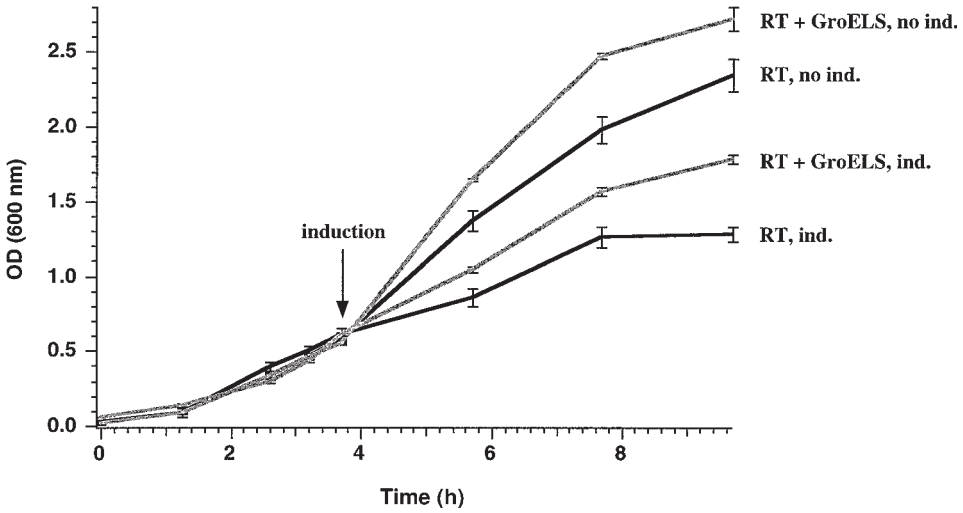

Fig. 3. Time-dependent growth of *E. coli* cells that express HIV-1 RT with and without GroELS. RT, growth curves obtained with the strain JM109 that harbors the plasmids pGST-p51/Arg (Fig. 1) and p66His *(15)*; RT + GroELS, growth curves obtained with the strain JM110 that harbors pGST-p51/Arg and p66H/Gro (Fig. 1); ind. and no ind., induction with IPTG (1 m*M*) at OD_{600nm} = 0.6 and no induction with IPTG, respectively. The time point of induction is indicated by an arrow.

As expected, the noninduced cells grow to higher density compared to the induced cultures.

Figure 4 shows the effect of GroELS on the accumulation of HIV-RT. To differentiate better the effect of GroELS on the cell growth (demonstrated in Fig. 2) from the increased accumulation of HIV-RT (*see* Fig. 4), equal amounts of cell mass were applied on each lane of the SDS-PAGE. Soluble and insoluble fractions were analyzed separately. The lysate was centrifuged. The supernatant and the resuspended pellet were applied on a gel. Although the yield of HIV-1 RT subunits in the insoluble fraction (pellet) was essentially the same regardless of whether GroELS was coexpressed or not, it differed in the soluble fraction (supernatant), as comparison of lanes 3 and 6, and lanes 2 and 5, respectively, in Fig. 4A indicates. The densitometric analysis (Fig. 4B) of lanes 1 and 4 of Fig. 4A permitted us to quantify the enhancement of the yield of HIV-1 RT subunits. p66-His expression was higher by a factor of 1.5 ± 0.2 and GST-p51 expression by a factor of 2.8 ± 0.3 when GroELS was coexpressed. This experiment was performed three times and yielded the same result with slight variation, as indicated by error bars.

The molecular basis for the enhancement of HIV-1 RT subunit accumulation is not clear in detail. One reason is improvement of the stability of the subunits toward proteolytic degradation. It is a well-recognized problem that p66 degrades into smaller products during expression in *E. coli* and that the degradation products (PR) having a molecular size of 66 kDa < PR

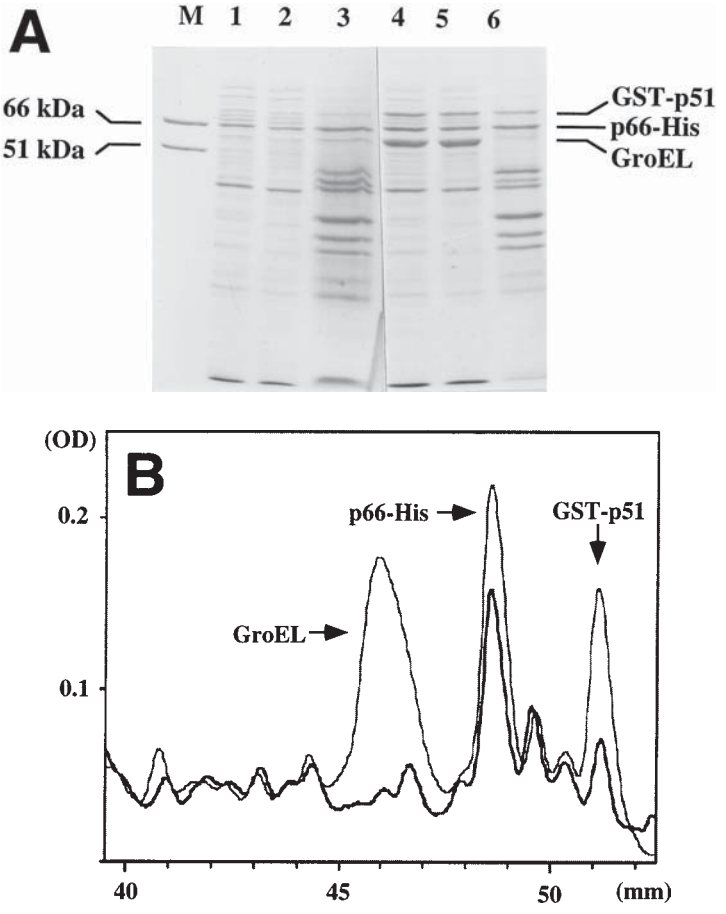

Fig. 4. **(A)** Effect of GroELS coexpression on the solubility of HIV-1 RT subunits. *E. coli* cells expressing HIV-1 RT subunits in the absence (lanes 1–3) and presence (lanes 4–6) of overproduced GroELS are analyzed by SDS-PAGE. Lanes 1 and 4 (with GroELS) show the lysate, lanes 2 and 5 (with GroELS) the supernatant after centrifugation of the lysate, and lanes 3 and 6 (with GroELS) the resuspended pellet. The cells were induced by IPTG (1 m*M*) at OD_{600nm} = 0.6 and were harvested 5 h after induction. Equal amounts of cells were applied equivalent to 50 µL of cell culture at OD_{600nm} = 1. M, molecular weight markers. **(B)** Densitometric analysis of the yield of HIV-1 RT subunits coexpressed with and without overproduced GroELS. There are intensity scans of the lanes 1 and 4 in (A).

≤ 51 kDa can form heterodimers with p66 *(14)*. We have shown that GroELS can suppress formation of these degradation products. Figure 1 shows expression of His-tagged p66 coexpressed with and without GroELS using plasmid p66H/Gro and plasmid p66His *(15)*, respectively. p66-His from both strains was purified by Ni-NTA affinity chromatography and analyzed on SDS gel, as shown in Fig. 5, lanes 1–4 (without GroELS) and lanes 5–8 (with GroELS). Ni-NTA affinity chromatography ensures that only C-terminally His-tagged p66 and its degradation products are found in the eluate. While the C-terminal fragments are usually small (up to

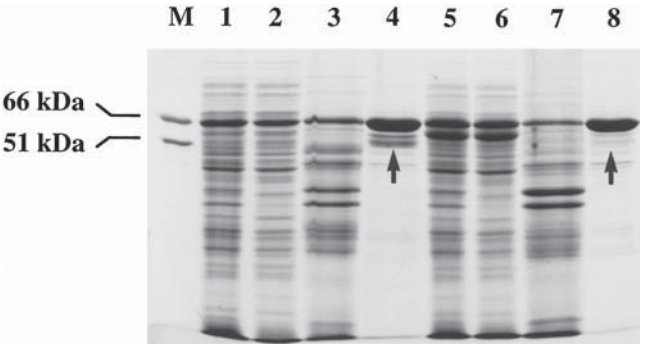

Fig. 5. Effect of overproduced GroELS on the stability of p66. HIV-1 RT subunit p66-His was expressed in the absence (lanes 1–4) and presence (lanes 5–8) of overproduced GroELS and analyzed on SDS gel. Whole-cell lysates were applied on lane 1 and 5, respectively, and the supernatant is shown in lanes 2 and 6, and insoluble fractions after resuspension in lanes 3 and 7, respectively. The amounts applied in each lane were equivalent to 100 μL of cell culture at OD_{600nm} = 1. Lanes 4 and 8, respectively, show samples (5 μg of protein each) of the eluate from Ni-NTA column that is the first purification step (*see* Materials and Methods). Arrows indicate the proteolytic products in lane 4 and their reduction by GroELS overproduction (lane 8). M, molecular weight markers.

approx 15 kDa *[14]* and probably further degraded into smaller products), the N-terminal fragments only can appear in the eluate if they form stable heterodimers with p66. That the fragments shown by the arrow in Fig. 5, lane 4 are carboxyterminal proteolytic cleavage products has been shown previously by sequencing *(14)*. Comparison of lanes 4 and 8 in Fig. 5 shows that these cleavage products (see arrows) disappear if HIV-1 RT is coexpressed with GroELS. This result is relevant not only with respect to improvement of the yield of p66 subunit. It is even more important for reconstitution experiments using mutant HIV-1 RT subunits. Proteolytically cleaved p66 can bind instead of authentic p51 and thus serves as a counterpart of p66 in the heterodimeric enzyme.

Lanes 1 and 5 in Fig. 5 confirm the previous result, namely, that coexpression of GroELS improves the yield of p66 by a factor of 1.5 (Fig. 5, lanes 1 and 5), and lanes 2, 3, 6, and 7 show that the yield of the insoluble HIV-1 RT fraction remains unchanged on coexpression with GroELS.

Densitometric analysis of crude extract shows that about 10% of the totally expressed protein is the heterologous RT corresponding to 15–20 mg of RT/1 L of cell culture (Fig. 4A,B). Using our purification protocol, about 50% of the expressed RT is recovered as purified enzyme. This value is substantially higher than reported values of 5–20% (*see* ref. *27* and references therein).

In Vitro Analysis of RT-GroELS Interaction Using Band-Shift Assays

The enhancement of the stability of p66 toward degradation in the presence of GroELS, as already reported, suggests a direct interaction of GroELS with the coexpressed HIV-1 RT or its subunits. This in vivo finding

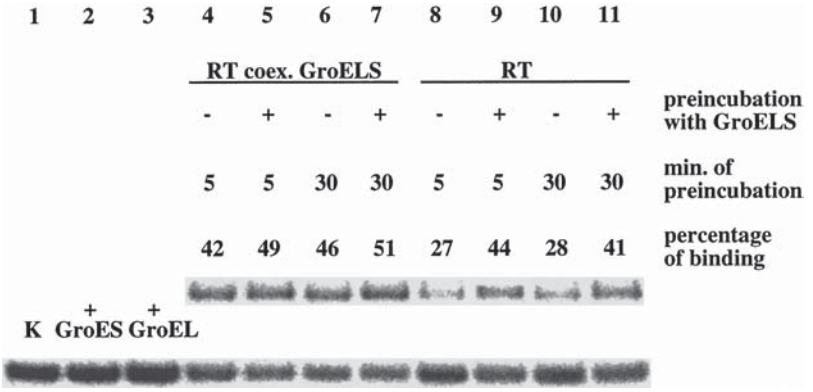

Fig. 6. Effect of GroELS on the DNA-binding affinity of HIV-RT expressed with and without GroELS. Complexes of purified HIV-1 RT (100 n*M*) and dsDNA primer/template (100 n*M*) were incubated at 37°C in a volume of 10 μL (50 m*M* Tris-HCl, pH 7.8, 5 m*M* NaCl, 6 m*M* $MgCl_2$, 10 m*M* ATP, 0.5 m*M* ddATP, and 30 m*M* KCl) at the time intervals indicated and were applied on a nondenaturing gel as described in Materials and Methods. HIV-1 RT was obtained from cells with (**left**, lanes 4–7) and without (**right**, lanes 8–11) coexpressing GroELS. The binding reaction was performed with (+) and without (–) addition of purified GroELS. The molar ratio of HIV-1 RT to primer/template was kept constant at 1:1, and the molar ratio of HIV-1 RT to GroELS asymmetric complex was 1:1.5 (150 n*M* $GroEL_{14} \cdot GroES_7$). Lanes 1–3 are controls: lane 1, free primer/template; lane 2, primer/template + GroES; lane 3, primer/template + GroEL.

is confirmed by an in vitro study of the interaction of primer/template and HIV-1 RT with and without purified GroELS using a band-shift assay, as described in Materials and Methods. HIV-1 RT purified from cells expressing HIV-1 RT alone or in combination with GroELS was incubated with primer/template at a 1:1 ratio for 5 and 30 min, respectively, and analyzed on a nondenaturing gel. The 5- and 30-min values are the same within the error margins, indicating that the binding reaction is completed within 5 min. We refer to the 30-min values. Lane 10 in Fig. 6 shows that approx 28% of the HIV-1 RT fraction was bound when the enzyme was purified from cells that do not coexpress GroELS. With GroELS coexpressed, the specific binding activity of HIV-1 RT was enhanced, yielding 46% active enzyme (lane 6). Most interesting is the finding that the binding affinity of HIV-1 RT obtained from cells without coexpressing GroELS could be partially restored. By adding purified GroELS, the specific binding activity increased from 28 (lane 10) to 41% (lane 11). This experiment was repeated three times, indicating an average increase of 1.6 ± 0.2. This is close to the level of HIV-1 RT coexpressed with GroELS (46%) (lane 6). That the GroELS effect is a specific effect in vivo is supported by the finding that HIV-1 RT obtained from cells coexpressing GroELS could only be marginally stimulated from 46% (lane 6) to 51% (lane 7) by adding GroELS.

These experiments suggest that GroELS enhances the nucleic acid binding activity of HIV-1 RT by an interaction of HIV-1 RT with GroELS,

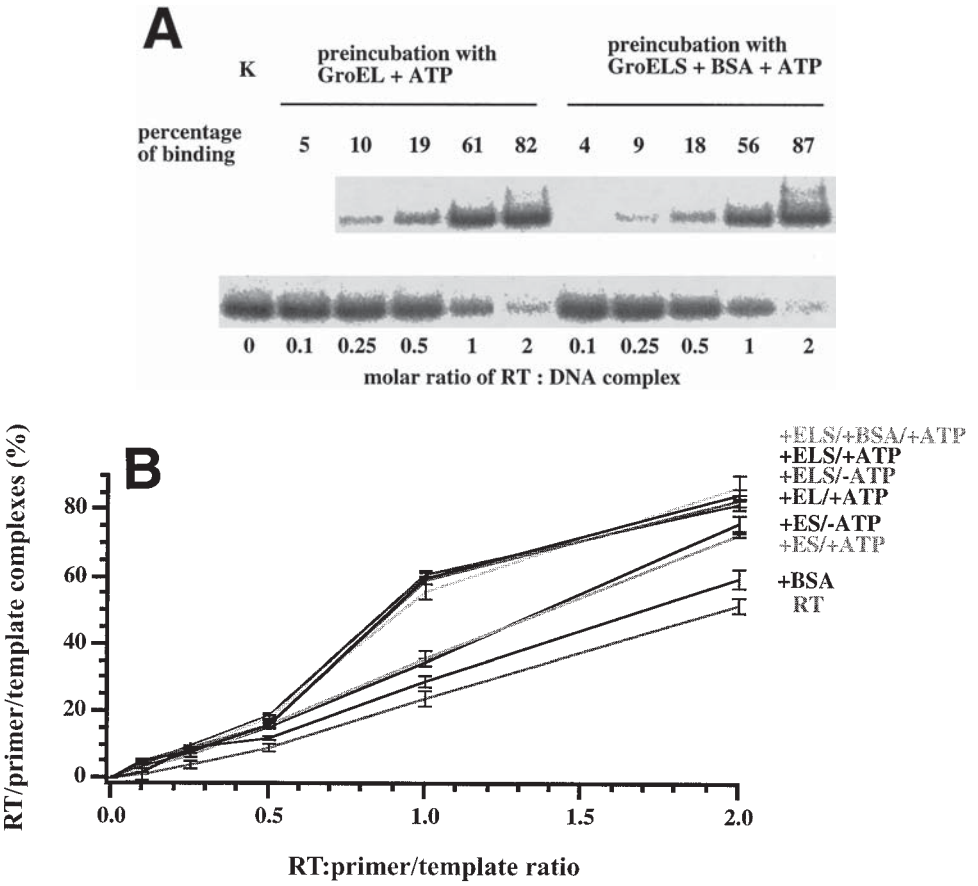

Fig. 7. Analysis of the influence of GroEL, GroES, and ATP on the yield of active HIV-1 RT using nondenaturing gel electrophoresis. HIV-1 RT was obtained from cells without coexpressing GroELS. HIV-1 RT and the analyzed components (GroEL, GroES, ATP, and BSA) were incubated for 5 min at 37°C at the indicated molar ratios with 100 n*M* primer/template in a total volume of 10 μL (50 m*M* Tris-HCl, pH 7.8; 5 m*M* NaCl; 6 m*M* $MgCl_2$; 0.5 m*M* ddATP; and 30 m*M* KCl) and applied on a nondenaturing gel as described in Materials and Methods. The concentration of GroELS asymmetric complex was 200 n*M*, that of ATP was 10 m*M*, and that of BSA was 200 n*M*. **(A)** Gel analysis of the effect of GroEL + ATP (**left**) and GroELS + BSA + ATP (**right**). Annealed primer/template substrate (100 n*M*) was incubated for 5 min with HIV-1 RT. Lane designated K is a control containing primer/template, GroELS, BSA, and ATP but not HIV-1 RT. **(B)** Plot of the complex yield vs the molar ratio of HIV-1 RT/DNA. The components that were analyzed are indicated at the left side of plot. The error margins show the statistical error from two or three independent measurements.

probably by supporting folding of HIV-1 RT into the active conformation. It is well established that the folding activity of GroELS is an ATP-dependent process *(1)*. But it has also been reported that the main chaperonin GroEL alone can assist reactivation of denatured protein even without ATP *(28)*. To analyze which of the components of the chaperonin cycle—GroEL,

GroES, or ATP—contributes to the enhanced binding affinity of HIV-1 RT in vitro, HIV-1 RT-primer/template affinity was determined at different molar ratios of RT and primer/template at constant chaperonin concentrations.

Figure 7A shows how binding affinity was determined by nondenaturing gel analysis, and Fig. 7B summarizes the results when complex formation was plotted vs the ratio of HIV-1 RT:primer/template using the conditions described in Fig. 7.

To analyze whether the chaperonin effect is specific, the binding reaction was performed in the presence and absence of BSA. Figure 7B shows that BSA had a minor influence, which is in accordance with previously published results *(29)*. In the presence of chaperonin GroEL, there was a significant increase in the HIV-1 RT DNA-binding affinity above a molar ratio of 0.5. This sigmoidal increase in the binding affinity suggests that the interaction between GroEL and HIV-1 RT is specific. The shape of the curve would be compatible with the assumption that two binding equilibria are superimposed: HIV-1 RT with DNA and HIV-1 RT with GroEL. The chaperonin-dependent enhancement of the binding affinity of HIV-1 RT does not require ATP, indicating that GroEL interacts with HIV-RT without performing the classical folding cycle that requires ATP *(1)*.

The stimulation effect is the same with GroEL alone and with GroEL combined with GroES (Fig. 7B). The cochaperonin GroES is obviously not required in the folding cycle when HIV-1 RT is the substrate. GroES without GroEL stimulates HIV-1 RT binding, but to a lesser extent than GroEL alone and significantly higher than BSA. This finding is compatible with the assumption that HIV-1 RT interacts with GroES, but only in the isolated state. The shape of the stimulation curve is linear, as observed with BSA, suggesting that the interaction of HIV-1 RT with BSA as well as with GroES is weak.

Discussion

Coexpression of *E. coli* chaperonin GroELS enhanced the yield and nucleic acid binding affinity of heterologously expressed HIV-1 RT by a factor 4 to 5 and 1.6, respectively, and protects HIV-1 RT toward proteolytic attack during maturation. We were able to show that the major factor contributing to the enhanced yield of HIV-1 RT is the increase in HIV-1 RT subunit accumulation in the cells, more precisely a 2.8-fold increase of GST-p51, which is the subunit limiting the yield of HIV-1 RT heterodimer formation in our expression system. One reason for the observed increase in subunit synthesis, especially of GST-p51, could be a storage function of the overproduced GroELS by binding of HIV-1 RT subunits.

Binding of HIV-1 RT subunits to GroELS might also be the reason for the enhanced stability of HIV-1 RT subunits toward bacterial protease attack. A similar effect was observed with procollagenase coexpressed with GroELS *(4)*. This protective effect of GroELS is especially valuable in obtaining pure populations of HIV-1 RT, mixed reconstituted from mutated

and wild-type subunits. Thus, contamination by HIV-1 RT molecules containing the same mutation or wild-type sequence in both subunits is suppressed.

The in vitro data suggest that the chaperonin-dependent stimulation of the DNA-binding affinity of HIV-1 RT is owing to interaction of HIV-1 RT with the main chaperonin GroEL. But a complex between the chaperonin GroEL and HIV-1 RT subunits could not be demonstrated, although the stimulation effect could be attributed specifically to GroEL. Probably the lifetime of the HIV-1 RT–GroEL complex is too short to survive the band-shift procedure.

There are few studies about the effect of GroELS overexpression on the bacterial growth rate. But note that coexpression of GroELS can have opposite effects on the growth rate depending on the type of expressed recombinant protein. Whereas the growth rate increases when HIV-1 RT is expressed (our study), it decreases when β-glucosidase is expressed *(9)*. A further difference between these two substrate proteins is the differential effect of GroELS coexpression on the solubility of these enzymes. Whereas the insoluble fraction of β-glucosidase is reduced by coexpression with GroELS, it remains constant with HIV-1 RT. The reason for this difference is not known but might reflect differences in the folding pathway of both enzymes. We suggest that the chaperonin-dependent folding pathways of recombinant proteins—maybe owing to differences in the requirement for ATP in the folding cycle—affect the concentration of free intracellular protein, which limits the growth rate.

Acknowledgment

This work was supported by Deutsche Forschungs-Gemeinschaft (DFG) grant He 1285/8-3.

References

1. Hartl, F. U. (1996), *Nature* **381,** 571–580.
2. Beissinger, M. and Buchner, J. (1998), *Biol. Chem.* **379,** 245–259.
3. Goloubinoff, P., Gatenby, A. A., and Lorimer, G. H. (1989), *Nature* **337,** 44–47.
4. Lee, S. C. and Olins, P. O. (1992), *J. Biol. Chem.* **267,** 2849–2852.
5. Wynn, R. M., Davie, J. R., Cox, R. P., and Chuang, D. T. (1992), *J. Biol. Chem.* **267,** 12,400–12,403.
6. Dale, G. E., Schönfeld, H.-J., Langen, H., and Stieger, M. (1994), *Protein Eng.* **7,** 925–931.
7. Amrein, K. E., Takacs, B., Stieger, M., Molnos, J., Flint, N. A., and Burn, P. (1995), *Proc. Natl. Acad. Sci. USA* **92,** 1048–1052.
8. Jeong, W., Shin, N.-K., and Shin, H.-C. (1997), *Biotechnol. Lett.* **19,** 579–582.
9. Machida, S., Yu, Y., Singh, S. P., Kim, J.-D., Hayashi, K., and Kawata, Y. (1998), *FEMS Microbiol. Lett.* **159,** 41–46.
10. Skalka, A.-M. and Goff, S. P., eds. (1993), *Reverse Transcriptase*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
11. Arts, E. J. and Le Grice, S. F. J. (1998), in *Prog. Nucleic Acid Res.*, vol. 58, Moldave, K., ed., Academic Press, pp. 339–393.
12. Lightfoote, M. M., Coligan, J. E., Folks, T. M., Fauci, A. S., Martin, M. A., and Venkatesan, S. (1986), *J. Virol.* **60,** 771–775.

13. Stahlhut, M. W. and Olsen, D. B. (1996), *Methods Enzymol.* **275,** 122–133.
14. Lowe, D. M., Bradley, C., Darby, G. K., Larder, B. A., Powell, K. L., Purifoy, D. J. M., Tisdale, M., and Stammers, D. K. (1988), *Biochemistry* **27,** 8884–8889.
15. Maier, G., Dietrich, U., Panhans, B., Schröder, B., Rübsamen-Waigmann, H., Cellai, L., Hermann, T., and Heumann, H. (1999), *Eur. J. Biochem.* **261,** 10–18.
16. Müller, B., Restle, T., Weiss, S., Gautel, M., Sczakiel, G., and Goody, R. S. (1989), *J. Biol. Chem.* **264,** 13,975–13,978.
17. Jonckheere, H., De Vreese, K., Debeyser, Z., Vanderkerckhove, J., Balzarini, J., Desmyter, J., De Clercq, E., and Anné, J. (1996), *J. Virol. Methods* **61,** 113–125.
18. Schröder, B. (1993), PhD thesis, Heinrich-Heine-University, Düsseldorf, Germany.
19. Laemmli, U. K. (1970), *Nature* **227,** 680–685.
20. Chen, M. and Christen, P. (1997), *Anal. Biochem.* **246,** 263, 264.
21. Choi, J.-K., Yoon, S.-H., Hong, H.-Y., Choi, D.-K., and Yoo, G.-S. (1996), *Anal. Biochem.* **236,** 82–84.
22. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989), *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor University Press, Cold Spring Harbor, NY.
23. Gründemann, D. and Schömig, E. (1996), *BioTechniques* **21,** 898–903.
24. Boyle, J. S. and Lew, A. M. (1995), *Trends Genetics* **11,** 8.
25. Fayet, O., Louarn, J.-M., and Georgopoulos, C. (1986), *Mol. Gen. Genetics* **202,** 435–445.
26. Stegmann, R., Manakova, E., Rößle, M., Heumann, H., Nieba-Axmann, S. E., Plückthun, A., Hermann, T., May, R. P., and Wiedemann, A. (1998), *J. Struct. Biol.* **121,** 30–40.
27. Koller, G., Graumann, W., Kramer, W., Sara, M., and Jungbauer, A. (1995), *J. Chromatogr. B* **664,** 107–118.
28. Wang, J. D., Michelitsch, M. D., and Weissman, J. S. (1998), *Proc. Natl. Acad. Sci. USA* **95,** 12,163–12,168.
29. Yagi, T. (1990), *Kansenshogaku Zasshi—J. Jpn. Assoc. Infect. Dis.* **64,** 847–853.
30. Le Grice, S. F. J. (1990), *Methods Enzymol.* **185,** 201–214.