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Laboratory-scale production and purification of recombinant HIV-1 reverse transcriptase

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Abstract

HIV-1 reverse transcriptase from the HIV-1 strain WMF 1.13 was expressed in *Escherichia coli* JM 105 using a pKK233-2 vector. The bacteria were cultivated in a 20-l fermentor with 14-l net volume using M9ZB medium containing bactotryptone and yeast extract. After induction of reverse transcriptase (RT) expression by addition of isopropyl-β-D-thiogalactopyranoside the enzyme concentration was monitored. Both soluble and inclusion-body deposited RT were detected by Western blots. Inclusion-body formation was confirmed by transmission electron microscopy. Further purification of soluble and insoluble RT was investigated. After cell desintegration by enzymatic treatment combined with osmotic shock and centrifugation, the supernatant was desalted by size-exclusion chromatography and further purified by DEAE-Sepharose FF, AF-Heparin Toyopearl 650 M and Fractogel EMD TMAE 650 (S). The results of the purification steps were monitored by SDS-PAGE with silver staining, non-radioactive RT assay and protein determination with Coomassie Blue. The sediment was extracted with 6 *M* GuHCl and after clarification and conventional refolding, treated in the same manner as soluble RT. This method is well suited for studying fermentation conditions as well as purification conditions. The RT is expressed in approximately equal amounts as soluble and insoluble enzyme.

1. Introduction

HIV-1 reverse transcriptase (RT) is the only enzyme of the human immunodeficiency virus that is necessary for formation of proviral DNA from viral RNA. Selective inhibition of HIV-1 RT is very popular for the screening of chemotherapeutics [1–3].

HIV-1 RT is a product of the pol gene and is characterized as a dimer of p66 and p51. The amino termini of both polypeptide chains are identical. The difference between the subunits originates from proteolytic cleavage of a small peptide (p15) from the C-terminus of p66; p66 consists of 560 amino acids and the subunits of the heterodimer are not covalently linked. The theoretical isoelectric point (IEP) is 9.2; the practical IEP was determined by chromatofocussing to be at ca. pH 8.0 [1,3–5].

HIV-1 RT can be expressed as active enzyme in *E. coli*. HIV-1 RT could be found both in the cytoplasm and in an insoluble form. Bhikhabhai [5] summarized a series of purification procedures. Common to all these purification schemes are the complexity and the low overall yield. Many purification procedures consist of a pre-

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cipitation by ammonium sulfate or poly(ethylene glycol) (PEG); an alternative precipitation method is chromatography over phosphocellulose or a combination of both methods. After this initial purification step a series of chromatographic methods is applied. These include combinations of ion-exchange chromatography and affinity chromatography using heparin or ssDNA. Size exclusion chromatography is used as final purification step.

The aim of the present work was to compare the production of cytoplasmatic and paracrystalline RT.

2. Experimental

2.1. Clone

E. coli JM105 transfected with the plasmid pKKRT66 expressing active HIV-1 reverse transcriptase was a gift from X. Wolf (Institute of Virology, University of Regensburg, Regensburg, Germany) [6]. Briefly, the RT 66-gene was isolated from the HIV-1 strain WMF 1.13 and cloned into a pKK233-2 expression vector [7]. pKK233-2 contains an ampicillin resistance gene and a strong trc promotor. The promotor is controlled by the lac repressor and it can be induced by IPTG (isopropyl-β-D-thiogalactopyronside).

2.2. Fermentation

Starting from a pure culture the *E. coli* JM105 (pKKRT66) cells were grown at 37°C in a 20-l fermenter with 14-l net volume in M9ZB-medium (10 g/l bactotryptone, 5 g/l yeast extract, 5 g/l NaCl, 1 g/l NH_4Cl, 3 g/l KH_2PO_4, 6 g/l Na_2HPO_4, 4 g/l glucose, 0.25 g/l $MgSO_4$) to an absorbance at 600 nm of 1.0. The cells were induced with 1 m*M*/l IPTG at 37°C for 4–5 h and then harvested by centrifugation. The cells were stored at −80°C.

2.3. Transmission electron microscopy

Ultrathin sectioning of whole cells was performed as described previously by Messner et al. [8].

2.4. Purification

Disintegration

The cell pellet (5 g) was suspended in 45 ml of lysis buffer [5 m*M* EDTA, 20 m*M* Tris-HCl (pH 8.0), 10 m*M* $MgCl_2$, 10 m*M* monothioglycerol (MTG), 500 μl/l β-mercaptoethanol, 0.1% PMSF] containing lysozyme (1 mg/ml), RNAse A (15 μg/ml) and DNAse I (15 U/ml). After incubation for 10 min at 37°C, Triton X-100 (final concentration, 1%) was added. The mixture was incubated for 10 min at 37°C and then treated with NaCl to a final concentration of 0.5 *M*. The mixture was again incubated for 10 min at 37°C and then centrifuged (40 000 *g*, 30 min, 4°C). The sediment was stored at −30°C. The following purification procedure was carried out at 4°C.

Supernatant

Desalting. The cell-free supernatant was desalted with a Sephadex G-25 Coarse-column (Pharmacia, Uppsala, Sweden; 28.5 × 5 cm I.D., flow-rate 8 ml/min) equilibrated with buffer A (50 m*M* histidine, 1 m*M* EDTA, 1 m*M* MTG, 2% glycerol, 90 m*M* NaCl, pH 7.0, $\kappa = 6.2$ mS/cm).

Anion-exchange chromatography. The protein eluting in the void volume was applied to a column of DEAE Sepharose Fast Flow (Pharmacia, Uppsala, Sweden; 5 × 3.5 cm I.D., flow-rate 8 ml/min) equilibrated with buffer A. After the sample was loaded, the column was washed with 2.5 column volumes of buffer A. The RT was collected in the flow-through.

Affinity chromatography. The RT pool was applied to an AF-Heparin Toyopearl 650 M column (TosoHaas, Philadelphia, PA, USA; 2.6 × 1.5 cm I.D., flow-rate during sample application 8 ml/min, flow-rate during elution 2.5 ml/min) equilibrated with buffer B (10 m*M* sodium phosphate, 1 m*M* EDTA, 1 m*M* MTG, 2% glycerol, pH 7.3, $\kappa = 1.6$ mS/cm) containing 150

mM NaCl. After the sample was loaded the column was washed with equilibration buffer until the UV absorbance at 280 nm returned to baseline. RT was eluted with buffer B containing 450 mM NaCl.

Desalting. The RT-pool was further purified by gel permeation chromatography on Sephadex G-50 Coarse (Pharmacia; 36 × 2.6 cm I.D., flow-rate 3 ml/min) equilibrated in buffer C (50 mM glycine, 5% glycerol, pH 9.0, $\kappa = 0.8$ mS/cm).

Anion-exchange chromatography. The protein eluting in the exclusion volume was applied to a Fractogel EMD TMAE 650 (S) column (Merck, Darmstadt, Germany; 1.5 × 1 cm I.D., flow-rate during sample application 1.5 ml/min, flow-rate during elution 1 ml/min) equilibrated with buffer C. After the sample was loaded, the column was washed with buffer C until the UV absorbance at 280 nm reached the baseline. The column was eluted stepwise with buffer C containing 50 mM, 100 mM, 200 mM and 1 M NaCl.

Sediment

Solubilisation and refolding. The sediment was suspended in 45 ml of solubilising buffer [6 M GuHCl, 100 mM Tris-HCl (pH 8.0), 100 mM β-mercaptoethanol, 1 mM EDTA, 20 mM NaCl] and stirred overnight at room temperature. After centrifugation (40 000 g, 30 min, 4°C) the supernatant was dropwise added to refolding buffer [70 mM Tris-HCl (pH 8), 20 mM NaCl, 1 mM EDTA, 1 M guanidinium hydrochloride (GuHCl), 2 mM red. glutathione, 0.2 mM ox. glutathione]. The ratio protein solution/refolding buffer was 1:25. The refolding solution was stirred gently for 88 h at 4°C.

Hydrophobic-interaction chromatography. The refolding solution was concentrated by means of Phenyl Sepharose Fast Flow (Pharmacia; 3 × 2.6 cm I.D., flow-rate during sample application 8 ml/min, flow-rate during elution 2.5 ml/min) equilibrated with buffer B containing 2 M $(NH_4)_2SO_4$. As GuHCl prevents binding of protein to the column the sample was applied repetitively to the column. A volume corresponding to 0.25 V_t was loaded onto the column and followed by equilibration buffer. After sample application the column was washed with buffer B containing 0.8 M $(NH_4)_2SO_4$. Elution was effected with buffer B.

RT containing fractions were applied to AF-Heparin Toyopearl 650 M and then treated in the same manner as soluble RT.

2.5. Reversed-phase HPLC

The refolding was monitored by reversed-phase HPLC. One milliliter of refolding buffer was applied to a Nucleosil 5C_8 column (Seibersdorf, Austria, 125 × 4.6 mm I.D., flow-rate 1.0 ml/min). The column was run with 0.1% TFA in distilled water (buffer D). RT was eluted with a linear gradient of 0.1% TFA in acetonitrile (buffer E) from 5% E to 60% E within 50 min.

2.6. Determination of protein concentration

Protein concentration was determined by a modified Bradford assay technique using a test kit from BioRad (Richmond, CA, USA). Bovine serum albumin was used for calibration [9].

2.7. SDS-PAGE

SDS-PAGE was performed with the PhastSystem™ using PhastGel 8–25% gradient gels (Pharmacia). The gels were stained either with Coomassie Brilliant Blue or by silver staining [10,11].

2.8. Western blotting

After electrophoresis proteins were transferred to nitrocellulose paper (BioRad) and then incubated with mouse monoclonal antibody against p66-HIV-RT (Du Pont, Bad Homburg, Germany). Colorimetric determination of immunoreactive polypeptides was accomplished with an alkaline phosphatase coupled second antibody (Sigma). For quantification the Western blots were scanned with PhastImage (Pharmacia) at 613 nm.

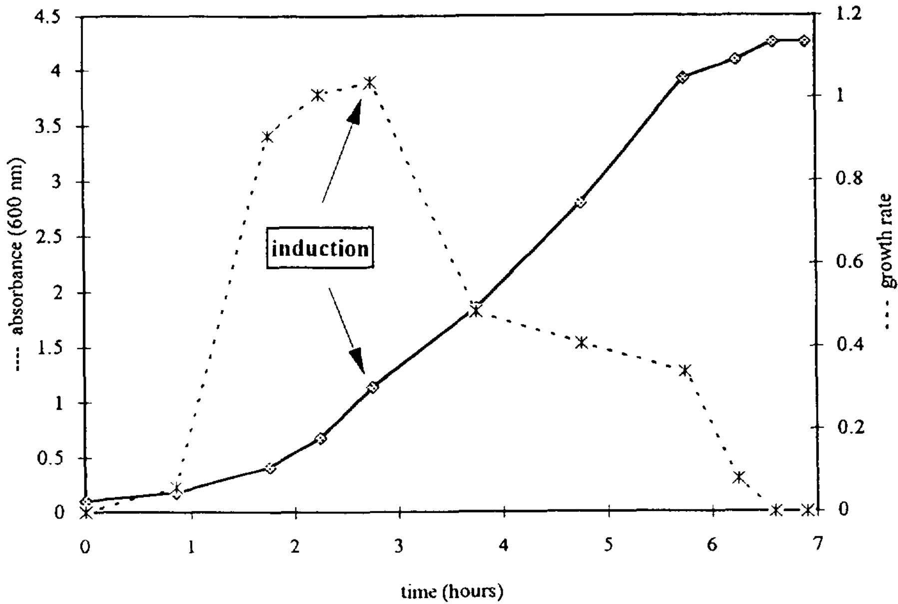

Fig. 1. Fermentation of *E. coli* JM105 (pKKRT66) expressing HIV-1 RT. (- - -) Growth-rate of *E. coli* cells, (——) UV absorbance at 600 nm as a measure of cell density.

2.9. Enzyme assay

The RT enzymatic activity was determined by a non-radioactive Reverse Transcriptase Assay (Boehringer Mannheim, Mannheim, Germany) [12,13].

3. Results

3.1. Fermentation

For identification of the enzymatic activity and the dimer formation of the soluble and inclusion

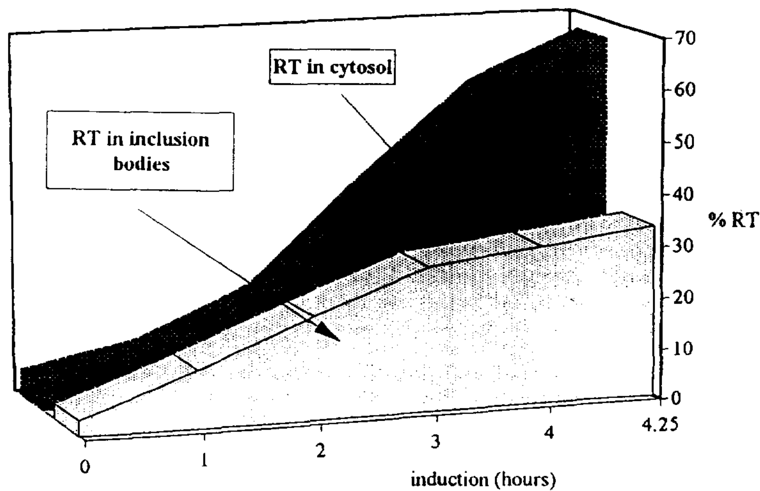

Fig. 2. Expression level of RT after induction by IPTG measured by semiquantitative Western blots.

body derived RT a controlled fermentation was carried out with a constant pH of 7.0, constant temperature of 37°C and constant pO_2 at 30% of saturation. Preliminary experiments indicated that *E. coli* JM105 cells transfected with a pKKRT66 plasmid express correctly folded enzyme as well as RT deposited in inclusion bodies.

Starting from a pure culture, a shaken culture was used to inoculate a 14-l laboratory scale fermentor. The cells were harvested 4–5 h after induction by IPTG. The yield was 6.6 g wet biomass/l culture medium. This corresponds to about 1.3 g dry mass/l. Growth-rate and UV absorbance at 600 nm during fermentation are shown in Fig. 1.

After centrifugation of the cells the sediment was resuspended in lysis buffer. After lysis of the cells the suspension was centrifuged and washed 3 times. RT present in the sediment was defined as insoluble form and RT present in the supernatant was defined as soluble form. Starting from induction until harvesting RT expression was quantified by Western blots (Fig. 2). Only a minimal amount of RT expression could be detected before induction.

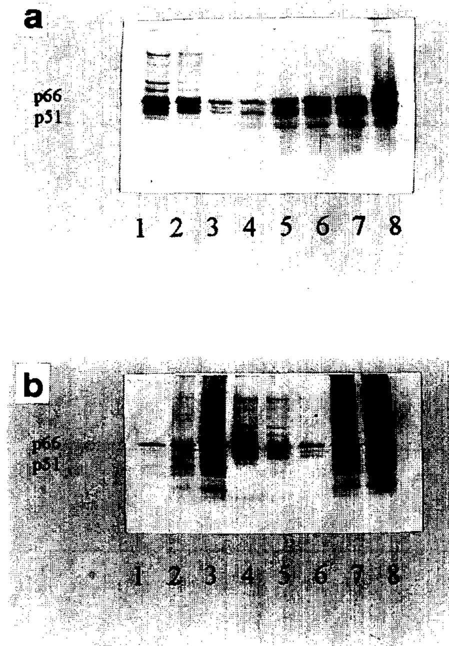

Fig. 3. Semiquantitative Western blot showing RT expression during induction. (a) Expression of soluble RT. Lane 1: RT standard (80 μg/ml); lane 2: RT standard (40 μg/ml); lane 3: RT standard (16 μg/ml); the following lanes show RT detected in the supernatant after disintegration of *E. coli* cells; lane 4: at induction by IPTG; lane 5: 1 h after induction; lane 6: 2 h after induction; lane 7: 3 h after induction; lane 8: 4 h after induction. (b) Expression of inclusion body deposited RT. Lane 4: RT standard (80 μg/ml); lane 5: RT standard (40 μg/ml); lane 6: RT standard (16 μg/ml); the following lanes show RT detected in the sediment after disintegration of *E. coli* cells; lane 1: at induction by IPTG; lane 2: 1 h after induction; lane 3: 2 h after induction; lane 7: 3 h after induction; lane 8: 4 h after induction.

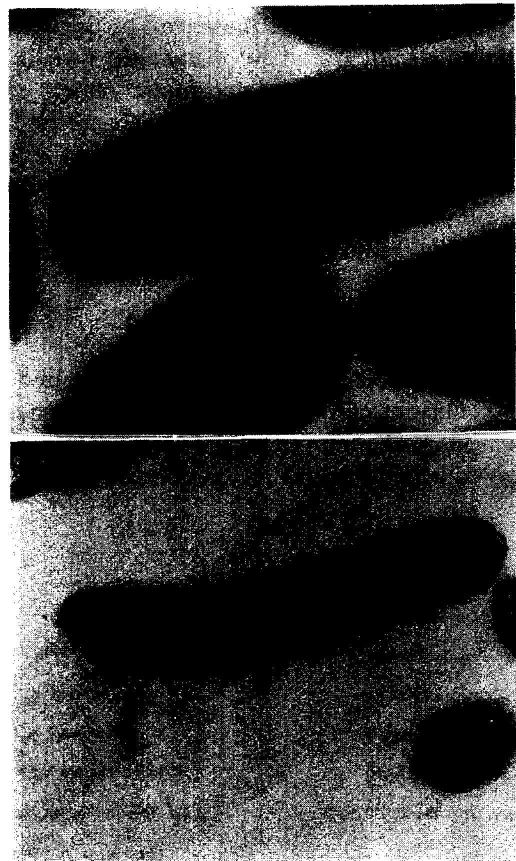

Fig. 4. Electron micrograph of *E. coli* JM105 (pKKRT66) expressing HIV-1 RT after induction. The dark spots represent the inclusion bodies.

In cytosol p66 is prominent over p51, whereas RT originating from inclusion bodies consists of polymeric and partially degraded enzyme (Fig. 3). The specific yield of RT present in cytosol and inclusion bodies as determined from Western blot analysis was 3 mg/g wet mass. About two thirds could be found as cytoplasmic RT.

For proof of inclusion-body formation transmission electron microscopy (TEM) of *E. coli* cells before and after induction was carried out. In the majority of the induced cells amorph aggregates could be found, whereas none could be detected in non-induced cells. The aggregates are relatively small and are deposited at the cell poles (Fig. 4).

3.2. Protein purification

The *E. coli* cells were harvested by centrifugation and disintegrated by enzymatic lysis using lysozyme combined with osmotic shock. The suspension containing the lysed bacteria was centrifuged. The sediment was frozen at −30°C and the supernatant was immediately processed. The purification scheme of recombinant HIV-1 RT from cytoplasm and inclusion bodies is shown in Fig. 5.

Purification of cytoplasmatic RT

The opalescent supernatant was desalted by size-exclusion chromatography on a Sephadex G-25 Coarse column. The desalted protein solution was applied on DEAE Sepharose Fast Flow.

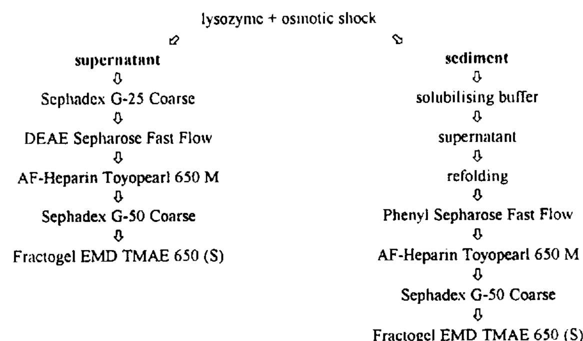

Fig. 5. Purification scheme of recombinant HIV-1 RT secreted into the cytoplasm or deposited in inclusion bodies.

Under these conditions (pH 7.0, $\kappa = 6.2$ mS/cm) about 60% of RT passed the column (Fig. 6a). The flow-through was collected and further

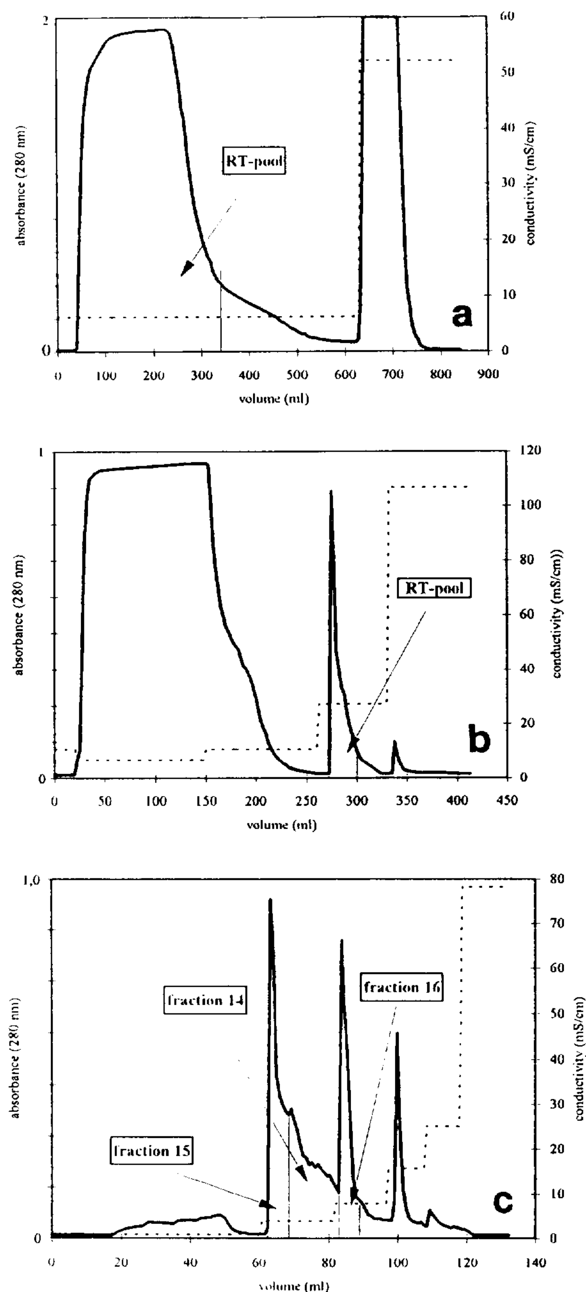

Fig. 6. Purification of soluble HIV-1 RT. (a) DEAE-Sepharose Fast Flow; (b) AF-Heparin Toyopearl 650 M; (c) Fractogel EMD TMAE 650 (S). (—) UV absorbance at 280 nm; (- - -) conductivity.

purified by AF-Heparin Toyopearl 650 *M* (Fig. 6b). RT was eluted by a step gradient using 450 m*M* NaCl. The eluate from AF-Heparin Toyopearl 650 *M* was desalted by Sephadex G-50 Coarse and then applied to Fractogel EMD TMAE 650 (S) (Fig. 6c). Elution was effected by a step gradient using 50 m*M*, 100 m*M*, 200 m*M* and 1 *M* NaCl. RT activity only could be detected in the 50 m*M* and 100 m*M* eluates and only traces (~25 ng) in the 200 m*M* eluate.

The purification steps were also monitored by

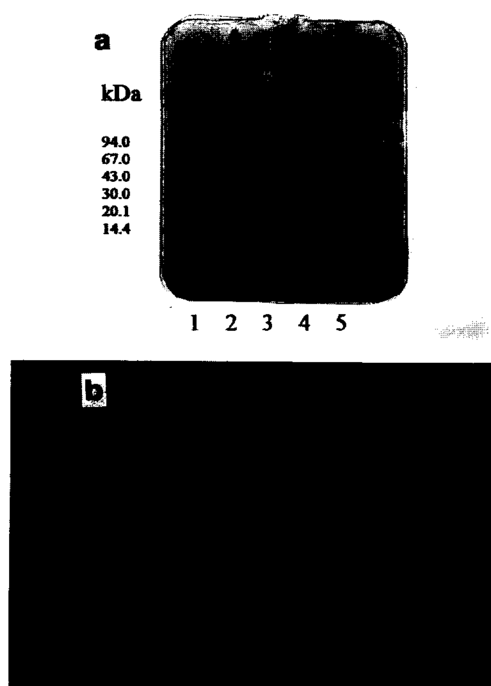

Fig. 7. Purification of soluble HIV-1 RT. (a) PhastGel 8-25 stained by Coomassie brilliant blue; lane 1: low molecular mass marker (Pharmacia); lane 2: supernatant after disintegration; lane 3: eluate of Sephadex G 25 Coarse; lane 4: eluate of DEAE Sepharose FF; lane 5: eluate of AF-Heparin Toyopearl 650 M (flow-rate during sample application: 30 cm/h; at a flow-rate of 90 cm/h during sample application the 14 kDa protein hardly binds to the column–see Fig. 7b); (b) PhastGel 8-25 stained with silver; lane 1: eluate of Sephadex G 50 Coarse; lanes 2 and 3: tailing of Sephadex G50 Coarse eluate; lane 4: Fractogel fraction 14; lane 5: Fractogel fraction 15; lane 6: Fractogel fraction 16; lane 7: Fractogel fraction 18; lane 8: eluate of AF-Heparin Toyopearl 650 M (in comparison with lane 4 of Fig. 7a, an additional RT active bond between p66 and p51 can be detected; this can be due to the longer storage of *E. coli* cells at −80°C).

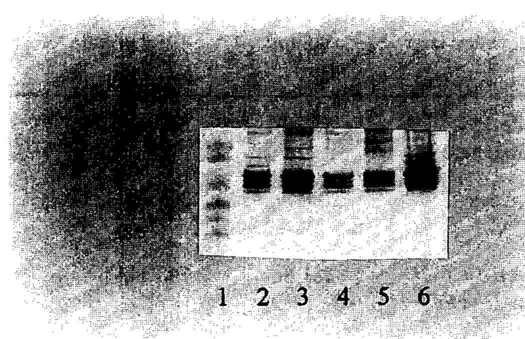

Fig. 8. Purification of soluble RT detected in the supernatant after disintegration. Lane 1: prestained SDS-PAGE standard, low range (BioRad); lane 2: eluate of Sephadex G 50 Coarse; lane 3: Fractogel fraction 14; lane 4: Fractogel fraction 15; lane 5: Fractogel fraction 16; lane 6: eluate of AF-Heparin Toyopearl 650 M.

PAGE (Fig. 7a,b), Western blots (Fig. 8) and RT enzymatic assay. The Western blot shows that the protein band between p66 and p51 is also a degradation product of p66. A balance of the purification is shown in Table 1.

RT does not withstand storage in partial purified state. To check the enzyme degradation reversed-phase chromatography was carried out.

Purification of inclusion-body deposited RT

After lysis the insoluble material was treated with solubilising buffer overnight at room temperature. Refolding of RT was carried out in presence of all contaminants. The protein solution was slowly dropped into an excess volume of refolding buffer. The correct folding is only obtained by high dilution (1:25) and low temperature (4°C). Refolding was monitored by reversed-phase HPLC and RT enzymatic assay. The correctly refolded RT is eluted a little earlier than the denaturated enzyme (Fig. 9). The different peaks were collected and identified by Western blot analysis (results not shown). The indicated peaks showed positive results. According to the fundamental work of Lin and Karger [14] active proteins adsorbed on hydrophobic surfaces elute later in the unfolded form than in the folded conformation.

Table 1
Purification of soluble HIV-1 RT expressed in *E. coli* JM105 (pKKRT66)

Purification step	Volume (ml)	Total protein	Specific activity (U/mg) [a]	RT [b]	Yield (%)
Crude homogenate	55	337 mg	[c]	10.15 mg	100
Sephadex G-25 C	180	306 mg	[c]	[d]	n.v.
DEAE-Sepharose FF	281.8	76.7 mg	[c]	[d]	n.v.
AF-Heparin Toyopearl	19	4.7 mg	6063.8	1.5 mg	15
Stored overnight at 4°C					
Sephadex G-50 C	34	1.7 mg	3200	1.06 mg	10.4
Fractogel fraction 14	6	312 μg	2885	290.3 μg	Fractogel fractions combined
Fractogel fraction 15	14.8	236 μg	1563	215.1 μg	6.7
Fractogel fraction 16	5.5	192 μg	2875	17.4 μg	

After fermentation the cells were harvested by centrifugation and desintegrated by lysozyme treatment combined with osmotic shock.
[a] One unit is the amount of enzyme required for the incorporation of 1 nmol of labeled dNTP in 10 min at 37°C using poly(A).oligo$(dT)_{15}$ as template/primer hybrid.
[b] Quantification by Western blot analysis.
[c] Due to the large amount of contaminating proteins the total amount of RT could not be estimated by enzymatic assay.
[d] The total amount of RT could not be estimated by Western blot analysis reproducible.
n.v.: no value due to the fact that RT-assay cannot be carried out in a crude homogenated (compare R. Bhikhabai [5]).

Since GuHCl interferes with the enzymatic assay, the samples were desalted with PD-10 columns (Pharmacia). After ca. 90 h of refolding low RT activity could be observed which could not be increased by further incubation. The whole protein solution was concentrated by Phenyl Sepharose Fast Flow. Since GuHCl prevents binding of protein to the HIC column, the sample was applied repetitively (Fig. 10a). After sample application the column was washed with buffer B containing 0.8 *M* $(NH_4)_2SO_4$. RT was eluted with buffer B. The column was run in the reverse direction. The pooled RT fractions were diluted with buffer B to 10.5 mS/cm and applied to AF-Heparin Toyopearl 650 M. Desalting and anion-exchange chromatography were carried out in the same manner as for the cytoplasmatic RT purification (Fig. 10b). The purity was checked by SDS-PAGE (Fig. 11). A balance of the purification is shown in Table 2.

4. Discussion

4.1. Fermentation

For production of sufficient enzyme we decided to grow the *E. coli* clone JM105 transfected with the plasmid pKKRT66 in a fermentor in order to enhance the reproducibility, since sig-

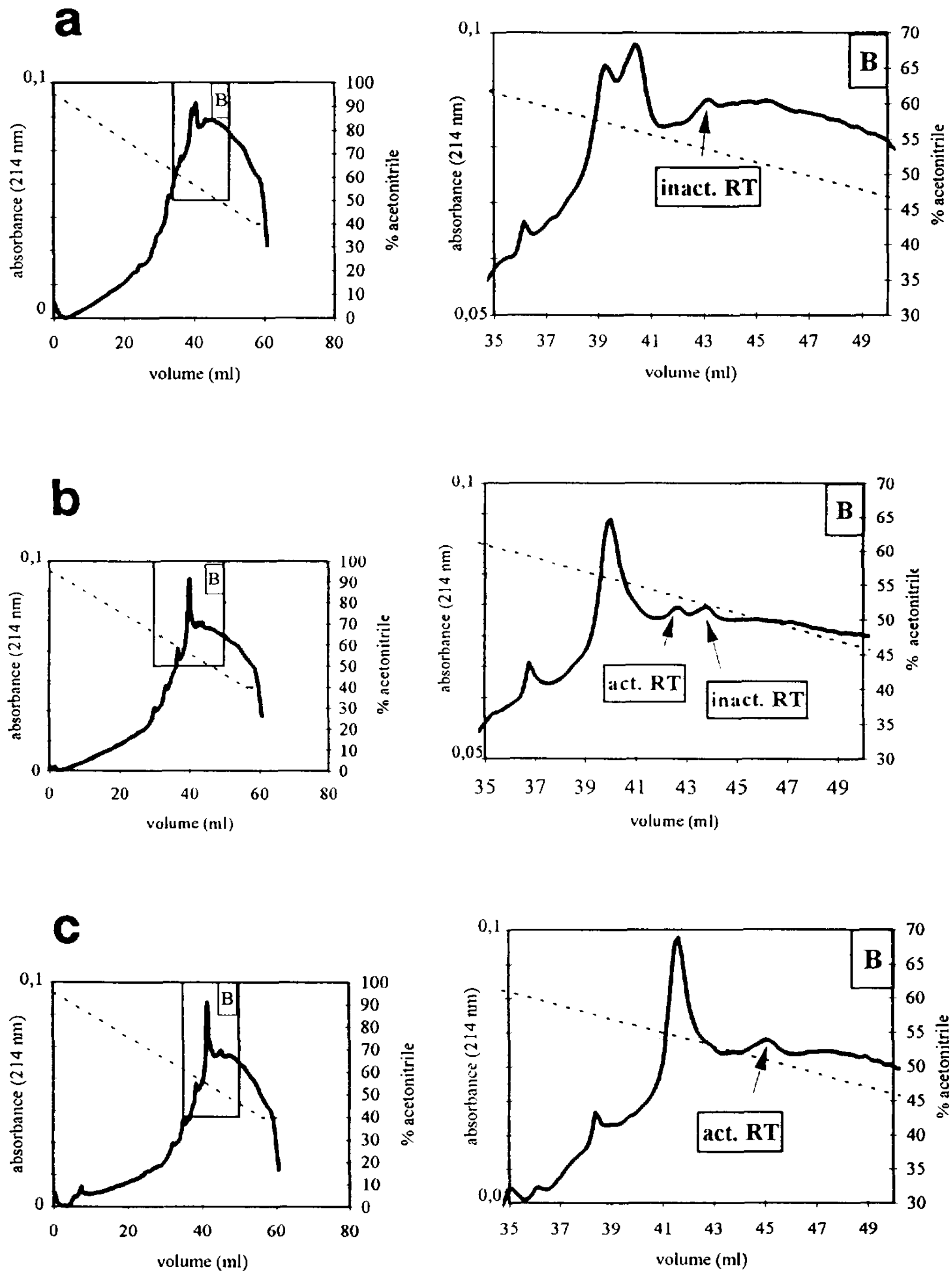

Fig. 9. Monitoring of refolding of inclusion-body deposited RT by reversed-phase chromatography: During the time course of investigation a slight shift in the retention time is observed. (a) Peak profile at the beginning of refolding; (b) peak profile after 48 h of refolding; (c) peak profile after 90 h of refolding. (——) UV absorbance at 214 nm; (- - -) % acetonitrile.

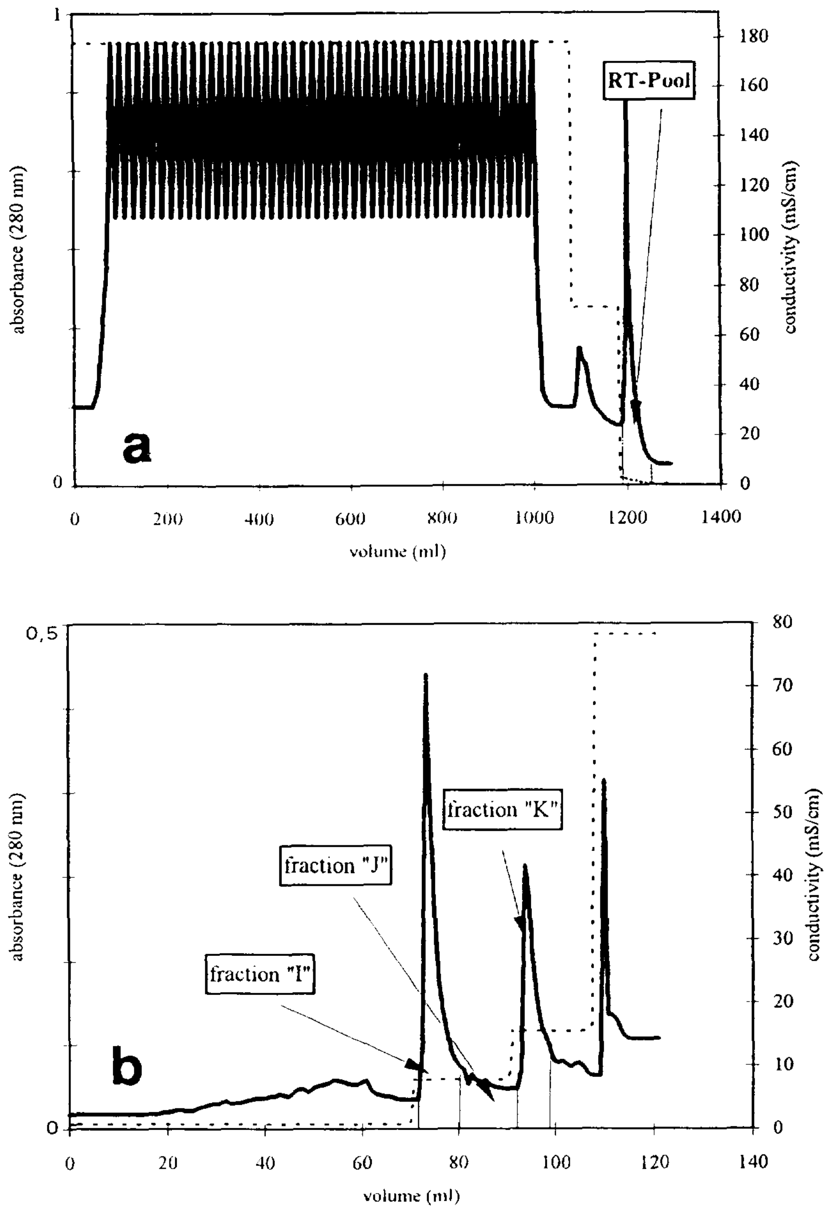

Fig. 10. Purification of inclusion-body deposited RT after 90 h of refolding. (a) Phenyl Sepharose Fast Flow; the sample was applied repetitively to the column; (b) Fractogel EMD TMAE 650 (S). (——) UV absorbance at 280 nm; (- - -) conductivity.

nificant differences in solubility were observed with RT produced in shaken cultures. The specific yield of enzyme calculated as mg RT per mg total *E. coli* protein was about 5% of total protein. Due to the fact that in crude solutions (cell homogenates) the RT assay does not work, the RT content was measured by semi-quantitative Western blots using a commercially available standard. Total *E. coli* protein was determined by the Biorad protein assay. TEM micrographs clearly demonstrated that inclusion bodies were formed.

Fig. 11. Purification of inclusion-body deposited RT after 90 h of refolding; PhastGel 8-25 stained with silver; lane 1: low molecular mass marker (Pharmacia); lane 2: eluate of Phenyl Sepharose Fast Flow; lane 3: eluate of AF-Heparin Toyopearl 650 M; lane 4: eluate of Sephadex G 50 Coarse; lane 5: Fractogel fraction I; lane 6: Fractogel fraction J; lane 7: Fractogel fraction K; lane 8: Fractogel fraction 14 of purification of soluble RT.

Since RT activity was also detected in cytoplasm, we assume that the enzyme is present in two different modifications, i.e. in a native and a paracrystalline form. Western blots showed more pronounced degradation of RT in inclusion bodies. This is conform the theory of Mizrahi et al. [1] that RT is an excellent substrate for *E. coli* proteases during the phase of protein synthesis. RT formation in cytoplasm and formation of inclusion bodies take place concurrently in the early phase of induction (up to 2 h). In the second phase more RT is produced in the cytoplasm than in the inclusion bodies. We conclude that correct folding of recombinant HIV-1 RT in microorganisms is a slow process and formation of insoluble folding intermediates is likely.

4.2. Purification

Soluble RT can be harvested after disintegration from the supernatant after centrifugation at 40 000 *g*. Further purification must be accomplished immediately after harvesting, since nearly complete degradation will take place within 24 h even at 4°C. For maintaining the enzymatic activity all purification steps were carried out at 4°C. To all buffers 2–5% glycerol was added. For protection of -SH groups MTG was added. If necessary the protease inhibitor PMSF was added to the eluates. As the first purification step after desalting Q-Sepharose Fast Flow and DEAE Sepharose Fast Flow at different pH values (6.0, 7.0, 7.5, 7.9, 8.1) and different salt concentrations (50 m*M*, 75 m*M*, 90 m*M* NaCl) were examined. The highest purification connected with the highest yield showed a negative purification using DEAE Sepharose Fast Flow at pH 7.0 and 90 m*M* NaCl. Nevertheless, RT partially bound to the ion-exchange sorbent, but the purification efficiency was so high that these losses were acceptable. Since most *E. coli* proteins have an IEP in the acidic range, about two thirds of all contaminating proteins could be separated in this particular step. Possibly the binding of RT to the column is effected by RNA and DNA fragments, which also bind to the gel under these conditions. Further purification was effected by AF-Heparin Toyopearl 650 *M* and Fractogel EMD TMAE 650 (S). Just storing the

Table 2
Purification of inclusion-body deposited RT

Purification step	Volume (ml)	Total protein	Specific activity (U/mg)
Phenyl Sepharose FF	60	3.45 mg	0.8
AF-Heparin Toyopearl	15	645 μg	1.2
Sephadex G-50 C	37.5	225 μg	12.5
Fractogel fraction I	6.5	46.8 μg	Total: 7
Fractogel fraction K	5.8	38.3 μg	

Prior to purification of the protein the solution was diluted in order to refold HIV-1 RT.

heparin eluate at 4°C overnight led to a significant degradation of active enzyme.

The higher specific activity after AF-Heparin Toyopearl can be explained by the fact that RNA- and DNA-polymerases interfere with the RT activity assay in both cases, the radioactive and the non-radioactive assay. This is one reason why the enzymatic assay cannot be carried out in crude lysates and after the first purification steps. In contrast SDS-PAGE with silver staining shows an increase in purity and specific activity after each purification step assuming that the bands representing RT (checked by Western blots) are active.

Assuming that a purification cycle is completed within one day a higher concentration of active enzyme in the final purified preparation up to 20% overall yield can be expected. The overall yield was determined by dividing the initial RT mass as measured by semi-quantitative Western blots of crude lysate, by the enzyme activity obtained in the final purification step. For calculation we assumed that the soluble RT fraction in the crude homogenate is completely active. Overall yields of RT purification schemes recently published are in the order of 5% [5,15].

Purification of inclusion-body deposited RT could only be effected with a very low yield of active enzyme. As the refolding takes about 4 days and the further purification by column chromatography is not simpler than that for cytoplasmatic RT, we recommend a purification strategy utilising cytoplasmatic RT.

During the course of purification a lot of enzyme degradation products as well as oligomers are generated. This influences the overall yield. In addition all cleavage fragments may interact with RT, thereby effecting the chromatographic properties and creating problems in the performance of a reliable enzyme activity assay.

It may be concluded that the described purification scheme is suitable for laboratory-scale production of HIV-1 RT. Further optimization primarily should be done on the fermentation and cloning site to achieve a higher productivity during fermentation.

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