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Characterization of Human Immunodeficiency Virus-1 (HIV-1) Rev by (Time-Resolved) Fluorescence Spectroscopy

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Fluorescence spectroscopy has been applied to the single tryptophan-containing regulatory protein Rev of human immunodeficiency virus (HIV-1). The fluorescence emission was found to have a maximum at 336 nm which refers to a surrounding of the chromophore of intermediate polarity. Fluorescence transients recorded at the maximum of fluorescence were found to decay nonexponentially. A bimodal lifetime distribution is obtained from exponential series analysis (ESM) with centers at 1.7 and 4.5 ns. Two microenvironments for tryptophan are suggested to be responsible for the two lifetime distributions. No innerfilter effect occurred in a Rev solution up to a concentration of 40 μM. A data quality study of ESM analysis as function of collected counts in the peak channel maximum (CIM) showed that, for reliable reconvolution, at least 15,000 CIM are necessary. The widths of the two distributions are shown to be temperature dependent. The broadening of the lifetime distributions when the temperature is raised to 50°C is interpreted as extension of the number of conformational substates which do not interconvert on the fluorescence time scale. The thermal deactivation (temperature quenching) is reflected in a constant decrease in the center of the short-lived lifetime distribution.

KEY WORDS: Protein fluorescence; distributional analysis; AIDS.

INTRODUCTION

The human immunodeficiency virus (HIV-1) regulatory gene product Rev (regulator of expression of virion proteins) posttranscriptionally induces cytoplasmatic accumulation of incompletely spliced viral RNA encoding for viral structural proteins [1–4]. The 116-amino acid (aa) protein Rev functions through a high-affinity, high-specificity interaction (aa 34–51) with a region of the nascent RNA transcript located within the envelope gene of the virus [5–10] (Rev-responsive element; RRE) and through an activation domain necessary to mediate Rev effector functions (aa 78–93) [11–16]. *In vivo* mutational analysis has demonstrated that Rev defective in RRE or cellular factor binding is biologically inactive. Both domains therefore represent attractive targets for chemotherapy [17–19]. HIV-1 Rev contains one tryptophan (TRP) at position 45 located within a stretch of 10 arginines contained within the 18 amino acids of the RRE binding region and two tyrosines (TYR) at position 23 and 63 [20,21].

Fluorescence spectroscopy was shown to yield valuable insights into protein structure and dynamics [22]. TRP and TYR, the natural intrinsic chromophores in proteins, have different spectral properties and a high quantum yield in proteins, which allows investigations in the micromolar to nanomolar range. The topological influence of the protein matrix on the excited-state properties of the fluorophores gives way to evaluate quali-

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tatively and (semiquantitatively) the local chromophore environment. Moreover, time-resolved fluorescence spectroscopy monitors the protein dynamics, which interferes with the chromophore on a fluorescence time scale (femtosecond to nanosecond range). In this study we confine ourselves to the presentation and discussion of the dependence of the exponential series (ESM) analysis [23] of Rev fluorescence on the data quality and on the variation of temperature. An extended study of Rev fluorescence characterization will be presented elsewhere (Kungl *et al.*, manuscript in preparation).

MATERIALS AND METHODS

Expression and purification of Rev were done according to Daly *et al.* [24,25]. All fluorescence measurements were done in 10 m*M* Tris–HCl, pH 7.0, and 1 *M* NaCl, 20°C, if not indicated otherwise in the text. Steady-state fluorescence measurements were performed on a SLM 8000C fluorometer (SLM Instruments, Urbana, IL). Time-resolved fluorescence measurements and data analysis were performed as described previously [23,26].

RESULTS AND DISCUSSION

The steady-state emission spectrum of aqueous Rev is dominated by TRP fluorescence and has a maximum at 336 ± 1 nm (corrected) which is nearly excitation wavelength independent (Rev λ_{Ex}, 295 nm). Exciting at 275 nm, only up to 12% (corrected) of the overall emission is due to TYR fluorescence, with a maximum at 303 nm. The TRP fluorescence emission maximum of 336 nm is an indication of an intermediate polarity of the TRP environment. At the excitation wavelength 295 nm, TRP in Rev has a fluorescence quantum yield (ϕ) of 0.09 ± 0.01, which was determined relative to the quantum yield of the free aa TRP (ϕ = 0.13). Measurement of the ratio of relative fluorescence intensity (λ_{ex} = 285 nm, λ_{em} = 340 nm) to the optical density at 285 nm of the Rev solution proved that no inner filter effect occurred up to a protein concentration of at least 40 μM (Fig. 1).

Recording the time-resolved fluorescence of Rev at λ_{ex} = 285 nm and λ_{em} = 340 nm yielded a nonexponential fluorescence decay. Analysis of these data by an exponential-series algorithm (ESM) [23] revealed a bimodal lifetime distribution, with the respective centers located at 1.7 ns and at 4.5 ns (Fig. 2). This pattern is interpreted as two microenvironments for the tryptophan, which represent the distributional centers of local conformations of the fluorophore in the protein, noninterconverting on a fluorescence time scale. Salt- and pH-dependent measurements (data not shown) indicated that the long-lived lifetime center at 4.5 ns is associated with electrostatic interaction parameters in the arginine-rich region surrounding the TRP. However, the main contribution to the fluorescence decay arises from a more hydrophobic environment, leading to a quenched lifetime center at 1.7 ns.

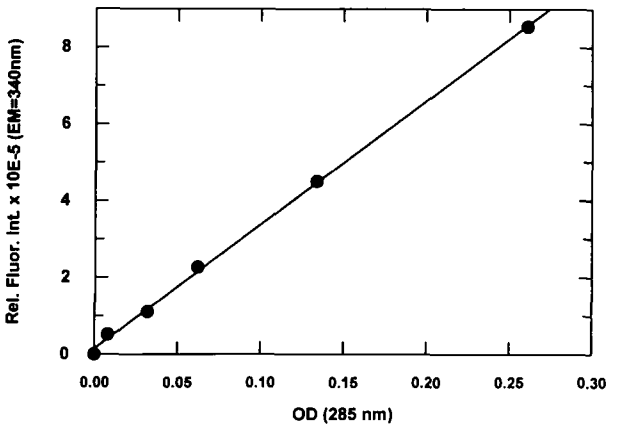

Fig. 1. Control of innerfilter effects in HIV-1 Rev protein solutions at concentrations up to 40 μM.

To investigate the reliability of extraction of lifetime distributions from fluorescence data by the ESM analysis, data sets with increasing quality were collected (Figs. 2a–c). We know already from synthetic data that a certain amount of counts should be in the peak channel maximum (CIM) in order to restore a known input distributional shape [23]. Analysis of Rev fluorescence with data collected up to 14,000 CIM gave an ESM pattern with a center at 1.7 ns and a shoulder starting at 3 ns (Fig. 2a). Improving the data quality to 34,000 CIM (Fig. 2b), and, in addition, to 68,000 CIM (Fig. 2c), results in two well-resolved peaks, located at 1.7 and 4.5 ns. Furthermore, the width of the distributions decreases with increasing data quality. This means that the information concerning the maxima of the distributions is already inherent in data sets with approximately 15,000 CIM. If, however, the best resolution between distributional modes is essential for the elucidation of a photophysical model, the highest possible data quality should be obtained. From these results we conclude that for time-efficient recording of Rev time-resolved fluorescence and reliable ESM data analysis, at least 15,000 CIM should be collected.

On raising the temperature in the system two effects on the fluorescence lifetime distributions become appar-

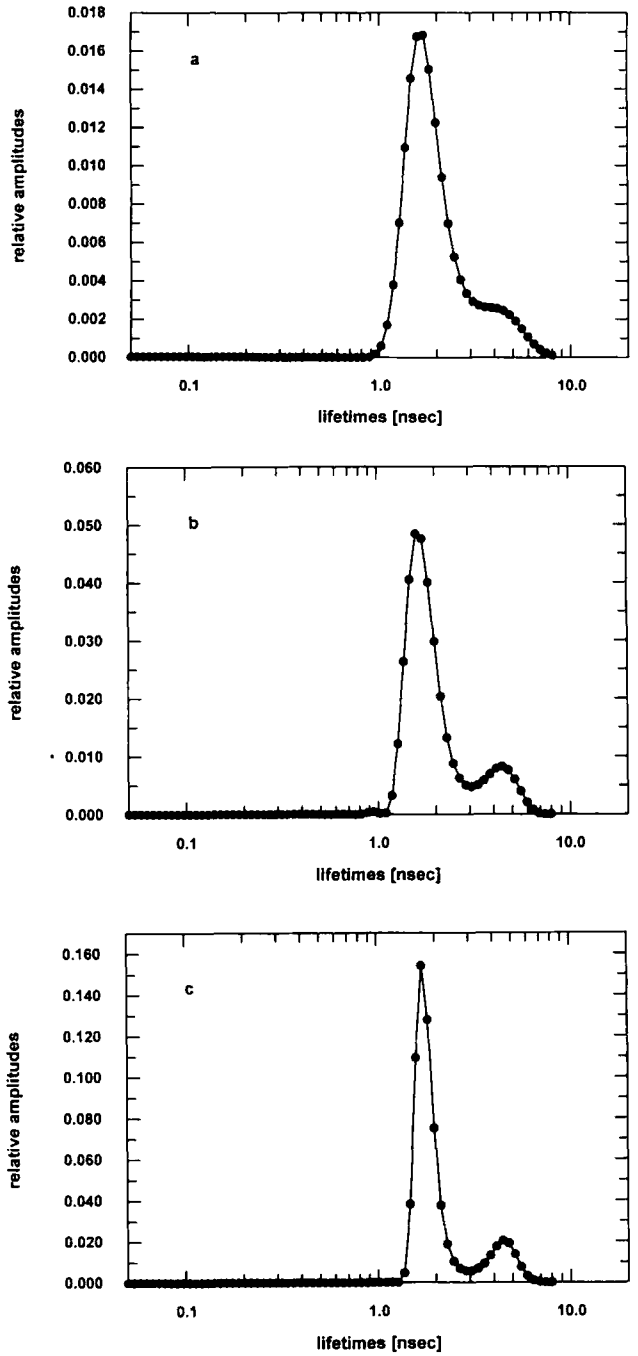

Fig. 2. (a) ESM analysis of a time-resolved fluorescence measurement on a 10.8 μM solution of HIV-1 Rev with a data quality of 14,000 counts in the peak channel maximum (CIM). (b) Analysis of the experiment described in a at 34,000 CIM. (c) Analysis of the experiment described in a at 68,000 CIM.

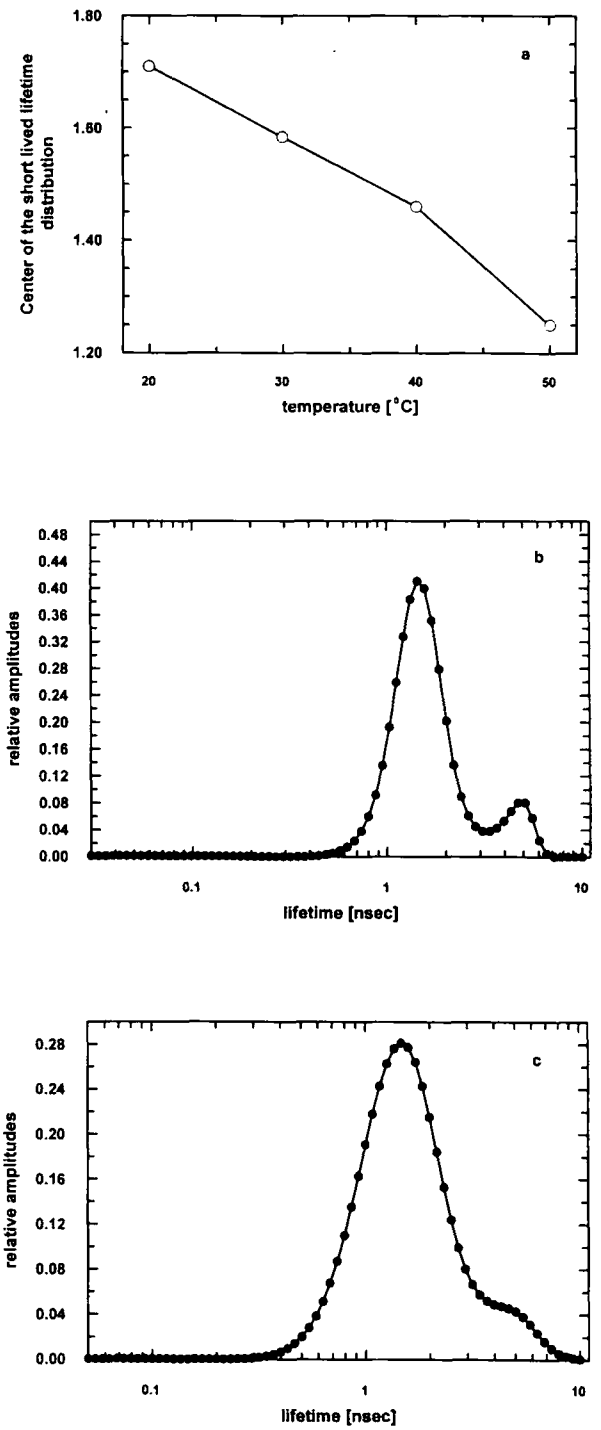

Fig. 3. (a) Thermal deactivation of HIV-1 Rev at a concentration of 10 μM demonstrated by the dependence of the maximum of the short-lived lifetime distribution as function of the temperature between 20 and 50°C. (b) Exponential-series analysis of Rev time-resolved fluorescence data recorded at λ_{ex} = 285 nm/λ_{em} = 340 nm, T = 30°C, 10 μM. Seventy lifetimes were spaced logarithmically over a time scale ranging from 0.05 to 10 ns. (c) Exponential-series analysis of Rev time-resolved fluorescence data recorded at λ_{ex} = 285 nm/λ_{em} = 340 nm, T = 40°C, 10 μM. Seventy lifetimes were spaced logarithmically over a time scale ranging from 0.05 to 10 ns.

ent (A). The two lifetime centers at 1.7 and at 4.5 ns are shifted to shorter wavelengths due to thermal deactivation of the excited states (temperature quenching). This is demonstrated in Fig. 3a, showing the shift of the po-

sition of the maximum of the short-lived mode as function of temperature. (B) The width of the distributions increases with temperature, leading to a considerable overlap of the two modes already at 40°C (see Fig. 2a for analysis at 20°C and Figs. 3b and c for analysis at 30 and 40°C). Due to this overlap a reliable interpretation of the change of the full width at half-maximum of the two distributional peaks is not possible. Therefore, only the center of the peak at 1.7 ns was used for demonstrating thermal deactivation. The broadening of the distributions with two still-resolvable modes up to 50°C is interpreted as extension of the conformational substates [27] which do not interconvert on a fluorescence time scale [28]. This increase in the number of discrete conformations due to extended Brownian dynamics leads to a broadening of the overall shape of the curve, which envelopes the numerous individual substate contributions around their mean values. Since the overall shape of the ESM patterns has not been destroyed up to 50°C, and the two maxima have not yet collapsed into one (narrower) distribution, we conclude that Rev is still (at least partially) folded and not fully denatured at this temperature. In time-resolved fluorescence measurements on Rev at concentrations between 2 and 20 μM, no substantial effect on the ESM pattern could be detected. Broadening of lifetime distributions due to entropically driven aggregational effects known to occur in Rev solutions [8,20] can therefore be excluded.

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