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Fluorescence study of the nucleic acid binding site of vimentin

Martin Kooijman[a], Michael Bloemendal[b,*], Peter Traub[c] and Rienk van Grondelle[a]

[a]*Free University of Amsterdam, Department of Biophysics, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands,* [b]*Free University of Amsterdam, Department of General and Analytical Chemistry, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands* and [c]*Max Planck Institute for Cell Biology, D6802 Ladenburg/Heidelberg, Germany*

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A selective tyrosine fluorescence quenching is found on interaction of vimentin with poly(dT) and poly(rA). However, addition of poly(dA) does not result in tyrosine quenching. The number of nucleotides covered by vimentin upon binding (n) of poly(dT) (50 ± 4) appeared to be approximately the same as for poly(rA) (44 ± 4), while the apparent binding constant (K_{app}) of the latter is slightly larger ($5.0 \pm 2.0 \times 10^7$ $M^{-1} \cdot cm^{-1}$ vs. $2.5 \pm 0.5 \times 10^7$ $M^{-1} \cdot cm^{-1}$). The finding that there exists a specific strong interaction between vimentin and nucleic acids could help in the search for cellular functions of intermediate filament proteins.

Intermediate filament protein; Vimentin; Protein fluorescence; Nucleic acid binding protein

1. INTRODUCTION

Intermediate-size filaments (IF) and IF-like proteins are prominent components of the cytoskeleton and nuclear envelope of most eukaryotic cell types [1]. The IF protein vimentin is found in mesenchymal tissue and many cultured cells. It consists of a central α-helical 'rod' domain flanked by non-α-helical amino- and carboxy-terminal domains. The α-helical stretch exists as two segments of nearly equal length of approximately 21 nm, separated by a non-α-helical spacer. Two protein chains are involved in the formation of a coiled-coil molecule [2]. Two of these, of which the precise arrangement is not exactly known, form a tetramer at low ionic strength [3,4]. At increasing ionic strengths vimentin assembles into higher aggregates [3,4]. Vimentin has been shown to bind ssDNA and RNA, although it is still a matter of discussion, whether this has any physiological relevance [5–7]. It has been proposed that five tyrosine residues present in the N-terminal domain are involved in the binding of nucleic acids to the protein [6]. Eight additional tyrosines as well as one tryptophan residue are found in the rod domain of the vimentin monomer from Ehrlich ascites tumor (EAT) cells [8], which gives a Tyr/Trp ratio of 13:1 [8]. The involvement of tyrosine in binding nucleic acids can be monitored with fluorescence spectroscopy, since the various features of protein fluorescence are often modified by direct ligand interactions with the fluorophore, or by subtle ligand binding-induced conformational changes in the protein. For instance, the binding of the gene 5 protein of fd bacteriophage to ssDNA or ss homopolynucleotides is accompanied by a dramatic quenching of the tyrosine fluorescence, and in general such a quenching is interpreted as a partial stacking of the aromatic residues of the protein with the DNA bases [9]. Our aim is to study the interaction of vimentin with nucleic acids using the fluorescence quenching technique. Hopefully, these experiments can contribute to the general discussion concerning the cellular functions of IF proteins.

2. MATERIALS AND METHODS

Vimentin was purified from EAT cells as previously described [10]. Poly(dT) was from P-L Biochemicals, poly(dA) and poly(rA) were purchased from Pharmacia. The concentrations of the polynucleotides (on a nucleotide basis) were calculated by using the following molar extinction coefficients: poly(rA), $\varepsilon_{260\ nm}$=9,400 $M^{-1} \cdot cm^{-1}$; poly(dA), $\varepsilon_{257\ nm}$=8,600 $M^{-1} \cdot cm^{-1}$; poly(dT), $\varepsilon_{264\ nm}$=8,520 $M^{-1} \cdot cm^{-1}$ [11]. The molar extinction coefficient of tetrameric vimentin was calculated by determination of protein concentrations using a modified Lowry protein assay [12] and the Bradford assay [13]. An average value of $114 \times 10^3 (\pm 4 \times 10^3)$ $M^{-1} \cdot cm^{-1}$ was obtained. A determination of the extinction coefficient by means of amino acid analysis led to a value of 99×10^3 $M^{-1} \cdot cm^{-1}$. In this study, $\varepsilon_{280\ nm} = 114 \times 10^3$ $M^{-1} \cdot cm^{-1}$ is used. All measurements were performed in 10 mM Tris–HCl, pH 7.4, 0.1 mM EDTA at 20 ± 0.1°C. Fluorescence data were collected on a Aminco SPF-500 spectrofluorometer. A square 3-ml quartz fluorimeter cuvette (pathway 1.0 cm) containing a stirring bar for sample mixing was used and emission was recorded perpendicular to the direction of the excitation beam. The bandwidth was 0.5 and 7.5 nm for the exciting and emitted light, respectively. Fresh solutions of vimentin with concentrations ranging from 0.32 to 0.76 μM were prepared in the cuvette and were allowed to come to thermal equilibrium prior to the measurements. Small aliquots of a concentrated

*Temporarily on leave at the Royal Free Hospital School of Medicine, University of London, England.

Correspondence address: M. Kooijman, Free University of Amsterdam, Department of Biophysics, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands.

nucleic acid solution (690–740 μM) were added to the vimentin solution, and emission spectra were measured using excitation wavelenghts of 280 and 295 nm. Assuming that tyrosine is not excited at 295 nm, the contribution of the tyrosine residues to the total fluorescence was calculated by subtraction of the vimentin emission obtained at an excitation wavelength of 295 nm from that obtained at 280 nm, after normalizing the two spectra above 380 nm (where tyrosine emission is practically zero [14]).

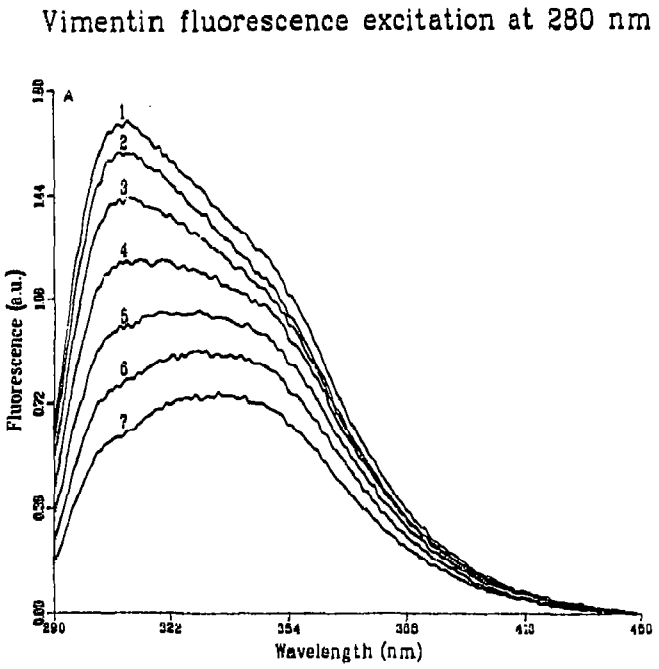

Fig. 1. Fluorescence emission spectra of vimentin (0.59 μM) with increasing amounts of pdT (stock 740 μM): 1, 0 μl; 2, 8.5 μl; 3, 24.5 μl; 4, 48.5 μl; 5, 78.5 μl; 6, 118.5 μl; 7, 189.5 μl. (A) Excitation wavelength 280 nm. (B) Excitation wavelength 295 nm. Spectra are corrected for dilution, the starting volume is 2,000 μl.

3. RESULTS

Fig. 1A and B show the fluorescence emission spectra of vimentin determined after the addition of increasing amounts of poly(dT). The vimentin emission at an excitation wavelength of 280 nm (A) shows a maximum at 310 nm, suggesting a large contribution of tyrosine fluorescence to the total signal. The intensity of the blue side of the spectrum (290–320 nm) is significantly decreased, while the red side of the spectrum (360–450 nm) is hardly affected when poly(dT) is added. Upon excitation with 295 nm light, a peak with a maximum at 346 nm due to tryptophan fluorescence is observed. The intensity as well as the maximum emission wavelength are practically unaffected upon titration with poly(dT). When the pure tyrosine emission is calculated via subtraction of the tryptophan emission (see section 2) one observes that the tyrosine signal is quenched upon addition of poly(dT) (Fig. 2). When relatively large amounts of poly(dT) are added (Fig. 2), saturation occurs and the tyrosine fluorescence remains at a constant level, about 48% of the level in the absence of poly(dT).

Assuming that the observed tyrosine fluorescence quenching is caused by binding of the nucleic acid to the protein, the stochiometry of binding n, which is the number of nucleotides covered by each vimentin tetramer in the complex, can be determined from a fluorescence titration curve, in which the quenching of tyrosine fluorescence is plotted against the ratio of the added nucleotide to the protein tetramer concentration (Fig. 3). This is accomplished by drawing two straight lines

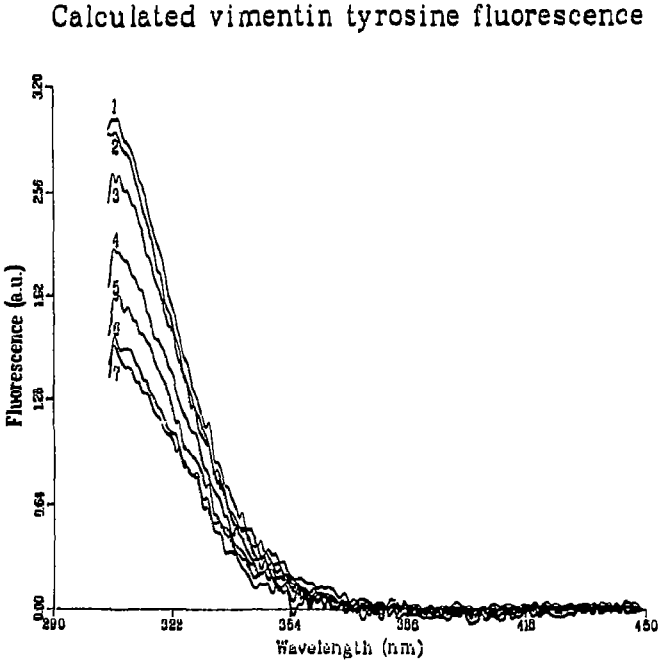

Fig. 2. Calculated tyrosine contribution to the fluorescence emission after excitation at 280 nm (see text). For explanation of the numbers near the spectra: see Fig. 1.

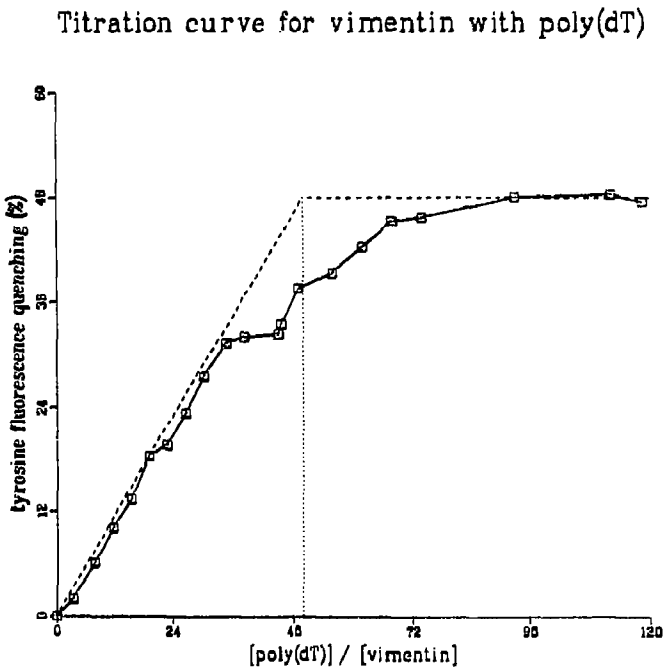

Fig. 3. Fluorescence titration curve for the binding of pdT to vimentin. The fluorescence due to tyrosine measured at 307 nm is plotted against the ratio of nucleotides and protein tetramers.

representing the initial and final slopes of the curve. The ratio at the intersection of these lines corresponds to n. For the vimentin–poly(dT) complex, $n=50 \pm 4$ is obtained. Assuming Scatchard binding to independent sites, the apparant binding constant (K_{app}) can be achieved from the titration curve using the expression [15]:

$$K_{app}=\theta/\{(1-\theta)^2[P_0]\}$$

K_{app}=apparent binding constant (M^{-1}), θ=ratio between protein fluorescence change at a particular ligand concentration and fluorescence change at a saturating ligand concentration and $[P_0]$=total protein concentration (M). Because this approximation does not correct for the accumulation of 'gaps' (which are smaller than the binding site size) between stretches of contiguously bound vimentin molecules, this binding constant is a minimum estimate only [16]. However, K_{app} is a useful parameter to compare binding constants of different polynucleotides. For the binding of vimentin to poly(dT), $K_{app}=2.5 \pm 0.5 \times 10^7$ M^{-1} was calculated.

Table I

Values of the number of nucleotides covered, n, the apparent binding constant, K_{app} and the maximum tyrosine quenching due to the interaction of vimentin tetramers with nucleic acids

	n	K_{app} (M^{-1})	Max. quenching (%)
poly(dT)	50 ± 4	2.5 ± 0.5 × 10^7	48 ± 2
poly(rA)	44 ± 4	5.0 ± 2.0 × 10^7	32 ± 4
poly(dA)	–	–	–

– = no measurable tyrosine fluorescence quenching

Similar binding experiments as those reported for poly(dT) were also performed for vimentin binding to poly(dA) and to poly(rA). The parameters n, K_{app} and the total tyrosine quenching of poly(dT) and poly(rA) are listed in Table I. In case of poly(rA), n is found to be similar to poly(dT), while K_{app} of poly(rA) is slightly larger. Interstingly, titration with poly(dA) did not result in a measurable fluorescence quenching (data not shown).

4. DISCUSSION

The specific quenching of tyrosine fluorescence in the titration experiments with poly(dT) and poly(rA) is interpreted as an interaction between tyrosyl rings of the protein and the heterocyclic bases of the nucleic acids. Since it has been shown that the N-terminus of vimentin is essential for its interaction with nucleic acids [6], it is obvious that some or all tyrosine residues located in this region are involved in this process. The interaction could be caused by intercalation of aromatic residues of amino acids between nucleotide bases, as has been shown for other ssDNA binding proteins such as gene 5 protein of fd bacteriophage and phage T4gp32 [9,17].

Crosslinking experiments of vimentin with tetranitromethane (Traub, unpublished results), which showed prevention from nitration of the tyrosyl residues by rRNA or fd DNA also indicate a shielding of tyrosine due to the nucleic acid. Shape and emission maximum of the tryptophan spectrum remain constant upon the addition of saturating amounts of nucleic acids, indicating that no conformational changes in the tryptophan region occur and that the tryptophan residue, which is located in the central rod domain [8], does not play a role in the nucleic acid binding process either.

The high affinity for poly(dT) and the absence of binding of poly(dA) are in reasonable agreement with the results of investigations via a quantitative filter binding assay [7]. The advantage of the fluorescence technique is that only specific binding in which tyrosine residues are involved, is detected. Moreover, the binding site size n and the degree of fluorescence quenching, which yields information about the binding process, can be determined. In this study, it is shown that the binding site size for poly(dT) and poly(rA) is approximately the same, suggesting the involvement of the same number of tyrosine residues in the binding process. The apparent binding constant is somewhat larger for poly(rA). At saturating amounts of poly(dT), however, the tyrosine quenching is significantly larger than at saturating conditions of poly(rA). This could be due to two different modes of these nucleic acids to the protein. The striking difference in binding properties of poly(rA) and

poly(dA) suggests that not only base composition but backbone properties of the nucleic acids as well play a role in the process. The physiological meaning of nucleic acid–vimentin interaction, if existent, is poorly understood. An active role for IF subunit proteins in modulating DNA replication, recombination, and repair, as well as in gene expression, has been postulated [18]. Vimentin has been identified as a DNA attachment protein within nuclei of Chinese Hamster Ovary cells [19]. However, more experiments are needed to fully characterize the nucleic acid binding properties of vimentin under several conditions. The present study, which shows a strong specific interaction between vimentin and some types of ssDNA and RNA, may be helpful in the search for the (possible) physiological role of the binding.

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