Acta Biochimica Polonica

Vol. 46 No. 3/1999

615-629

QUARTERLY

Fluorescence decay time distribution analysis of cyclic enkephalin analogues. Influence of the solvents and configuration of amino acids in position 2 and 3 on changes in conformation*°

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Received: 14 June, 1999

Key words: enkephalin, fluorescence, lifetime distribution, energy transfer, conformation, configuration

The lifetime distribution calculations were applied to study the influence of configuration of amino-acid residues in positions 2 and 3 on changes in conformation of the peptide chain of cyclic analogues of enkephalins containing a fluorescence energy donor and acceptor in different solvents. In all the solvents studied the lifetime distributions were bimodal. This testified to the presence of two families of conformations. In this paper the relationship between the population of each conformation and configuration of the residues in position 2 and 3, and the solvent used is discussed.

The application of fluorescence spectroscopic methods to biological samples often involves an analysis of the intensity and anisotropy of decay kinetics. The decay times result

*Presented at the symposium "Conformation of peptides, proteins and nucleic acids" held in Rynia, Poland, on 26-29 May, 1999.

° This work was supported by Grant 1210/T09/99/16 from the State State Committee for Scientific Research (KBN, Poland).

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Abbreviations: D-Dab, α,γ-diaminobutyric acid; F-L,L-EN, Phe-*cyclo*(Dab-Pro-Nal-Leu); F-L,D-EN, Phe-*cyclo*(Dab-D-Pro-Nal-Leu); F-D,L-EN, Phe-*cyclo*(D-Dab-Pro-Nal-Leu); F-D,D-EN, Phe-*cyclo*(D-Dab-D-Pro-Nal-Leu); F(NO_2)-L,L-EN, Phe(p-NO_2)-*cyclo*(Dab-Pro-Nal-Leu); F(NO_2)-L,D-EN, Phe(p-NO_2)-*cyclo*(Dab-D-Pro-Nal-Leu); F(NO_2)-D,L-EN, Phe(p-NO_2)-*cyclo*(D-Dab-Pro-Nal-Leu); F(NO_2)-D,D-EN, Phe(p-NO_2)-*cyclo*(D-Dab-D-Pro-Nal-Leu); HBTU, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; Me_2SO, dimethyl sulfoxide; RP-HPLC, reversed phase high performance liquid chromatography; FAB-MS, fast atom bombardment mass spectrometry; ^1H NMR COSY, hydrogen nuclear magnetic resonance correlation spectroscopy.

from molecular features of the sample and provide information about the existence of two or more conformations of the biomolecules. Usually, they are also sensitive to the polarity of the environment surrounding the fluorophore. The intensity decays are generally more complex than a single exponential, and can be analyzed in terms of the sum of discrete exponential decays (multi-exponential model) [1–5]. Recently, continuous distribution of decay times has been proposed [6–23] as method alternative to the sum of discrete exponentials of the decay times.

So far many systems have been described from the results of which one can state with confidence that the decay is complex in nature and cannot be described by a mono-exponential function. A variety of phenomena may be reflected by occurrence of a decay time distribution. These include: transient effects in quenching, energy transfer [11], conformational heterogeneity of proteins [9, 10, 12, 15, 22], conformational differences between native and denatured state of a protein [12, 16], a protein conformation modification due to ligand binding [15, 23], or peptide interaction with proteins and phospholipids [20]. Also a single chromophore in heterogeneous environment [13, 14, 17] can exhibit a distribution of fluorescence lifetimes.

We used the decay time distribution method in conformational analysis of opioid peptides that have a fluorescence energy donor (β-naphthylalanine) and a fluorescence energy acceptor (Phe(p-NO_2)) and differ in configuration of amino-acid residues in position 2 and 3. The cyclic enkephalin analogues X-*cyclo*[Y-Z-Nal-Leu], where X = Phe or Phe(p-NO_2), Y = D- or L-Dab, Z = D- or L-Pro, have been studied (Fig. 1).

THEORY

Fluorescence intensity decays are usually described as the sum of individual exponentials. The intensity decay following infinitesimal pulse (δ-function) excitation is described by:

$$I(t) = \sum_i \alpha_i e^{-t/\tau_i} \tag{1}$$

where τ_i are the individual decay times and α_i the associated pre-exponential factors. The fractional contribution of the i-th component to the total emission is

$$f_i = \frac{\alpha_i \tau_i}{\sum_i \alpha_i \tau_i} \tag{2}$$

It is a common practice to normalize the α_i and f_i values so that $\sum_i \alpha_i = 1.0$ and $\sum_i f_i = 1.0$. Now let us consider an alternative model in which the α_i values are not discrete ampli-

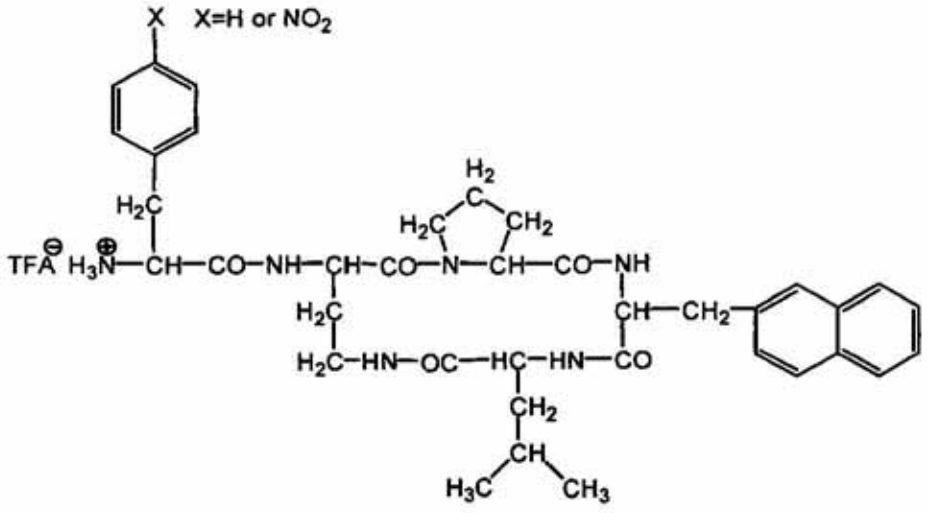

Figure 1. Structure of cyclic enkephalin analogues.

tudes at τ_i, but rather are described by a continuous distribution $\alpha(\tau)$. Then intensity decay contains components of each lifetime τ with amplitude $\alpha(\tau)$. The component with each individual τ is given by:

$$I(t)=\int_{\tau=0}^{\infty}\alpha(\tau)e^{-t/\tau}d\tau \qquad (3)$$

where $\int\alpha(\tau)d\tau=1.0$. At present we do not have a theoretical model to predict the $\alpha(\tau)$ distributions, nor do we have experimental data justifying the use of a particular function. Hence, arbitrarily selected Gaussian or Lorentzian distributions have been used [11–16, 19, 20]. An alternative approach is to use $\alpha(\tau)$ distributions which are not described by any particular function. This approach is superior in that it makes no assumptions about the shape of the distribution. Two general algorithms: maximum entropy [18, 21, 24–27] or exponential series are used for such calculations [28, 29].

For time-correlated single photon counting the measured fluorescence decay $F(t)$ is a convolution of instrument response function $R(t)$ and intensity decay function $I(t)$:

$$F(t)=\int_{0}^{t}R(s+\delta)I(t-s)ds \qquad (4)$$

in which δ is the time shift parameter which takes care of experimental or computational artifact which causes an artificial time-shift of the calculated function with respect to experimental data. $I(t)$ is the theoretical intensity decay function, a continuous distribution of lifetimes, as described in equation (3). $\alpha(\tau)$ is the distribution function which requires to be determined. A numerical method of analysis requires the τ space to be discretized in an appropriate manner. Discretization is preferred in the log(τ) space, which also makes the initial choice of a flat distribution physically meaningful [27]. A hundred to 150 discrete lifetime values uniformly spaced in the log(τ) space are computationally manageable for covering the range from 10 ps to 10 ns. With this approximation $I(t)$ is a multi-exponential function,

$$I(t)=\sum_{i=1}^{N}\alpha_i\exp(-t/\tau_i) \qquad (5)$$

with the important property that α_i(i = 1,N) represent a continuous, smooth function. α_i(i = 1,N) has to satisfy the condition that the experimental data are correctly fitted. That is, the intensity calculated by equation (4), $F_c(t_i)$, and the experimental value, $F_c(t_i)$ should satisfy the χ^2 statistic.

$$\chi^2=(1/M)\sum_{i=1}^{N}\{F_c(t_i)-F_e(t_i)\}^2/\sigma_i^2\approx 1.0 \qquad (6)$$

where δ_i is the standard deviation for the i-th data point and M is the degrees of freedom. Usually the good criterion of $\chi^2\approx 1.0$ could be obtained for many different distributions of $\alpha(\tau)$. The optimum is that which fits data adequately ($\chi^2\approx 1.0$) and maximizes the value of the Shanon-Jaynes entropy function S, as defined below.

$$S=-\sum p_i\log p_i \qquad (7)$$

where $p_i=\alpha_i/\sum\alpha_i$. If there is *a priori* knowledge about the distribution (m_i) then equation (7) is modified as follows

$$S=-\sum p_i\log(p_i/m_i) \qquad (8)$$

If the χ^2 criterion is satisfied for many distributions then the maximum entropy criterion selects that distribution which contains the minimum number of peaks of maximal width.

MATERIALS AND METHODS

Synthesis. Linear precursors of peptides (F-L,L-EN, F-L,D-EN, F-D,L-EN, F-D,D-EN, F(NO_2)-L,L-EN, F(NO_2)-L,D-EN, F(NO_2)-D,L-

Table 1. Fluorescence decay parameters (lifetimes τ and pre-exponential factors) for cyclic enkephalin analogues in different solvents, obtained using the discrete exponential model.

Analogue	Time [ns]	Preexp. factor	Time [ns]	Preexp. factor	Time [ns]	Preexp. factor	Chi square χ^2_R		
	τ_1	α_1	τ_2	α_2	τ_3	α_3	1 exp	2 exp	3 exp
H_2O									
F-L,L-EN	7.86	0.0417	39.09	0.9583			1.11	1.00	
F-L,D-EN	7.43	0.0319	41.51	0.9681			1.09	1.03	
F-D,L-EN	6.18	0.0417	39.25	0.9583			1.13	1.06	
F-D,D-EN	5.53	0.0722	40.40	0.9278			1.27	1.04	
F(NO_2)-L,L-EN	0.77	0.5725	3.94	0.3435	33.38	0.0840	60.18	3.28	1.00
F(NO_2)-L,D-EN	0.61	0.5149	4.03	0.4104	33.12	0.0747	58.65	4.29	1.09
F(NO_2)-D,L-EN	0.61	0.6536	3.15	0.2549	35.06	0.0915	65.20	3.12	1.03
F(NO_2)-D,D-EN	1.79	0.5094	4.50	0.3962	24.18	0.0944	23.96	1.33	1.08
				CH_3OH					
F-L,L-EN	6.97	0.0700	18.62	0.9300			1.23	1.08	
F-L,D-EN	5.69	0.0891	18.90	0.9109			1.39	1.03	
F-D,L-EN	5.14	0.0693	18.67	0.9307			1.29	1.06	
F-D,D-EN	5.55	0.0900	18.63	0.9100			1.45	1.04	
F(NO_2)-L,L-EN	1.85	0.5000	4.49	0.4808	10.83	0.0192	11.79	1.18	1.09
F(NO_2)-L,D-EN	1.24	0.5185	3.19	0.4259	8.97	0.0556	22.53	1.41	0.96
F(NO_2)-D,L-EN	1.98	0.5882	4.04	0.3922	14.02	0.0196	11.17	1.18	1.05
F(NO_2)-D,D-EN	1.72	0.4231	5.53	0.5385	14.11	0.0384	13.80	1.22	1.13
				CH_3CN					
F-L,L-EN	5.92	0.0792	16.97	0.9208			1.29	1.03	
F-L,D-EN	6.62	0.0808	17.38	0.9192			1.20	0.97	
F-D,L-EN	5.10	0.1188	17.56	0.8812			1.49	1.01	
F-D,D-EN	4.38	0.0700	17.04	0.9300			1.38	1.10	
F(NO_2)-L,L-EN	2.13	0.6863	5.21	0.3137			11.45	1.18	
F(NO_2)-L,D-EN	1.06	0.3273	2.36	0.6182	8.95	0.0545	20.08	1.31	1.06
F(NO_2)-D,L-EN	1.51	0.4528	3.54	0.4906	9.64	0.0566	17.37	1.28	1.05
F(NO_2)-D,D-EN	2.01	0.5686	6.41	0.4314			16.50	1.10	
				Me_2SO					
F-L,L-EN	18.05	0.2968	40.81	0.7032			2.71	1.21	
F-L,D-EN	16.69	0.2727	41.07	0.7273			2.84	1.14	
F-D,L-EN	12.69	0.2675	39.54	0.7325			4.26	1.25	
F-D,D-EN	16.33	0.2739	39.89	0.7261			2.90	1.20	
F(NO_2)-L,L-EN	0.58	0.2414	3.15	0.5000	7.15	0.2586	11.21	1.50	1.06
F(NO_2)-L,D-EN	0.52	0.1930	2.91	0.5789	8.27	0.2281	15.35	1.38	1.06
F(NO_2)-D,L-EN	0.53	0.2759	3.25	0.5000	8.92	0.2241	16.15	1.93	1.20
F(NO_2)-D,D-EN	3.56	0.451	9.94	0.5490			7.03	1.13	

EN, F(NO_2)-D,D-EN) were synthesized by solid-phase methodology using the fluorene-9-yl-methoxy-carbonyl chemistry [30]. Cyclization was performed using HBTU [31]. The peptides were purified by means of preparative RP-HPLC. The homogeneity and molecular constitution of the compounds was assessed by analytical RP-HPLC and FAB-MS, and ^{1}H NMR COSY spectroscopy.

Time-resolved fluorescence measurements. Fluorescence decay times were measured using a time correlated single-photon counting apparatus at Apparatus Laboratory (ŚLAS), Adam Mickiewicz University (Poznań, Poland). The excitation source (λ_{ex} = 280 nm) was a pico/femtosecond laser system (Ti:Saphire "Tsunami" laser pumped with an argon ion laser "BeammLock" 2060). The emission was detected with a magic angle polarizer at an emission wavelength of λ_{em} = 340 nm. A Ludox solution was used to collect the instrument response. All measurement were done at 20°C parallelly in water, methanol, acetonitrile and Me_2SO. For lifetime distribution calculation the software provided by Edinburgh Analytical Instruments was used.

RESULTS AND DISCUSSION

Fluorescence energy transfer between a donor and an acceptor can also result in a changes in distribution of decay times. Suppose that the energy donor has a mono-exponential fluorescence decay time. If the donor and acceptor remain at a fixed distance the decay of the donor is accelerated by energy transfer, without changing its mono-exponential time dependence. However, if there is a range of donor-acceptor distances a range of transfer rates, and hence a distribution of decay times should be observed. If two unique distance distributions are present one should expect a bimodal time distribution.

To check how different configurations of amino acids in positions 2 (Dab) and 3 (Pro) influence the distance between the donor in position 4 (β-naphthylalanine) and the acceptor in position 1 [p-Phe(NO_2)] in cyclic analogues of enkephalin we measured the fluorescence decay times of the donor in a donor-alone model (cyclic enkephalin without the acceptor, Phe in position 1) and in donor-acceptor pairs in different solvents. Data presented in Table 1 and 2 were obtained using two calculation schemes – discrete exponential analysis and lifetime distribution. According to the values listed in Table 1 in the absence of acceptor the donor fluorescence decays are biexponential in all solvents studied. The longest fluorescence lifetimes are observed in water, the most polar solvent used in our studies, and in Me_2SO. The biexponential fluorescence decay was also observed by Schiller's group [32] for β-naphthylalanine in peptides. Such heterogeneous fluorescence decay was explained by coexistence of two conformers one of which was highly quenched by the peptide backbone.

The configuration of amino acids in position 2 and 3 has little influence on the fluorescence decay times of β-naphthylalanine in all systems studied (Table 2). The longest fluorescence lifetimes are observed for the enkephalin analogues with amino-acid residues in positions 2 and 3 having alternating configurations (D-Dab^2,L-Pro^3, and L-Dab^2,- D-Pro^3) in all solvents studied except water; however, differences between eukephalin analogues are small.

The configuration of Dab in position 2 has generally rather small influence on the photophysical properties of β-naphthylalanine. Such behaviour can be easily explained taking into account the position of this amino acid in the peptide chain. The proline residue is directly connected with β-naphthylalanine and affects conformational freedom of the naphthyl chromophore whereas change of the Dab configuration, even when it changes the peptide skeleton conformation, does not influence the photophysical properties of β-naphthylalanine due to a considerable distance between these two amino acids.

Table 2. Fluorescence mean lifetimes and amplitudes for cyclic enkephalin analogues in different solvents, obtained using the lifetime distribution model.

Analogue	Mean lifetime [ns]	Amplitude	Mean lifetime [ns]	Amplitude	Mean lifetime [ns]	Amplitude	Chi square
	τ_1	f_1	τ_2	f_2	τ_3	f_3	χ^2_R
				H_2O			
F-L,L-EN	3.37	0.0113	39.73	0.9887			1.02
F-L,D-EN	3.64	0.0053	42.18	0.9947			1.05
F-D,L-EN	2.52	0.0116	39.87	0.9884			1.08
F-D,D-EN	3.37	0.0507	41.11	0.9493			1.07
F(NO_2)-L,L-EN	0.76	0.5244	3.88	0.3962	37.86	0.0794	0.94
F(NO_2)-L,D-EN	0.43	0.4365	3.69	0.4833	36.89	0.0802	1.01
F(NO_2)-D,L-EN	0.75	0.6369	3.55	0.2572	38.10	0.1058	1.04
F(NO_2)-D,D-EN			3.12	0.9155	35.51	0.0845	1.06
				CH_3OH			
F-L,L-EN			18.06	1.00			1.15
F-L,D-EN	2.58	0.0246	18.31	0.9754			1.11
F-D,L-EN	1.80	0.0141	18.18	0.9859			1.14
F-D,D-EN	2.03	0.0375	18.01	0.9625			1.10
F(NO_2)-L,L-EN	0.47	0.0239	3.34	0.9761			1.12
F(NO_2)-L,D-EN			2.53	1.00			0.96
F(NO_2)-D,L-EN	0.16	0.0261	2.88	0.9633			1.08
F(NO_2)-D,D-EN	1.75	0.3662	5.58	0.6338			1.23
				CH_3CN			
F-L,L-EN	2.48	0.0078	16.36	0.9922			1.11
F-L,D-EN	2.25	0.0031	16.73	0.9969			1.05
F-D,L-EN	2.87	0.0573	16.92	0.9427			1.08
F-D,D-EN	1.82	0.0215	16.56	0.9785			1.21
F(NO_2)-L,L-EN	0.09	0.1015	3.06	0.8985			1.16
F(NO_2)-L,D-EN	0.25	0.0801	2.05	0.8777	9.77	0.0423	1.06
F(NO_2)-D,L-EN	0.30	0.0767	3.06	0.9233			1.04
F(NO_2)-D,D-EN	1.70	0.4058	5.39	0.5942			1.10
				Me_2SO			
F-L,L-EN	3.76	0.0414	35.07	0.9586			1.18
F-L,D-EN	4.26	0.0552	35.77	0.9448			1.12
F-D,L-EN	5.07	0.1132	35.43	0.8868			1.18
F-D,D-EN	4.55	0.0528	34.83	0.9472			1.22
F(NO_2)-L,L-EN	0.76	0.2093	4.49	0.7844	75.72	0.0064	1.04
F(NO_2)-L,D-EN	0.17	0.1249	3.96	0.8650	69.56	0.0101	1.02
F(NO_2)-D,L-EN	0.39	0.2222	4.60	0.7675	69.42	0.0103	1.18
F(NO_2)-D,D-EN	2.84	0.2078	8.34	0.7900	84.12	0.0022	1.14

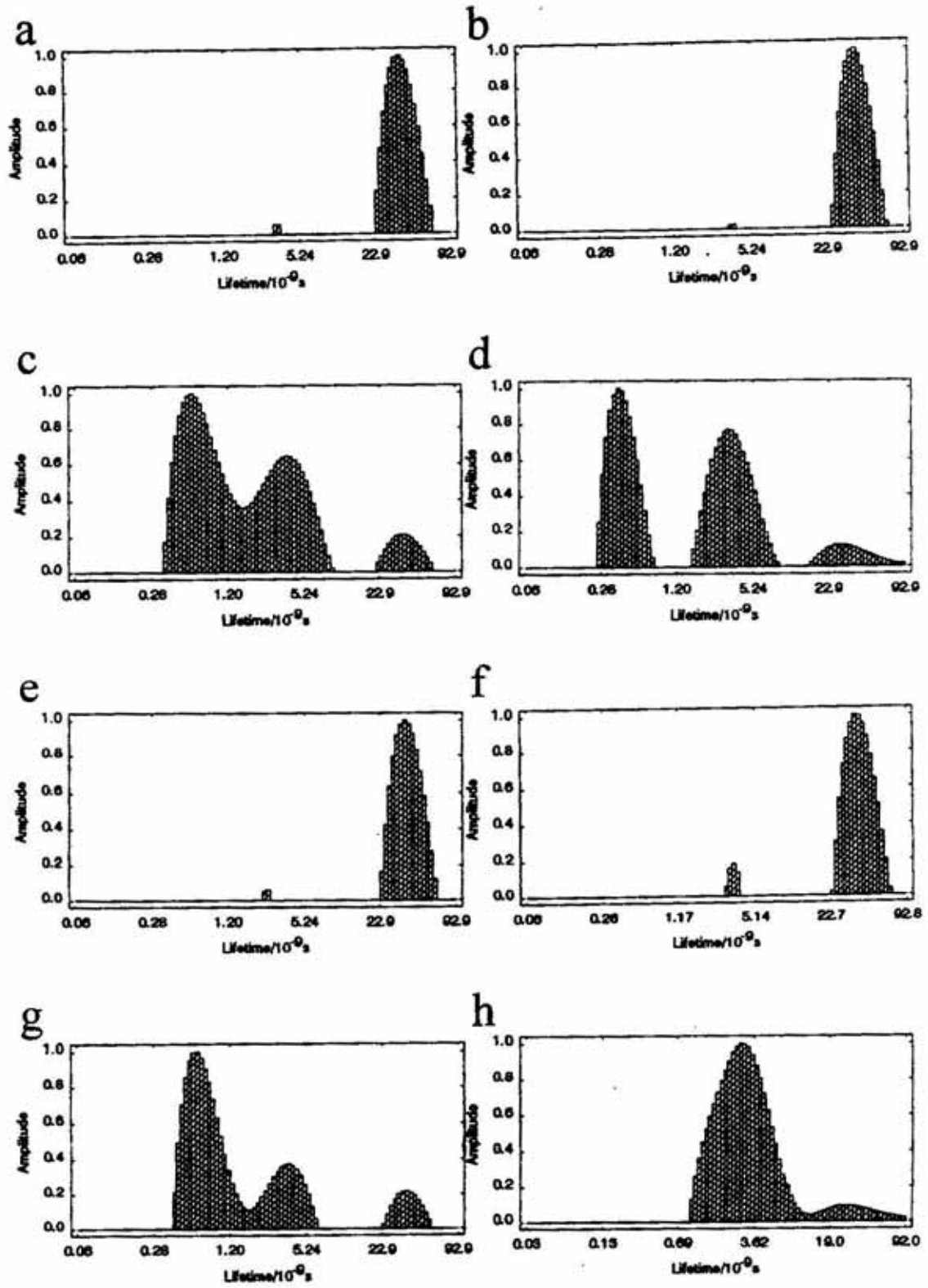

Figure 2. Fluorescence lifetime distributions of β-naphthylalanine in cyclic enkephalin analogues (a. F-L,L-EN; b. F-L,D-EN; c. F(NO_2)-L,L-EN; d. F(NO_2)-L,D-EN; e. F-D,L-EN; f. F-D,D-EN; g. F(NO_2)-D,L-EN; h. F(NO_2)-D,D-EN) in water.

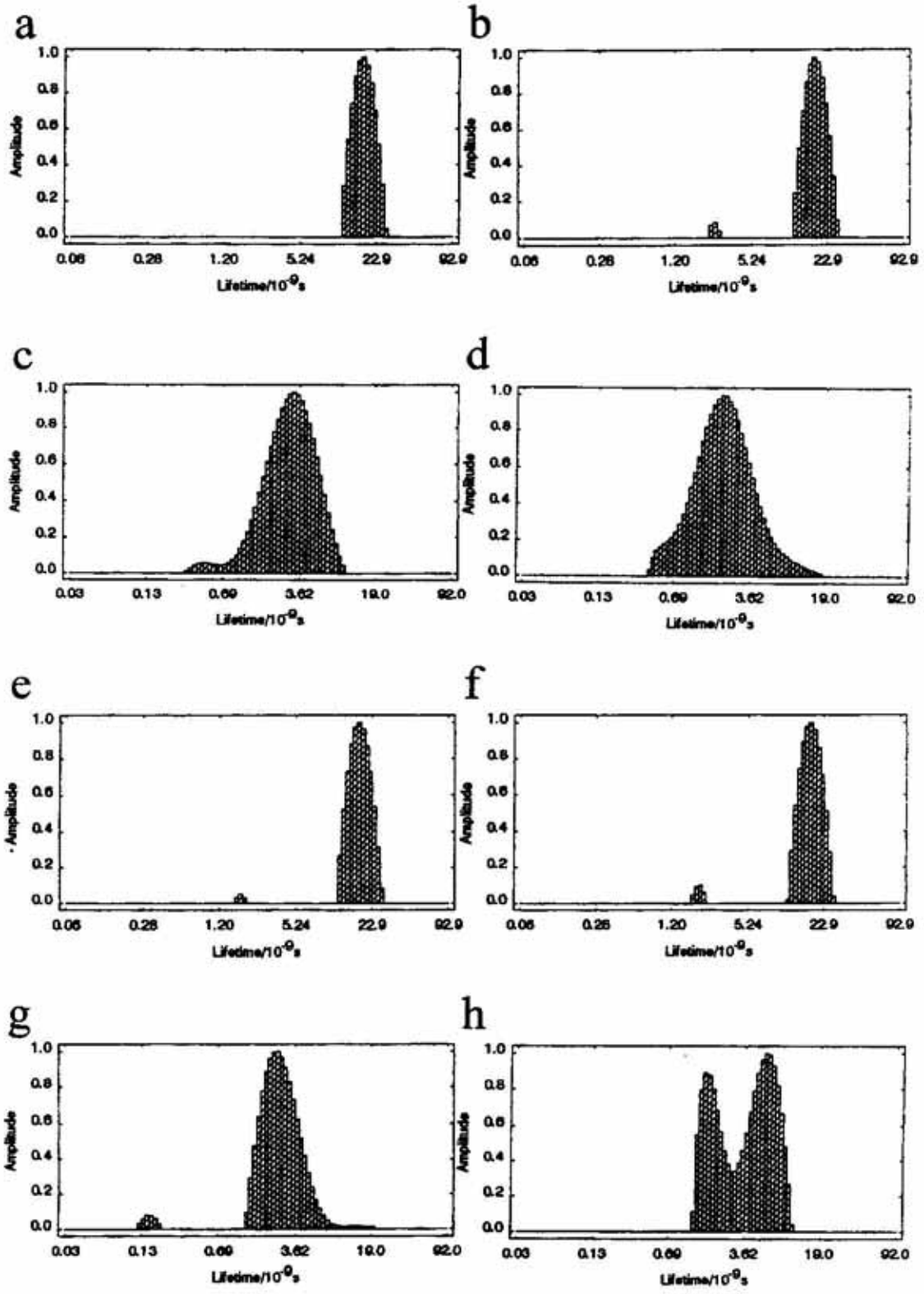

Figure 3. Fluorescence lifetime distributions of β-naphthylalanine in cyclic enkephalin analogues (a. F-L,L-EN; b. F-L,D-EN; c. F(NO_2)-L,L-EN; d. F(NO_2)-L,D-EN; e. F-D,L-EN; f. F-D,D-EN; g. F(NO_2)-D,L-EN; h. F(NO_2)-D,D-EN) in methanol.

The fluorescence decay of β-naphthylalanine in enkephalin analogues containing energy acceptor in position 1 [Phe(p-NO_2)] are considerably more complex and single or double exponential decays fitted the experimental data poorly (high values of χ^2_R, Table 1). The fluorescence decay for the D-A pair are adequately described by a triple exponential model (Table 1). Because of energy transfer between β-naphthylalanine and Phe(p-NO_2) the observed decay times of donor in donor-acceptor pairs are much shorter than for the donor-alone model. It should be noted that the decay would be expected to remain a single exponential if there were a unique conformation and a single D-A distance. The high heterogeneity of donor fluorescence decay in all enkephalin analogues containing Phe(p-NO_2) (in all studied solvents) provides thus evidence for the existence of a range of interchromophoric distances. The parameters obtained from the three-exponential fit to the intensity decays do not fully describe conformational changes in the systems studied, therefore, we have applied the lifetime distribution model. The mean lifetimes and their amplitudes are presented in Table 2 and in Figs. 2-5. Lifetime distributions of the donor decays in cyclic enkephalin analogues containing β-naphthylalanine as the only aromatic amino acid are narrow and bimodal, with mean lifetimes corresponding to the lifetimes obtained using the discrete exponential analysis. Certain deviation from the observed tendency are found for Me_2SO, where distributions are wider and asymmetric. For this reason the mean lifetimes and their amplitudes differ in Me_2SO considerably from those obtained by multi-exponential analysis.

Because of the energy transfer the lifetime distributions of β-naphthylalanine in enkephalin analogues containing the Phe(p-NO_2) acceptor, differ considerably from donor-alone enkephalin analogues. All distributions are asymmetric, multi-modal with mean lifetime shifted to smaller values. In low polarity solvents like MeOH and CH_3CN the major component of the distribution is located around 3 ns. A minor subnanosecond component with a small amplitude (2-3%) is also observed in all studied solvents. This type of distribution is a common future for all enkephalin analogues regardless of configurations of Dab and Pro, except for the analogue of enkephalin which contains both amino acids in D-configuration. For this particular compound, in all studied solvents except water, one can observe a clearly resolved bi-modal distribution with approximately equal amplitudes and mean lifetimes shifted towards higher values (about 2.0 and 5.5 ns, Table 2).

In the polar solvents, H_2O and Me_2SO, distributions of lifetimes are multi-modal. A third minor component with a long mean lifetime similar to that of the donor-alone, especially well visible in water is observed for all studied enkephalins. It is probably due to the presence of enkephalin without acceptor. Because of the lack of fluorescence quenching by energy transfer, a very small amount of the latter invisible by the RP-HPLC method with absorption detection, can give a substantial fluorescence signal in comparison to that of the quenched sample.

Thus, like in less polar solvents, in water and Me_2SO bimodal distributions are present but the width of distributions in these solvents is larger. In Me_2SO the distribution located in the subnanosecond range has a smaller amplitude than the component with mean lifetime around 4 ns. A quite different picture (larger amplitude of the shorter component) is observed in water. Like in less polar solvents, the lifetime distribution calculated for the analogues of enkephalin containing D-Dab and D-Pro are narrower than for the analogues containing one of these amino acids in L-configuration. The asymmetric uni-modal distribution is observed in water whereas a highly overlapping bi-modal in Me_2SO.

In less polar solvents one major peak in lifetime distribution indicates the presence of one major family of conformations. Intramolecular hydrogen bonds between carbonyl

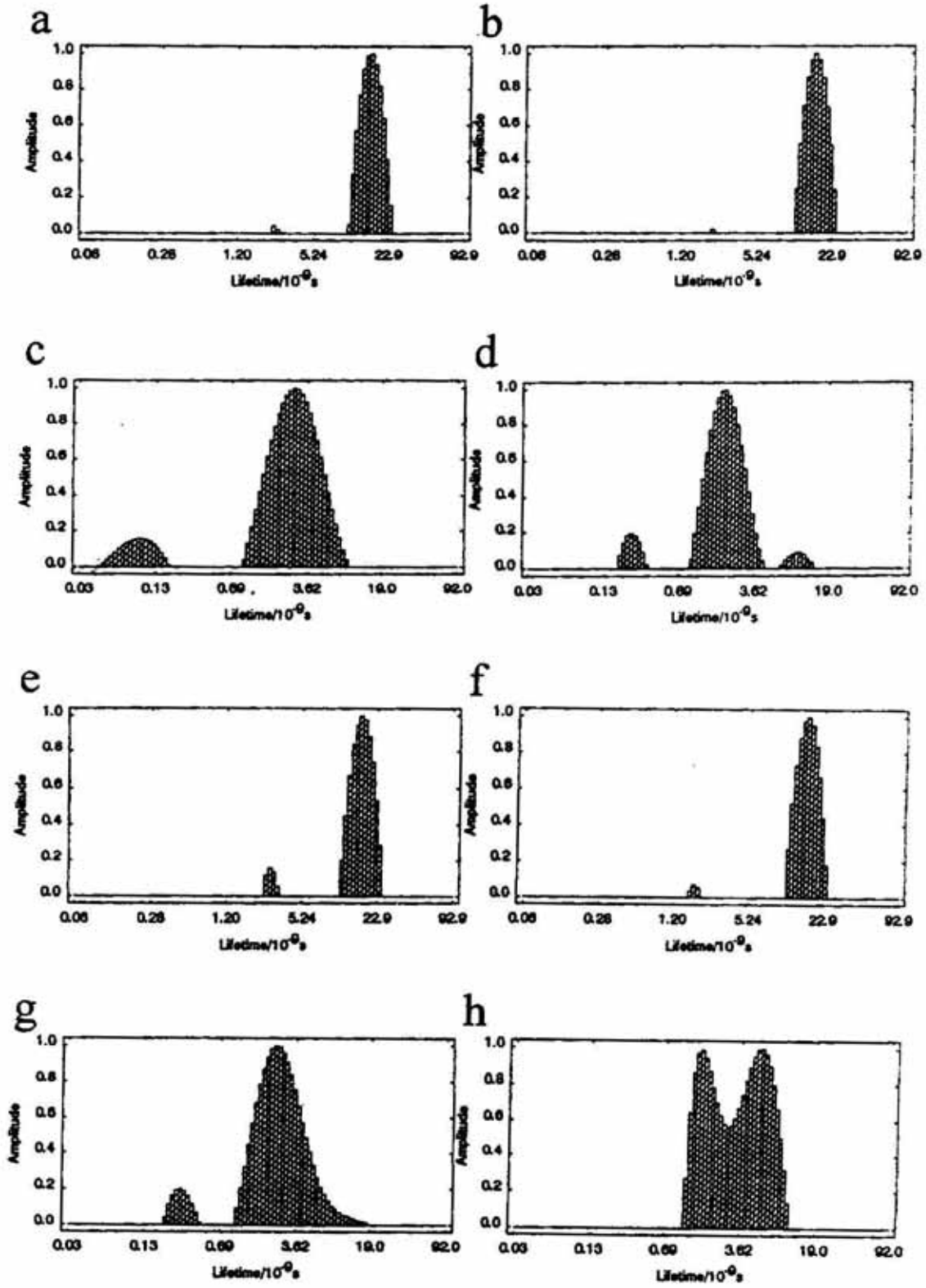

Figure 4. Fluorescence lifetime distributions of β-naphthylalanine in cyclic enkephalin analogues (a. F-L,L-EN; b. F-L,D-EN; c. F(NO_2)-L,L-EN; d. F(NO_2)-L,D-EN; e. F-D,L-EN; f. F-D,D-EN; g. F(NO_2)-D,L-EN; h. F(NO_2)-D,D-EN) in acetonitrile.

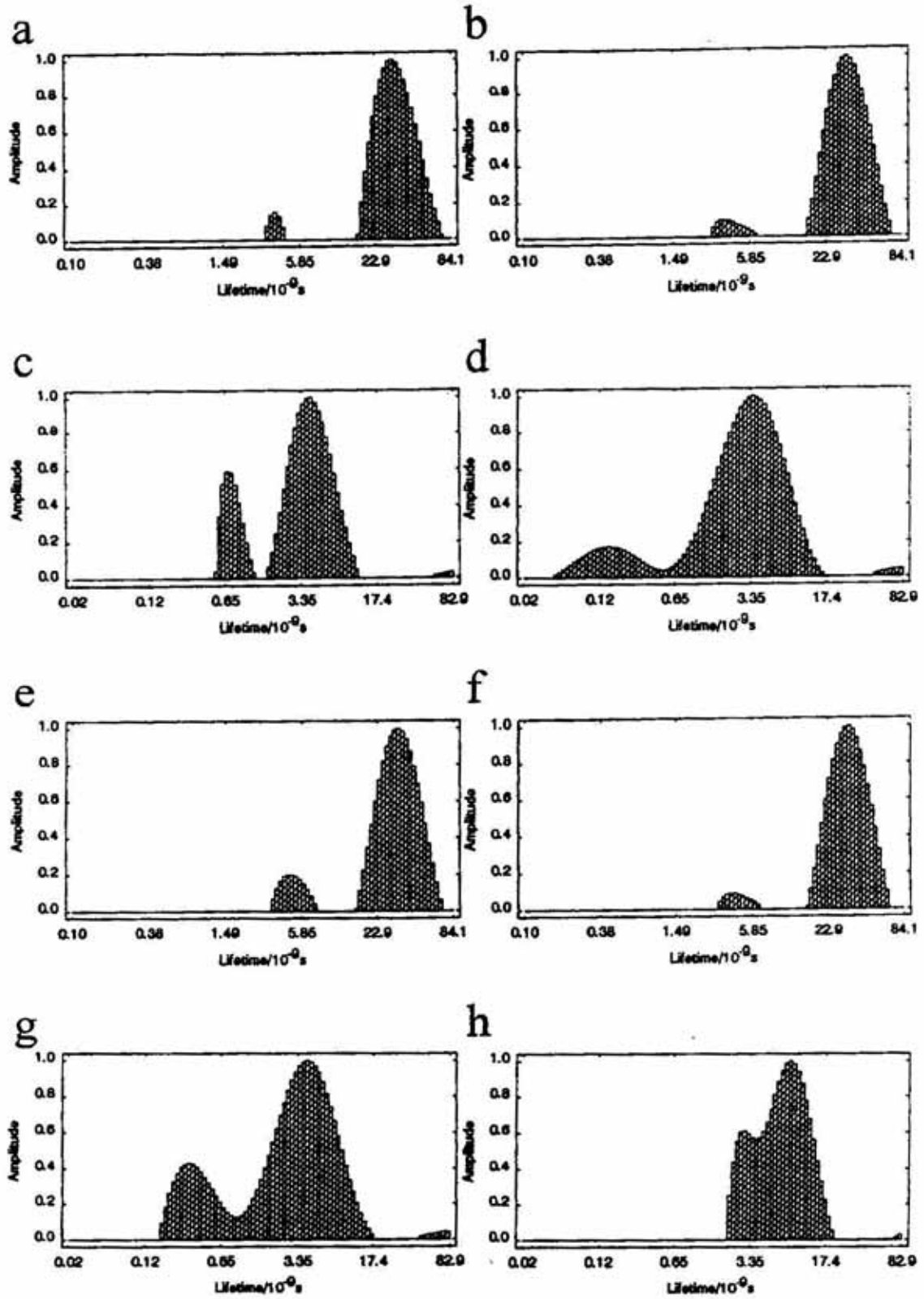

Figure 5. Fluorescence lifetime distributions of β-naphthylalanine in cyclic enkephalin analogues (a. F-L,L-EN; b. F-L,D-EN; c. F(NO_2)-L,L-EN; d. F(NO_2)-L,D-EN; e. F-D,L-EN; f. F-D,D-EN; g. F(NO_2)-D,L-EN; h. F(NO_2)-D,D-EN) in Me_2SO.

and amide NH groups of peptide chain stabilize the conformation of peptidic cyclic ring [33–37]. The width of the lifetime distribution peak can be related to the conformational freedom of aromatic amino acid residues. However, one should remember that the width of the lifetime distribution peak depends also on the quality of the data. The noisier the data, the wider will be the distribution [29]. Thus, caution should be taken in connecting the width of the distribution peak to the conformational freedom of the peptide or amino-acid residues.

In more polar solvents used in our studies (water, Me_2SO) enkephalins exist as two main conformation families. In Me_2SO like in methanol or acetonitrile, the most populated are conformations with relatively large interchromophoric distances. On the other hand, in water which forms strong intermolecular hydrogen bonds, part of intramolecular hydrogen interactions in enkephalin is disrupted allowing for more conformational freedom of the peptide skeleton. However, configuration of Dab in position 2 and Pro in position 3 have little effect on the conformation of enkephalin in all solvents used except when both amino acids have the D,D configuration. Such configuration restricts conformational freedom giving preference to those conformations in which intrachromophoric distances (regardless of the solvent) are larger.

While data for a donor-acceptor pair can be analyzed using the decay time distribution, we believe it is preferable to formulate the analysis in terms of parameters which directly characterize the distribution of distances [15, 38–41]. A general restriction concerning mathematical methods used at present is the *a priori* assumption of Gaussian [15, 40, 41] or Lorentzian [38, 39] dependence of distance distribution. This assumption can not give details of distance distribution, especially when it is multi-modal with small contribution of one of the components. To the best of our knowledge, at this time there is no software available which could give a detailed, unbiased information about interchromophoric distance distribution. The distance distribution calculations can be only made if energy transfer between a donor and an acceptor proceeds according to the long-range, Fŏrster mechanism.

Therefore, application of lifetime distribution analysis without the *a priori* knowledge of distribution function that characterizes the donor-acceptor pair in terms of population of conformations can be very useful. Additional advantages of lifetime distribution analysis is a possibility to obtain information about changes in conformational of peptides containing an energy donor-acceptor pair regardless of the mechanism of the energy transfer process. Lifetime distribution analysis can also yield useful information about the influence of different factors on conformation of peptides in solution not only from lifetime distribution analysis of donor-acceptor pair but also from lifetime distribution analysis of a donor-alone model compound. Finally, this method can be applied to verify distance distribution calculations (uni- or multi-modal).

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