ELSEVIER

Coordination Chemistry Reviews
185–186 (1999) 307–319

COORDINATION CHEMISTRY REVIEWS

Lanthanide ions as redox probes of long-range electron transfer in proteins

Ronald M. Supkowski, James P. Bolender, Wendy D. Smith, Lewis E.L. Reynolds, William DeW. Horrocks Jr *

Chemistry Department, Pennsylvania State University, University Park, PA 16802, USA

Received 3 August 1998; received in revised form 9 November 1998

Contents

Abstract

Occupancy of the two calcium-binding sites of codfish parvalbumin by the redox-active probe ions Yb^{3+} and Eu^{3+} causes the average tryptophan (Trp) fluorescence lifetime in this protein to decrease and become non-exponential from the single exponential values found for Ca^{2+} and La^{3+} of 4.45 ns. These observations are interpreted in terms of an electron transfer (ET) deexcitation mechanism wherein excited singlet state Trp transfers an electron to the Ln^{3+} ion, reducing it to the $+2$ oxidation state and producing a Trp cation radical. Back ET reestablishes the initial system. The driving forces, $\Delta G°$, for the Eu^{3+} and Yb^{3+} ET systems are different, whereas the nuclear rearrangement factor, λ, and electron donor-acceptor coupling, H_{AB}, of semiclassical ET theory should be nearly the same for both ions. This allows the λ-value to be determined from the measured rates (~ 2.05 eV). Temperature dependence studies show that the rate constant for the Eu^{3+} system is near the activationless

* Corresponding author. Fax: +1-814-865-3314.
E-mail address: wd2@psu.edu (W.D. Horrocks Jr)

PII: S0010-8545(98)00276-8

maximum value. Yb^{3+} and Eu^{3+} are established as redox probes of long-range ET in proteins making Ln^{3+}-substituted calcium-binding proteins convenient model systems for studying the distance-dependence of ET.

Keywords: Lanthanide; Electron transfer; Parvalbumin; Europium; Ytterbium; Tryptophan

1. Introduction

A major area of study in the field of bioinorganic chemistry is long-range electron transfer (ET) in proteins [1–4]. Biological electron transfer lies at the heart of many physiological processes: photosynthesis, production of deoxyribonucelotides for DNA, and respiration, to name a few. A common feature of a large percentage of these proteins is the presence of a metal ion binding site or a metal cluster moiety. Metal ion sites are useful because they provide an electron sink since metals can easily cycle between redox states. The ease with which metals can change redox states is determined by both the properties of the metal and the characteristics of the binding environment that the protein constructs for the metal [5]. The rates at which the electrons move to and from the metal sites are presumably controlled by the protein matrix. Detailed knowledge of the factors determining rates of ET is central to an understanding of how redox proteins function.

Experimental study of electron transfer requires a system that has an electron donor, an electron acceptor, and a method of initiating the electron transfer process. Photosynthetic proteins contain a naturally occurring set of electron donors and acceptors that do just this, the ET mechanism is started with the absorption of light. There have also been many ingenious semi-synthetic systems designed to date to study long-range electron transfer in proteins. Proteins that act as electron shuttles by accepting electrons from one protein and delivering them to others, such as cytochrome *c*, have had ruthenium complexes ligated to surface histidines on the proteins [6–8]. The electron transfer is monitored by instantaneously reducing the ruthenium by a number of methods and observing the transfer of an electron from the reduced ruthenium to, for example, an intrinsic iron(III) heme. Another method that has been used is monitoring the passage of an electron between two non-covalently bound heme-containing proteins. One protein of the couple has its heme substituted with a zinc or magnesium protoporphyrin IX, the triplet state of this closed-shell porphryin is obtained through photoexcitation and reduces the iron(III) heme in the adjoining protein [9]. Many biological electron transfer proteins have been studied using slight modifications of one of these methods and have added to the understanding of protein control of electron transfer.

Our recent discovery [10] that tryptophan (Trp) fluorescence in the calcium-binding protein parvalbumin from codfish is quenched by an electron transfer process when the lanthanide ions (Ln^{3+}) Eu^{3+} or Yb^{3+} are bound at the Ca^{2+}-binding sites introduces these ions as a new class of electron transfer redox probe for the study of long-range ET in proteins. The electron donor in this system is a Trp

photoexcited to a singlet state which then reduces the nearby bound Ln^{3+} to its +2 oxidation state. The electron then returns to the Trp radical cation which, in the case of Yb^{3+} substituted cod parvalbumin, may leave the Yb^{3+} in its electronically excited state. A schematic diagram of our proposed ET mechanism for Eu^{3+}-substituted and Yb^{3+}-substituted codfish parvalbumin is shown in Fig. 1. The quenching of the singlet state of Trp by electron transfer is not a new phenomenon, Trp fluorescence is quenched in Trp-containing azurins where the electron acceptor is a Cu^{2+} in a blue-copper site [11], and Eu^{3+} and Yb^{3+} are known to quench Trp fluorescence both in solution [12] and when the indole ring is covalently attached to an Ln^{3+} complex [13,14]. The substitution of Ln^{3+} ions for the spectroscopically silent Ca^{2+} ion in calcium binding proteins is also not new. It is well established that Ln^{3+} ions represent useful surrogate replacements for the spectroscopically-silent Ca^{2+} ion in calcium-binding proteins [15]. The luminescence of Tb^{3+} or Eu^{3+} brought about by either direct excitation or sensitized by energy transfer from nearby excited fluorophores has been exploited on numerous occasions in this laboratory and in others to provide information about the metal ion binding sites as well as other aspects of protein structure [16–19]. The ability of Ln^{3+} ions to serve as ET redox centers further expands the utility of these ions as biological probes.

Parvalbumin belongs to a larger class of calcium binding proteins which are all characterized by having similar metal binding sites [20–23]. Other proteins in this

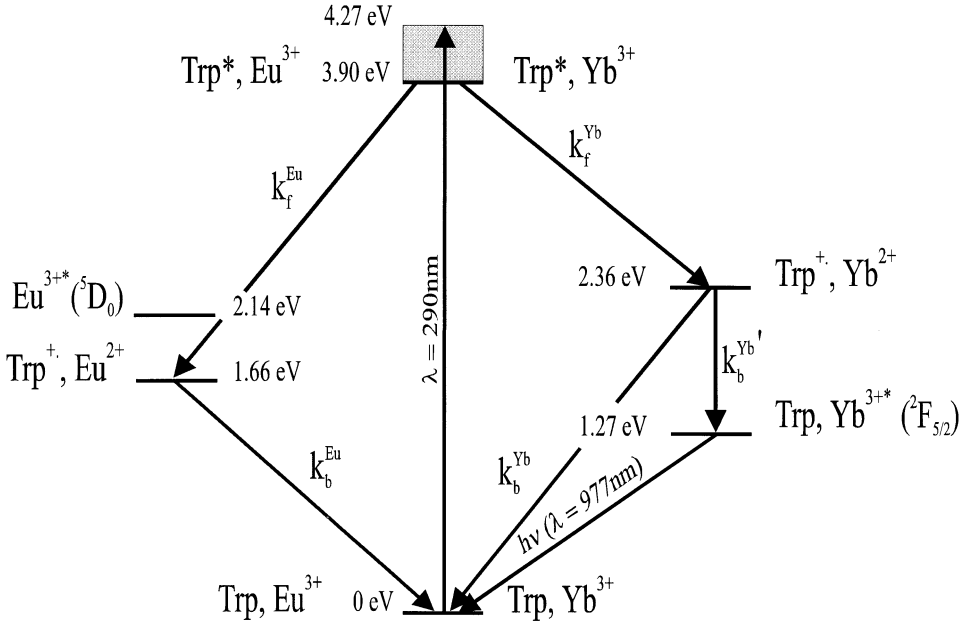


Fig. 1. Proposed electron transfer energy level schematic for Eu^{3+}- and Yb^{3+}-substituted codfish parvalbumin.

class include calmodulin, calbindin, troponin *c* and S-100β. The Ca^{2+} binding sites in these proteins generally consist of a 12-residue loop connecting two α-helices (known as EF hands). The metal ligating atoms at each site consist of various combinations of acidic, alcoholic and backbone carbonyl oxygens located in the loop as well as supplemental water oxygens. Parvalbumin is a relatively small protein (12 000 Da) found in the white muscle of vertebrates. Although its exact function is unknown, parvalbumin serves as a structural prototype for proteins in this class. It is made up of six α-helices labeled A through F with two calcium binding sites located between the CD and EF helices. Carp parvalbumin (pI = 4.25), whose X-ray crystal structure was determined early on [24,25], contains 10 phenylalanine residues, but no tyrosine or typtophan. Many isotypes of parvalbumin of similar structure are known; one from codfish (pI = 4.75) contains a single Trp and a single tyrosine as well as a complement of 10 phenylalanine residues [23,26] and its Ln^{3+} substituted forms have been thoroughly characterized [19,27–31], which makes codfish parvalbumin an excellent candidate for the study of Trp photophysics. The CD calcium binding site of cod parvalbumin resides in a loop consisting of residues 51–62 and the EF site is formed by residues 90–101. The single Trp is located at position 102 in the protein [26], which begins the F helix. The through-space distance from Trp 102 to each protein-bound metal ion is about 11 Å for both the CD and EF sites, as inferred from the carp parvalbumin crystal structure (PDB file 5CPV).

2. Materials and methods

All water used was deionized with a Nanopure system to a resistance of at least 18 MΩ cm^{-1}. Holo codfish parvalbumin was isolated from fresh codfish according to the method of Horrocks and Collier [31] and prepared in 0.5–1.0 mM stock solutions as determined by its molar absorptivity at 280 nm, $\varepsilon_{280\ nm}$, of 7180 $M^{-1}cm^{-1}$ [32]. Hydrated $LaCl_3$, $EuCl_3$ and $YbCl_3$ were all obtained from Aldrich chemicals and used to make ca. 20 mM stock solutions, the exact concentration determined by an EDTA titration with arzenazo II dye as the indicator [33]. All samples were 10 μM protein, 100 μM DTT (to prevent dimerization through the lone cysteine) and diluted to volume with a buffer containing 150 mM NaCl, and 50 mM HEPES adjusted to pH 7.0 by additions of a concentrated NaOH solution. All Ln^{3+}-substituted codfish parvalbumin samples were prepared by the addition of at least 3 equivalents of Ln^{3+} to the holoprotein.

Time-correlated single photon counting experiments to determine Trp fluorescence lifetimes were made using a mode-locked Nd:YAG laser synchronously pumping a cavity-dumped dye laser. The details of the laser system and time-correlated photon counting (TCPC) detection are described elsewhere [34]. The instrument function had a FWHM of about 50 ps. The channel widths for the multi-channel analyzer were 6.72 ps $(channel)^{-1}$ for La^{3+}- and Yb^{3+}-substituted codfish parvalbumin and 3.51 ps $(channel)^{-1}$ for Eu^{3+}-substituted parvalbumin; 2000 channels were used for all experiments. The largest number of counts in any

Table 1
Trptophan fluorescence lifetimes of metal substituted codfish parvalbumin

Temp (°C)	La^{3+}	Yb^{3+}			Eu^{3+}		
	τ (ns)	α_1[a] (%)	τ_1 (ns)	τ_2 (ns)	α_1[a] (%)	τ_1 (ns)	τ_2 (ns)
1	5.03	80.0	2.13	3.81	92.1	0.90	1.94
8	4.94	80.2	2.06	3.57	94.9	0.91	2.02
13	4.87	78.0	1.98	3.35	95.3	0.91	1.99
19	4.78	81.8	1.98	3.33	96.0	0.91	1.98
25	4.68	82.2	1.95	3.22	95.1	0.91	1.88
33	4.54	85.6	1.94	3.20	97.6	0.92	2.09

[a] α_1 is the percentage of the tryptophan fluorescence intensity at $t = 0$ due to the τ_1 tryptophan lifetime for Eu^{3+} and Yb^{3+} substituted proteins.

channel for the TCPC experiments was kept at about 20 000 for all experiments. The 296 nm excitation light was obtained by doubling the emission of rhodamine 6G dye. Since it is known that the fluorescence of the single Trp in codfish parvalbumin [19,26,35] and the similar whiting parvalbumin [36,37] decays non-exponentially over most of its emission envelope, all of our lifetime data was taken at an emission wavelength of 340 nm where the fluorescence decay of the holo protein can be fit well to a single exponential [35]. Temperature dependence data was obtained using a brass cuvette holder through which thermostated water was circulated. Each sample was allowed at least 5 min to come to thermal equilibrium in the holder.

3. Results

Our Trp fluorescence emission lifetime experiments reveal single-exponential fluorescence decays ($\lambda_{em} = 340$ nm) for the holo codfish parvalbumin (Ca_2cod) in good agreement with previous reports [35]. The substitution of La^{3+} for Ca^{2+} in cod parvalbumin (La_2cod) produces no change in the lifetime of the Trp fluorescence. Upon the substitution of Eu^{3+} or Yb^{3+} for the calcium, on the other hand, the decay of the Trp fluorescence becomes faster and decidedly non-exponential; it fits well to a sum of two exponential decays. Lifetime data is given in Table 1, and typical Trp fluorescence decays along with residuals from the fits are shown in Fig. 2.

To determine the origin of the double exponential lifetimes of the Eu_2cod and Yb_2cod we titrated Eu^{3+} and Yb^{3+} into holo parvalbumin (data not shown). As the Ln^{3+} is added the long holo lifetime disappears with the concomitant appearance of both shorter lifetimes. When the protein is fully saturated with Ln^{3+} (up to 10 equivalents of metal ion), the double exponential lifetime still remains. Since each Trp has both ET paths available to it in the fully metal-saturated protein, ET to each site would not be manifested as two lifetimes but as one corresponding to

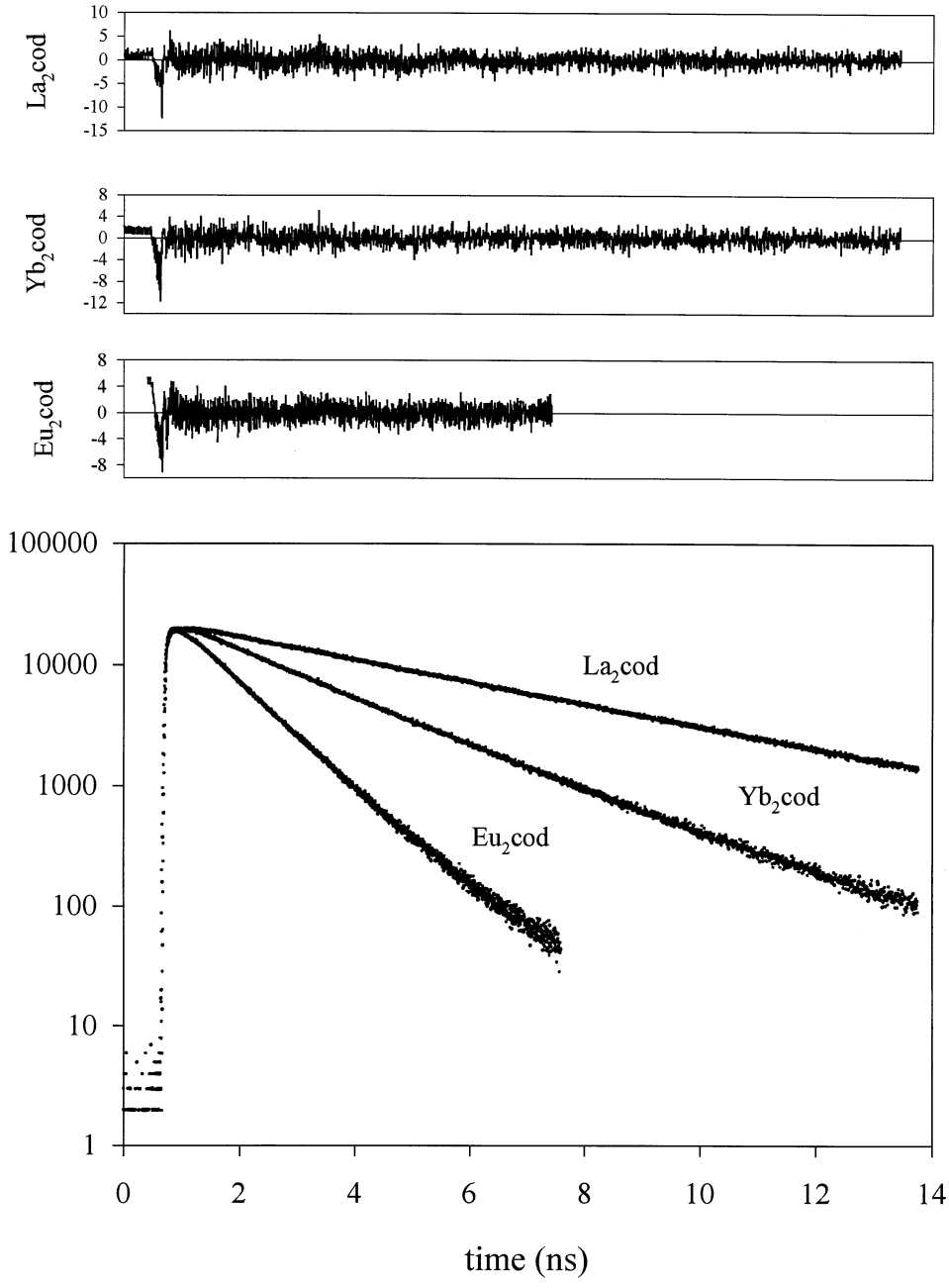


Fig. 2. Tryptophan fluorescence decays for La^{3+}, Yb^{3+} and Eu^{3+} substituted codfish parvalbumin at 25°C. Residuals are from fitting the data to the the single/double exponential decay functions of Table 1.

the sum of the ET rates to both sites. We hypothesize that two species of protein exist wherein the Trp is in two different environments which interconvert at a timescale longer than the ns regime. The reason for differing percentages of the fluorescence decay of the two species for Eu_2cod and Yb_2cod may be due to the proximity of the EF metal binding site to the Trp 102 pocket. Eu^{3+} and Yb^{3+} differ in size by about 0.07 Å, and this difference may perturb the Trp environment.

The rate constant of forward electron transfer, k_f, is calculated from Eq. (1):

$$k_f = 1/\tau\ (Eu_2\text{cod or } Yb_2\text{cod}) - 1/\tau\ (La_2\text{cod}) \quad (1)$$

Where the τ's are the Trp lifetimes of the Ln^{3+}-substituted cod parvalbumin. A plot of these rate constants versus temperature is shown in Fig. 3. According to semi-classical Marcus theory [38], ET rate constants, k_{ET}, are given by Eqs. (2a), (2b) and (2c).

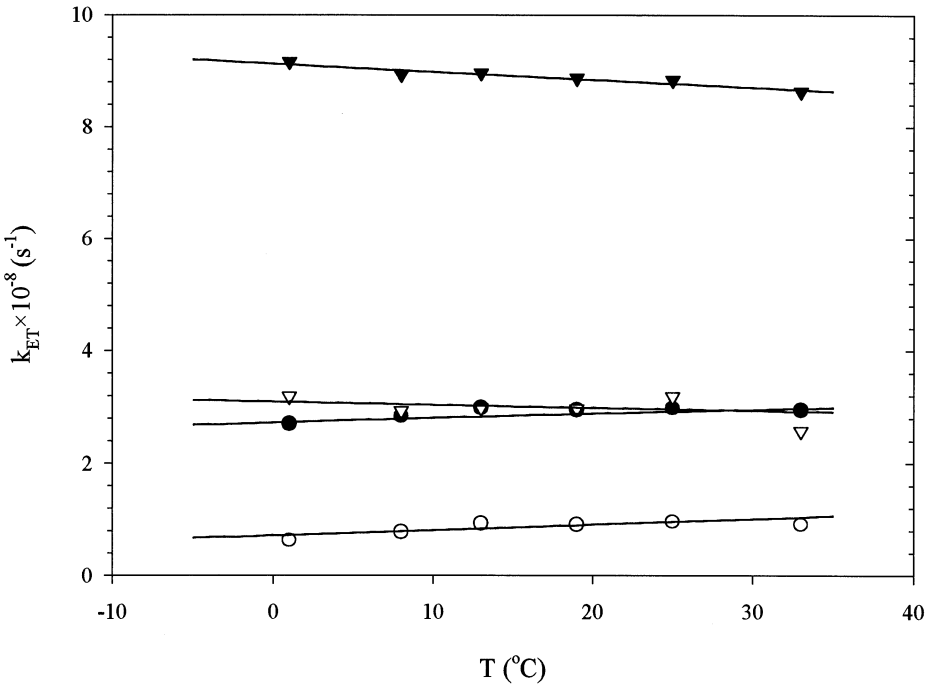
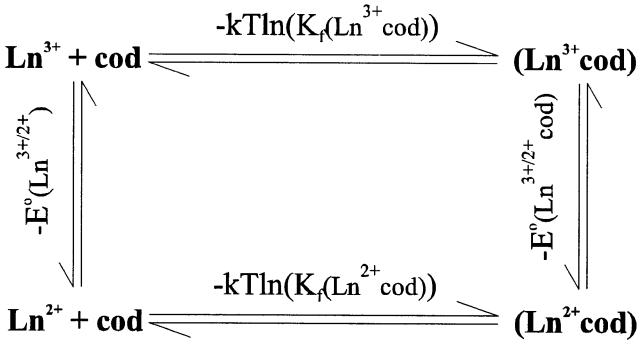


Fig. 3. Plots of the temperature dependence of the rate constants for electron transfer in metal substituted codfish parvalbumin. Circles are the Yb_2cod system and triangles are the Eu_2cod system. Filled symbols are the higher percentage component of the Trp fluorescence decay, empty symbols are the smaller percentage component. The solid lines are plots calculated from Eqs. (2a), (2b) and (2c) with the parameters of Table 3.



Scheme 1.

$$k_{ET} = k_0 e^{\frac{-\Delta G^\circ}{kT}} \tag{2a}$$

$$\Delta G^\circ = \frac{(\Delta G^\circ + \lambda)^2}{4\lambda} \tag{2b}$$

$$k_0 = H_{AB}^2 \sqrt{\frac{4\pi^3}{h^2 \lambda kT}} \tag{2c}$$

Key components of this equation are the nuclear reorganization energy, λ, the free energy of the reaction, ΔG°, and the electron donor–acceptor coupling, H_{AB}. ΔG° for both Yb_2cod and Eu_2cod is estimated from Eq. (3) [39].

$$\Delta G^\circ_{Ln} = E(Trp^{\bullet +}/Trp) - E_{Trp^*} - E^\circ(Ln^{3+/2+}cod) \tag{3}$$

Where the reduction potential of the Trp radical cation, $E(Trp^{\bullet +}/Trp)$, is estimated as 1.13 eV [11], and the energy of Trp in its first singlet excited state, E_{Trp^*}, is 3.90 eV. The reduction potential of the protein bound metal ion, $E^\circ(Ln^{3+/2+}cod)$, is estimated from the thermodynamic cycle in Scheme 1 with aqueous ion reduction potentials of $E^\circ(Eu^{3+/2+}) = -0.35$ eV and $E^\circ(Yb^{3+/2+}) = -1.05$ eV [40] and the assumption that formation constants for the divalent and trivalent metal binding sites are $K_f(Ln^{3+}cod) = 10^{11}\ M^{-1}$ and $K_f(Ln^{2+}cod) = 10^8\ M^{-1}$. The resulting reduction potentials are $E^\circ(Ln^{3+/2+}) - 0.18$ eV.

As the electron is transferred from the Trp to the Ln^{3+}, the local environment around the donor and acceptor will change to accommodate the different charge, size, polarity, etc. of each. The reorganization energy, λ, is defined as the energy needed to change the local environment of the donor and acceptor from its reactant state (Trp* and Ln^{3+}) to its product state ($Trp^{\bullet +}$ and Ln^{2+}) without actually moving the electron. H_{AB} measures the ease at which the electron is transferred from the donor to the acceptor; it accounts for the distance-dependence of the ET rate constant. With the reasonable assumption that H_{AB} and λ are the same, or nearly so, for the forward electron transfer process for both Yb_2cod and Eu_2cod it is easy to show that

$$\lambda = \frac{-(\Delta G^{\circ 2}_{Eu} - \Delta G^{\circ 2}_{Yb})}{4k_B T \ln(k_f^{Eu}/k_f^{Yb}) + 2\Delta G^\circ_{Eu} - 2\Delta G^\circ_{Yb}} \tag{4}$$

Table 2
Calculation of λ from Eq. (4)

Temp (°C)	$\frac{k_f^{Eu}(95\%)}{k_f^{Yb}(80\%)}$ λ (eV)	$\frac{k_f^{Eu}(5\%)}{k_f^{Yb}(20\%)}$ λ (eV)	$\frac{k_f^{Eu}(95\%)}{k_f^{Yb}(20\%)}$ λ (eV)	$\frac{k_f^{Eu}(5\%)}{k_f^{Yb}(80\%)}$ λ (eV)
1	2.06	2.12	2.31	1.91
8	2.05	2.08	2.27	1.89
13	2.05	2.06	2.25	1.89
19	2.05	2.06	2.26	1.89
25	2.05	2.07	2.26	1.90
33	2.06	2.05	2.27	1.87
Average $\pm$ 1 S.D.	2.05 ± 0.004	2.07 ± 0.025	2.27 ± 0.020	1.89 ± 0.013

The estimated driving forces for the forward ET from Eq. (3) are $\Delta G^{\circ}_{Yb} = -1.54$ eV and $\Delta G^{\circ}_{Eu} = -2.24$ eV for the Eu_2cod and Yb_2cod systems, respectively. Since both Eu_2cod and Yb_2cod give double exponentials, the question becomes which rates to enter in the k_f^{Eu}/k_f^{Yb} ratio. Table 2 shows the four resultant λ values obtained for all possible combinations.

Values of λ are within one standard deviation when the two predominant component lifetimes are ratioed and the two lesser component lifetimes are ratioed, whereas the cross ratios give very different values for λ. This result suggests that either the difference of having Eu^{3+} and Yb^{3+} in the metal binding sites shifts the equilibrium positions of the Trp slightly and doesn't significantly change λ, or that having Eu^{3+} and Yb^{3+} in the sites markedly changes the equilibrium and that each site has a very different λ value.

An independent method of determining λ is to fit the temperature dependence of the ET rate constants to Eqs. (2a), (2b) and (2c). The solid lines in Fig. 3 show the best fits of the data to Eqs. (2a), (2b) and (2c), and the results are presented in Table 3.

Since Eqs. (2a), (2b) and (2c) is quadratic in λ, two values of λ fit the data equally well. Data from Table 2 suggest that the ET is in the Marcus normal region for

Table 3
Calculation of λ and H_{AB} from the fitting of temperature dependent data to Eqs. (2a), (2b) and (2c)

	Yb τ_1	Yb τ_2	Eu τ_1	Eu τ_2
λ (eV)	2.05	2.51	2.14	2.24
H_{AB} (cm^{-1})	2.36	4.76	2.28	1.31
r^2 [a]	0.601	0.670	0.901	0.188

[a] r^2 is the coefficient of determination for the fit of Eqs. (2a), (2b) and (2c) to the data. For n data points:

$$r^2 = 1 - \left(\sum_{i=1}^{n} (y_i - \hat{y}_i)^2 \right) \Big/ \left(\sum_{i=1}^{n} (y_i - \bar{y})^2 \right)$$

where the y data points are y_i, the estimated y values are $\hat{y}_i$, and the mean of all y data is $\bar{y}$.

Yb_2cod, i.e. $-\Delta G^\circ_{Yb} < \lambda$, and near activationless for Eu_2cod, $-\Delta G^\circ_{Eu} \approx \lambda$. This governed our choice for the correct λ from the fit of Eqs. (2a), (2b) and (2c). The fits are rather poor ($r^2 = 1$ represents a perfect fit) due to the limited number of data points and the large amount of scatter, causing the error to be as high as 20%. Even so, the λ values do agree reasonably well with the results obtained from Eq. (3) and add some validity to the assumption that the λ and H_{AB} do not change drastically for each metal.

4. Discussion

The double exponential nature of the decay of Trp fluorescence upon the substitution of Eu^{3+} or Yb^{3+} for Ca^{2+} in cod parvalbumin may be due to the presence of two species wherein the Trp occupies different positions in its pocket. The origin of the non-exponential decay of Trp, either free in solution or in proteins, has been debated for some time [35,41–43] and the prevailing explanation is that the Trp is present in two or more equilibrium positions. An explanation of the two lifetimes in our present system is that the oxidation potential of the Trp may be slightly different for each species, by about 0.25 eV. The redox potential of Trp in proteins is hard to measure [11,44,45], and may change with pH [46]. A difference in redox potentials for Trp sidechains in non-identical environments would explain why a single lifetime is observed when non-redox active ions are in the binding sites of the protein. This interpretation would, of course, require different ΔG° values for each species, and this is the subject of ongoing experiments.

The unique secondary and tertiary structure of cod parvalbumin may allow it to be used to determine the mechanism of electron transfer from the Trp to the metal. Two competing theories for how an electron moves between a donor and acceptor exist. One theory states that the coupling of an electron donor to the acceptor in a protein decreases exponentially with distance, r, at a rate of β, i.e. $k \propto k_0 \exp(-r/\beta)$, without regard to the specifics of the protein structure [47–50]. This 'β theory' predicts ET from the Trp to either of the metal sites with about the same rate since both sites are the same distance from the Trp. The competing theory states that the donor is coupled to the acceptor through an intervening 'pathway' constructed of covalent bonds, H-bonds, and short through-space jumps, making the specifics of the protein structure very important [51–53]. The 'pathway' model predicts about three orders of magnitude better coupling to the EF site than the CD since the Trp 102 is next to the EF site, whereas ET to the CD site requires a more circuitous pathway for the electron. The pathway model, therefore, predicts that the ET reaction involves only the Trp and the EF site. Studies involving site-directed mutant proteins are in progress in an attempt to resolve this issue.

Our λ value of ~ 2 eV is relatively high with respect to current literature values of λ ranging from 0.74 to 1.15 eV [9]. One reason for our large λ value may be that parvalbumin is not a physiological ET protein, so it has not evolved to minimize the rearrangement of the environment around the Trp and metal ion caused by ET.

Most ET proteins have their metal sites buried in the protein to minimize rearrangement [5], yet both the CD and EF sites are rather solvent exposed; the Ln^{3+} at each site has at least one coordinated water [30]. Also, Eu and Yb go through a relatively large change in size on going from their 3+ to 2+ redox state, further increasing the value of λ. We are currently trying to determine the λ value for the $Ln^{3+/2+}$ self exchange reaction independently.

The introduction of Eu and Yb ions as a new class of electron acceptor probe opens the study of ET to a wide variety of calcium-binding and other proteins. While Ca^{2+} is not redox-active and neither Yb^{3+} nor Eu^{3+} are present in physiological redox centers, the latter nevertheless represent well-characterized probe species for the study of photophysical ET processes in proteins. Besides, the study of the rates of ET in non-redox active proteins may shed light on if and how physiological redox proteins have evolved to optimize λ and H_{AB}. Ln^{3+} ions are also known to substitute for Fe^{3+} in certain proteins, e.g. transferrin [54], so replacement of this ion in redox relevant situations may also prove useful. The electron donor, in our case a Trp residue in a singlet excited state, provides a reasonable model for this residue when it acts in the capacity of a ground state electron donor (to form a Trp cation radical) as has been established in the cases of ribonucleotide reductase [55,56], compound ES of cytochrome *c* peroxidase [57] and DNA photolyase [58]. Our experiments may be relevant to such situations and preliminary experiments with Yb^{3+}-substituted ribonucleotide reductase have already been carried out.

The present experiments have the advantage of relative simplicity compared to some other types of systems that have been used for the study of ET rates in proteins. In our case the electron donor is a naturally-occurring amino acid side chain that can be photoexcited to initiate the ET reaction. Site-directed mutagenesis can be used to provide a variety of species with different spatial positioning of the electron donor with respect to the metal ion, electron-acceptor site. The preparation of the electron-acceptor site requires only the replacement of a Ca^{2+} (or other) ion at an already existing site. Such replacements often occur spontaneously upon mixing with a solution of the appropriate Ln^{3+} ion, as is the case in the present study.

Acknowledgements

This work was supported by the National Institutes of Health. (Grant GM 23599). We thank Professor M. Maroncelli for making his picosecond laser apparatus available for this project and Dr J.J. Regan for supplying us with the Greenpath pathway analysis program.

References

[1] G. McClendon, R. Hake, Chem. Rev. 92 (1992) 481.
[2] H.B. Gray, J.R. Winkler, Annu. Rev. Biochem. 65 (1996) 537.

[3] C.C. Moser, J.M. Keske, K. Warncke, R.S. Farid, L.P. Dutton, Nature 355 (1992) 796.
[4] H.B. Gray, B.G. Malmstrom, Biochemistry 28 (1989) 7499.
[5] R.J.P. Williams, Electron transfer in biology and the solid state, American Chemical Society, Washington, DC, 1990, p. 3.
[6] S.L. Mayo, W.R. Ellis Jr, R.J. Crutchley, H.B. Gray, Science 233 (1986) 948.
[7] J.R. Winkler, H.B. Gray, Chem. Rev. 92 (1992) 369.
[8] J. Sun, C. Su, J.F. Wishart, Inorg. Chem. 35 (1996) 5893.
[9] B.M. Hoffman, M.J. Natan, J.M. Nocek, S.A. Wallin, Struct. Bonding 75 (1991) 85.
[10] W.D. Horrocks Jr, J.P. Bolender, W.D. Smith, R.M. Supkowski, J. Am. Chem. Soc. 119 (1997) 5972.
[11] J.W. Petrich, J.W. Longworth, G.R. Fleming, Biochemistry 26 (1987) 2711.
[12] R.W. Ricci, K.B. Kilichowski, J. Phys. Chem. 78 (1974) 1953.
[13] A. Abusaleh, C.F. Meares, Photochem. Photobiol. 39 (1984) 763.
[14] W.R. Kirk, W.S. Wessels, F.G. Prendergast, J. Phys. Chem. 97 (1993) 10326.
[15] W.D. Horrocks Jr, Advan. Inorg. Biochem. 4 (1982) 201.
[16] W.D. Horrocks Jr, D.R. Sudnick, Acc. Chem. Res. 14 (1981) 384.
[17] W.D. Horrocks Jr, Methods Enzymol. 226 (1993) 495.
[18] D. Chaudhuri, J.C. Amburgey, D.J. Weber, W.D. Horrocks Jr, Biochemistry 36 (1997) 9674.
[19] K. Sudhakar, C.M. Phillips, S.A. Williams, J.M. Vanderkooi, Biophys. J. 64 (1993) 1503.
[20] R.H. Kretsinger, CRC Crit. Rev. Biochem. 8 (1980) 119.
[21] J.J. Falke, S.K. Drake, A.L. Hazard, O.B. Peersen, Q. Rev. Biophys. 27 (1994) 219.
[22] S. Linse, S. Forsén, Adv. Second Messenger Phosphoprotein Res. 30 (1995) 89.
[23] H. Kawasaki, R.H. Kretsinger, Protein Profiles 1 (1994) 343.
[24] R.H. Kretsinger, C.F. Nockolds, J. Biol. Chem. 248 (1973) 3313.
[25] A.L. Swain, R.H. Kretsinger, E.L. Alma, J. Biol. Chem. 264 (1989) 16620.
[26] C.M.L. Hutnik, J.P. MacManus, A.G. Szabo, Biochemistry 29 (1990) 7318.
[27] P.J. Breen, K.A. Johnson, W.D. Horrocks Jr, Biochemistry 24 (1985) 4997.
[28] P.J. Breen, E.K. Hild, W.D. Horrocks Jr, Biochemistry 24 (1985) 4991.
[29] D.T. Cronce, W.D. Horrocks Jr, Biochemistry 31 (1992) 7963.
[30] C.W. McNemar, W.D. Horrocks Jr, Biochim. Biophys. Acta 1040 (1990) 229.
[31] W.D. Horrocks Jr, W.E. Collier, J. Am. Chem. Soc. 103 (1981) 2856.
[32] J.I. Closset, C. Gerday, Comp. Biochem. Physiol. 55B (1976) 537.
[33] J.S. Fritz, R.T. Oliver, D.J. Pietrzyk, Anal. Chem. 30 (1958) 1111.
[34] C.F. Chapman, R.S. Fee, M. Maroncelli, J. Phys. Chem. 94 (1990) 4929.
[35] M. Eftink, Z. Wasylewski, Biochemistry 28 (1989) 382.
[36] F. Castelli, H.D. White, L.S. Forster, Biochemistry 27 (1988) 3366.
[37] S.T. Ferreira, Biochemistry 28 (1989) 10066.
[38] R.A. Marcus, N. Sutin, Biochim. Biophys. Acta 811 (1985) 265.
[39] D. Rehm, A. Weller, Israel J. Chem. 8 (1970) 259.
[40] A.J. Bard, R. Parsons, J. Jordan, Standard potentials in aqueous solution, Marcel Dekker, NY, 1976.
[41] A. Vix, H. Lami, Biophys. J. 68 (1995) 1145.
[42] M.V. Gilst, C. Tang, A. Roth, B. Hudson, J. Fluorescence 4 (1994) 203.
[43] Y. Chen, F. Gai, J.W. Petrich, J. Phys. Chem. 98 (1994) 2203.
[44] M.R. DeFilippis, C.P. Murthy, M. Faraggi, M.H. Klapper, Biochemistry 28 (1989) 4847.
[45] G. Merényi, J. Lind, X. Shen, J. Phys. Chem. 92 (1988) 134.
[46] A. Harriman, J. Phys. Chem. 91 (1987) 6102.
[47] J.R. Miller, J.V. Beitz, R.K. Huddleston, J. Am. Chem. Soc. 106 (1984) 5057.
[48] J.R. Miller, J.A. Peeples, M.J. Schmitt, G.L. Closs, J. Am. Chem. Soc. 104 (1982) 6488.
[49] J.V. Beitz, J.R. Miller, J. Chem. Phys. 71 (1979) 4579.
[50] J.J. Hopfield, Proc. Nat. Acad. Sci. USA 71 (1974) 3640.
[51] D.N. Beratan, J.N. Betts, J.N. Onuchic, Science 252 (1991) 1285.
[52] J.N. Betts, D.N. Beratan, J.N. Onuchic, J. Am. Chem. Soc. 114 (1992) 4043.
[53] J.J. Regan, S.M. Risser, D.N. Beratan, J.N. Onuchic, J. Phys. Chem. 97 (1993) 13083.

[54] P. O'Hara, S.M. Yeh, C.F. Meares, R. Bersohn, Biochemistry 20 (1981) 4704.
[55] J.M. Bollinger Jr, W.H. Tong, N. Ravi, B.H. Huynh, D.E. Edmonson, J. Stubbe, J. Am. Chem. Soc. 116 (1994) 8024.
[56] F. Lendzian, M. Sahlin, F. MacMillan, R. Bittl, R. Fiege, S. Pötsch, B.-M. Sjöberg, A. Gräslund, W. Lubitz, G. Lassmann, J. Am. Chem. Soc. 118 (1996) 8111.
[57] J.E. Huyett, P.E. Doan, R. Gurbiel, A.L.P. Houseman, M. Sivaraja, D.B. Goodin, B.M. Hoffman, J. Am. Chem. Soc. 117 (1995) 9033.
[58] C. Essenmacher, S.-T. Kim, M. Atamian, G.T. Babcock, A. Sancar, J. Am. Chem. Soc. 115 (1992) 1602.