J Fluoresc (2011) 21:687–692
DOI 10.1007/s10895-010-0756-4

ORIGINAL PAPER

Interaction of Potassium Mono and Di Phosphates with Bovine Serum Albumin Studied by Fluorescence Quenching Method

S. Bakkialakshmi · B. Shanthi · D. Chandrakala

Received: 26 July 2010 / Accepted: 18 October 2010 / Published online: 12 November 2010

Abstract The interactions between potassium mono and di phosphates and bovine serum albumin (BSA) were studied using fluorescence spectroscopy (FS) and ultraviolet spectroscopy (UV). The experimental results showed that the potassium mono and di phosphates could insert into the BSA and quench the inner fluorescence of BSA by forming the potassium mono phosphate—BSA and pottassium di phosphate—BSA complexes. It was found that the static quenching was the main reason leading to the fluorescence quenching. It was conformed by XRD and SEM techniques.

Keywords Bovine serum albumin · Pottassium mono phosphate · Pottassium di phosphate · Fluorescence

Introduction

Serum albumin is the most abundant protein in animal's including human circulatory system. It is in charge of the transport of a variety of endogenous and exogenous substances in body and plays an important role in the transport and deposition of these substances. The most outstanding property of albumins is their ability to bind reversibly a larger variety of ligands [1].

The recognition of a large number of binding sites on bovine serum albumin (BSA) [2] for hydrophobic organic anions has led to several investigations [2–4]. Fluorescence spectroscopy is a sensitive and convenient method for the study of protein-ligand interactions [5].

Quenching measurement of albumin fluorescence is an important method to investigate the interactions of drugs with serum albumins. It can reveal the accessibility of quenchers to albumin's fluorophore groups, help understand binding mechanisms of albumin with drugs, and provide dues to the essential of the binding phenomenon [6]. Fluorescence quenching refers to any process which decreases the fluorescence intensity of a given substance. There are mainly two types of quenching. The collisional quenching (dynamic quenching) results from collision between fluorophores and a quencher. The static quenching is due to the formation of ground-state complex between fluorophores and a quencher. The quenching can be mathematically expressed by the stern-volmer equation, which allows for the calculation of quenching constants [7, 8]. Measuring fluorescent change is a practical method to study protein interactions with other substances [9, 10]. There are many reports on the study of interactions between proteins with ligands using fluorescence spectroscopic methods [11–16]. But there have been no reports on the interaction of BSA with potassium mono and diphosphates. Thus, Bovine serum albumin (BSA) and potassium mono and diphosphates were selected in this study.

In this paper, both the traditional spectroscopic methods (electronic absorption spectra and fluorescence specta) were

S. Bakkialakshmi (✉) · D. Chandrakala
Department of Physics, Annamalai University,
Annamalainagr 608 002 Tamilnadu, India
e-mail: bakkialakshmi66@yahoo.com

D. Chandrakala
e-mail: chandrakalad@yahoo.com

B. Shanthi
Centralized Instrumentation and Services Laboratory (CISL),
Annamalai University,
Annamalainagar 608 002, India

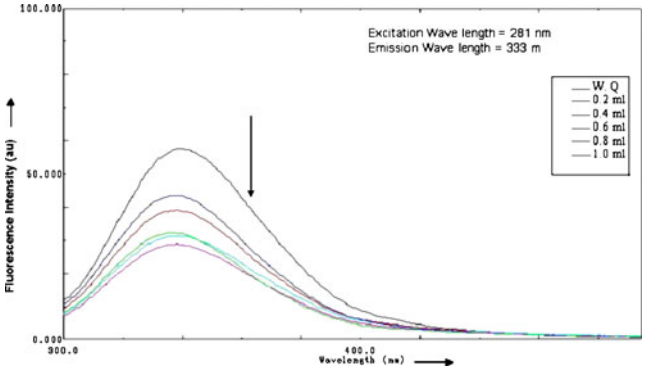

Fig. 1 Fluorescence quenching spectra of BSA in Water with Potassium mono phosphate

utilized to investigate binding interactions of the complex with bovine serum albumin (BSA).

Experimental Arrangements

Materials

Bovine Serum Albumin (BSA) was Obtained form Sigma Aldrich Company, Bangalore. All the other materials, were of analytical reagent grade and double distilled water was used throughout.

Apparatus

Fluorescence Measurements

Steady state Fluorescence measurements were performed on a spectrofluorimeter JASCO Model FP ~550 equipped with a 150 W Xenon lamp and a slit width of 10 nm, available at Centralized Instrumentation and Services Laboratory (CISL), Annamalai University, Annamalainagar.

The time-resolved fluorescence spectra were recorded using a HORIBA JOBIN YNON-SPEX F_{13}-111 Spectrofluorimeter available at Pondicherry University.

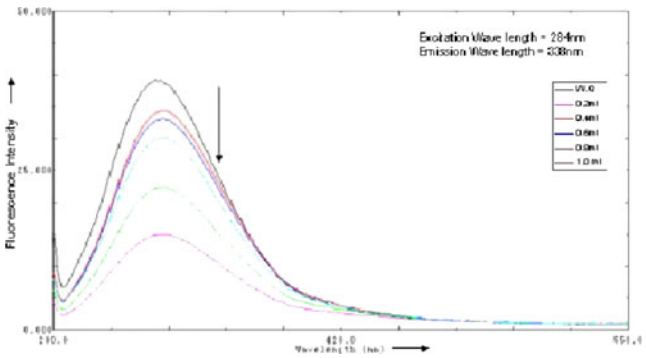

Fig. 2 Fluorescence quenching spectra of BSA in water with Potassium diphosphate

Table 1 Decay constant (K_{sv}), regression coefficient (r) by calculation and graphical methods for BSA

Quencher	Solvent	K_{sv}		r	
		By calculation	By graph	By calculation	By graph
KH_2PO_4	Water	118.2	115	0.99	0.95
K_2HPO_4		172.5	175	0.97	0.99
KH_2PO_4	NaCl	87.7	89	0.99	0.99
K_2HPO_4		179.4	180	0.95	0.96
KH_2PO_4	2-Propanal	112.2	110	0.96	0.99
K_2HPO_4		209.4	210	0.96	0.98

UV Measurements

The absorption spectra were recorded using a JASCO-UVIDEC-650, Spectrophotometer available at Instrumentation laboratory, Department of Chemistry, Annamalai University, Annamalainagar.

XRD Measurements

The X-ray diffraction Patterns were rescored on a PAN Analytical X'Pert Philip X-ray diffractometer, available at CIF, Pondicherry University, Pondicherry.

SEM Analysis

The scanning electron microscope JSM-5610 is used for the surface analysis is available at CISL, Annamalai University.

Results and Discussion

Steady-state and Time–resolved Fluorescence Studies

For macromolecules, the fluorescence measurements can give some information of the binding of small molecule substances to protein. Fluorescence intensity of a compound can be decreased by a variety of molecular interactions, viz., ground-

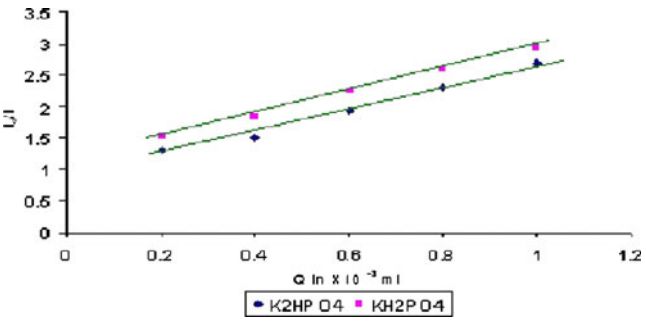

Fig. 3 Stern volmer plot for BSA in water with different quenchers

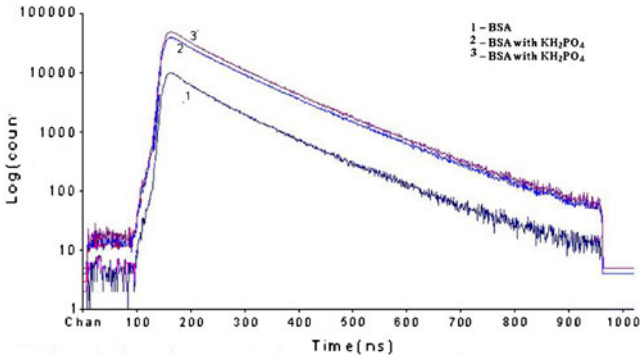

Fig. 4 Time–resolved fluorescence spectra for BSA with different quenchers

state Complex formation, excited-state complex formation, charge transfer, energy transfer, etc. Such decrease in intensity is called quenching.

The fluorescence spectra of BSA in the absence and presence of different concentrations of KH_2PO_4 and K_2HPO_4 were recorded in the range of 200–500 nm upon excitation at 284 nm and 287 nm respectively.

The emission maximum and shape of the peaks were not changed by the different concentrations of the both the quenchers KH_2PO_4 and K_2HPO_4 (Figs. 1 and 2). These results indicated that there were interactions between potassium salts and BSA. The interactions of potassium salts to BSA were further conformed by the UV–VIS absorption, XRD and SEM Analysis.

The fluorescence quenching data were analyzed by the stern-volmer equation.

$$\frac{I_o}{I} = 1 + K_{SV}\,[Q]$$

Where I_o and I are the steady-state fluorescence intensities in the absence and presence of quenchers respectively. K_{SV}, the stern-volmer quenching constant and [Q] is the concentration of the quenchers KH_2PO_4 and K_2HPO_4. The values of K_{SV} and r the regression coefficient are shown in Table 1. The linearity of I_o/I versus [Q] plots (Fig. 3) for both the quenchers revealed that the quenching type, static or dynamic.

The formation of complex was further confirmed from the values of quenching rate constants, kq (Table 2) from the eqn.

$$kq = \frac{K_{SV}}{\tau_o}$$

Table 2 Life time, relative amplitude, χ^2 and rate constant for BSA

Fluorescer	Quencher	Life time (τ)	Relative amplitude (A)	χ^2	Kq
BSA	W.Q	3.038×10^{-4}	25.73	1.2124	–
	KH_2PO_4	7.8988×10^{-4}	74.50	1.097	4.693×10^{5}
	K_2HPO_4	8.356×10^{-4}	49.58	1.1987	5.908×10^{5}

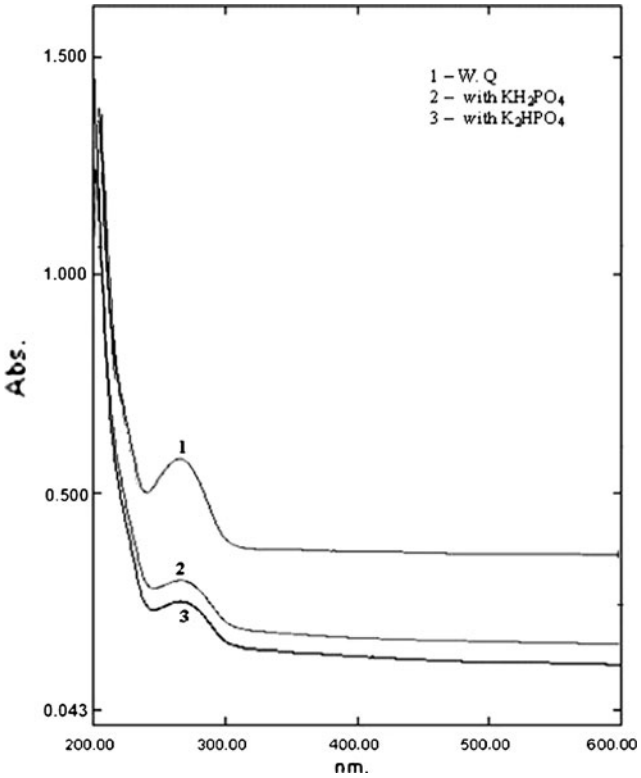

Fig. 5 Absorption spectra of BSA in water

Where τ_o is the life time of BSA without quencher. The life time spectra were shown in Fig. 4. Stoke's shift, Molar extinction coefficient, energy, ionization potential and electron affinity values were given in Table 3.

UV—VIS Absorption Studies

The complex formation between potassium salts—BSA was also evident from UV–VIS absorption spectra data, (Fig. 5). The UV-visible intensity of BSA decreased with the

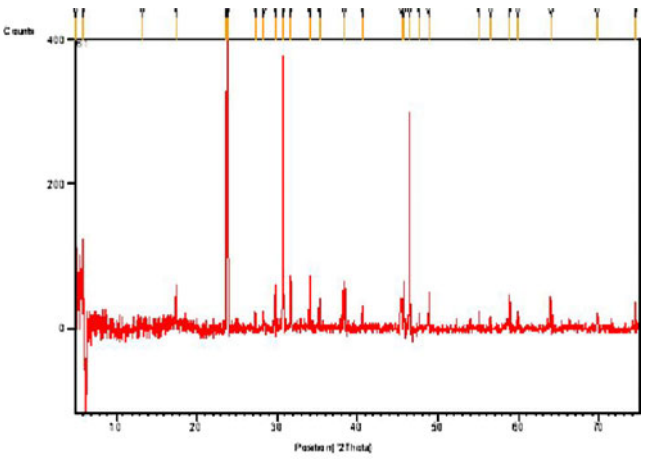

Fig. 6 X-ray diffractogram of Bovine serum albumin

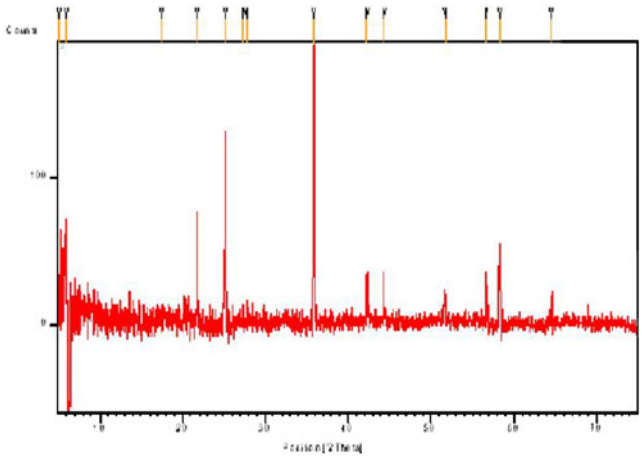

Fig. 7 X-ray diffractogram of Bovine serum albumin with potassium mono phosphate

addition of quencher (both). Blue shift of maximum peak position was noticed due to the complex formation.

XRD Analysis

The mixture of BSA & Potassium salts was subjected to x-ray diffraction analysis. The XRD pattern is shown in Figs. 6, 7, and 8. They were compared with the XRD pattern of pure BSA. JCPDS values were compared with our values and the hkl values are given in Table 4. It also conformed the complex formation.

No data were observed for BSA + Potassium diphosphate. This may be due to the nature of potassium diphosphate.

SEM Analysis

The fractured samples of BSA with and without KH_2PO_4 and K_2HPO_4 were subjected to SEM micrograph analysis to identify the surface morphology. The surface micrographs of BSA, BSA + KH_2PO_4 and BSA + K_2HPO_4 were shown in Figs. 9, 10 and 11 respectively. Changes in SEM photographs confirmed the formation of complex between BSA and potassium salts.

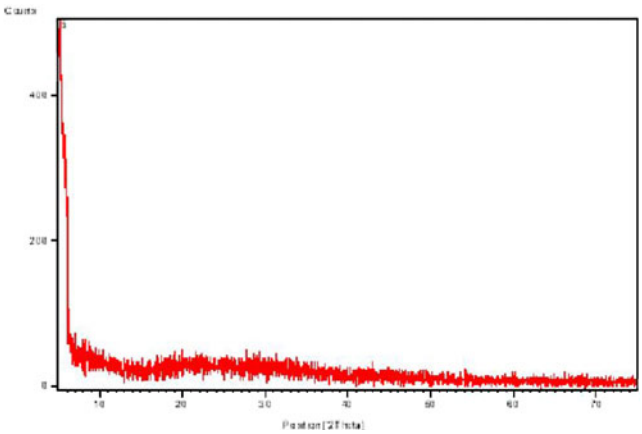

Fig. 8 X-ray diffractogram of Bovine serum albumin with potassium di phosphate

Table 3 Stoke's shift, energy, ionization potential and electron affinity values of BSA

Quencher	Solvent	λ_{abs} (nm)	λ_{flu} (nm)	Stoke's Shift	Log ε	E	I_D	EA
KH_2PO_4	Water	281.50	333	5493	7.4495	4.4133	10.766	−0.313
K_2HPO_4		289.00	332	4482	7.4608	4.2988	10.623	−0.195
KH_2PO_4	NaCl	281.00	340	6172	7.4487	4.4211	9.6711	−0.320
K_2HPO_4		288.00	338	5137	7.4592	4.3137	10.642	−0.211
KH_2PO_4	2-Proponal	286.00	341	5640	7.4563	4.5014	9.5014	−0.4038
K_2HPO_4		284.00	337	5538	7.4533	4.344	10.68	−0.242

Effect of Solvents

The absorption maxima, logε and fluorescence maxima of egg yolk were obtained in three different solvents. The relevant data were compiled in tables. When compared to potassium mono phosphate the absorption maxima of potassium di phosphate is slightly red shifted in any one

Table 4 XRD spectral data and hkl values of BSA

BSA				BSA + KH_2PO_4			
Pos. [°2Th.]	h	k	l	Pos. [°2Th.]	h	k	l
5.0716	1	1	0	5.8592	0	0	1
5.9963	1	0	1	7.1081	2	0	1
13.2499	1	2	0	17.3255	2	0	1
17.4472	1	1	1	18.7924	0	0	2
23.7915	2	0	0	23.7839	2	0	2
23.9510	2	1	0	29.5732	2	0	2
27.2971	1	2	1	30.6323	2	1	1
28.3688	1	3	0	33.9112	4	0	0
29.7558	2	0	1	35.1195	2	1	1
30.7882	2	1	1	38.1888	0	1	2
31.6762	0	0	2	45.6656	2	0	3
33.9995	2	2	1	46.3715	3	1	0
35.2582	0	1	2	47.5685	4	0	2
38.4252	0	4	0	54.9813	4	0	1
40.6079	1	0	2	58.3108	3	1	2
45.4883	1	1	2	58.7267	2	0	3
45.7525	0	4	1	63.9187	4	1	1
46.4195	2	3	1	69.5984	4	1	0
47.6537	3	0	1	71.2622	4	0	3
48.8447	1	4	1				
55.0817	3	1	1				
56.4363	2	1	2				
58.8196	0	3	2				
59.8728	2	4	0				
63.9664	3	2	1				
69.7117	3	3	0				
74.4610	2	2	2				

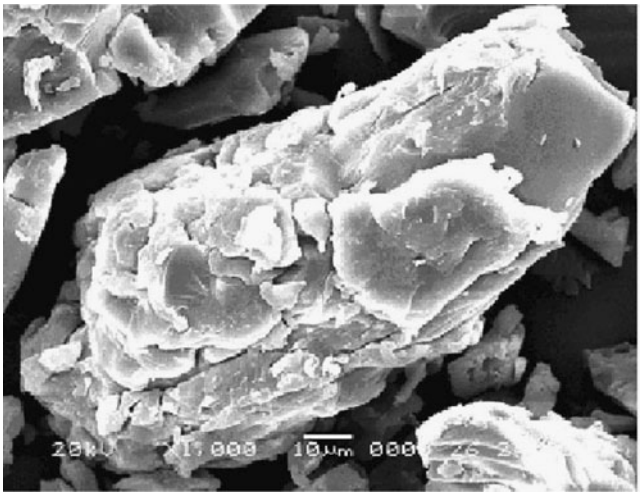

Fig. 9 SEM micrograph of BSA (X 1000)

solvent. This shows the intermolecular hydrogen bonding present is potassium di phosphate.

Conclusion

This study developed an approach to understand the interaction on Bovine serum albumin with potassium mono and di phosphates using fluorescence spectroscopic method. First, the interaction of BSA with potassium mono and di phosphates was proven by the fluorescence quenching experiments. The results may provide basic knowledge for a better understanding of the properties of the protein involved in the interactions. Time-resolved fluorescence studies conform this interaction. Changes in XRD patterns and SEM photographs confirmed the formation of complex between BSA and potassium mono and diphosphates. Potassium mono and di phosphates quench the fluorescence

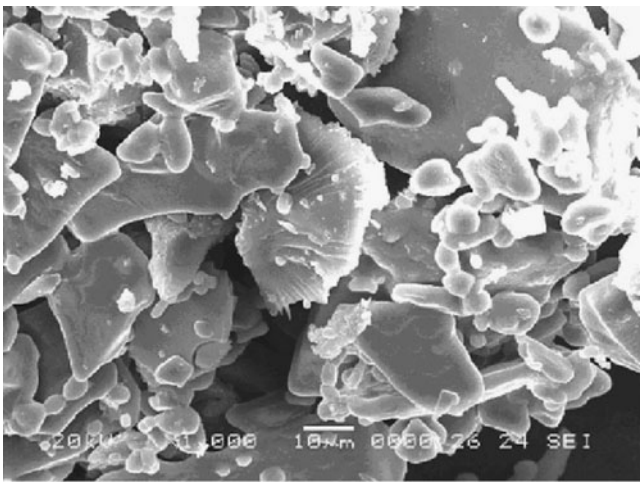

Fig. 10 SEM micrograph of BSA with KH_2PO_4 (X 1000)

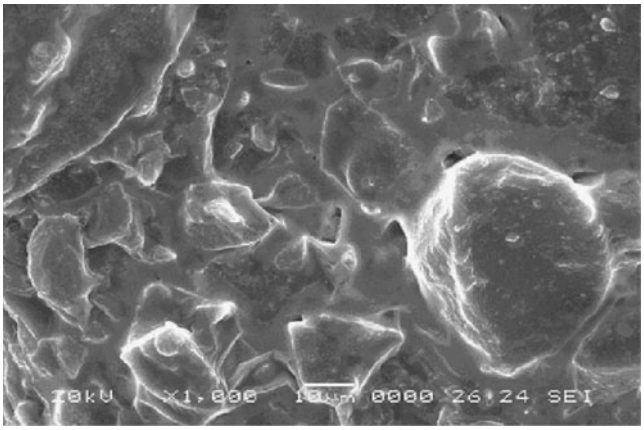

Fig. 11 SEM micrograph of BSA with K_2HPO_4 (X 1000)

of BSA by forming the complexes. These interactions yield a linear stern-volmer plot. Potassium di phosphate quench the fluorescence of BSA more than potassium mono phosphate.

References

1. Boghaci DM, Farvid SS, Gharagozlou M (2007) Interaction of copper (11) complex of compartmental schiff base ligand N, N'—bis (3-hydroxysalicylindene ethylenediamine with bovine serum albumin. Spectrochim Acta A 66:650–655
2. Foster JF (1960) The Plasma Proteins (F.W. Putnam. Ed.) Vol. 1. Academic Press, New York/London, pp 179–239
3. Spector AA (1975) Fatty acid binding to plasma albumin. J Lipid Res 16:165–179
4. Reed RG, Feldhoff RC, Clute OL, Peters T (1975) Fragments of Bovine serum albumin produced by limited proteolysis, conformation and Ligand Binding. Biochemistry 14:4578–4583
5. Brand L, Gohlke JR (1972) Fluorescence probes for structure. Annu Rev Biochem 41:843–868
6. Silva D, Cortez CM, Chunha-Bastos J, Loura Sonia RW (2004) Methyl parathion interaction with human and bovine serum albumin. Toxicol Lett 147:53–61
7. Athina P, Green Rebecca J, Frazier Richard A (2005) Interaction of flavonoids with bovine serum albumin: a fluorescence quenching study. J Agric Food Chem 53:158–63
8. Lakowicz JR (1999) Principles of fluorescence spectroscopy, 2nd edn. Kluwer Academic Publishers/Plenum Press, New York
9. Romer J, Bichkel MH (1979) Mehtod to estimate binding constants at variable protein concentrations. J Pharm Pharmacol 31:7–11
10. Bhattacharyya M, Chauahuri V, Poddar RK (1990) Evidence for co operative Binding of chloropromazine with hemoglobin: equilibrium dialysis, fluorescence quenching and oxygen release study. Biochem Biophys Res Commun 167:1146–1153
11. Zhao H, Su W, Luo YH, Li ZC, Jiu HF, Liang H, Chen B, Zhang QJ (2006) Rectification of excitation with bathochromic shift induced by intense absorption of organic ligands during emission. Spectrochim Acta A 65:846–851
12. Subbiah D, Ashok KM (2005) Fluorescence spectroscopic study of serum albumin-bromadiolone interaction: fluorimetric determination of bromadiolone. J Pharm Biomed Anal 38:556–563
13. Hirshfactd KM, Toptygin D, Grandhige G, Kim H, Packard BZ, Brand C (1996) Steady-state and time—resolved fluorescence

measurements for studying molecular interactions: interaction of a calcium—binding probe with proteins. Bio Phys Chem 62:25–38
14. Richeieri GV, Ancl A, Kleinfeld AM (1993) Interaction of long-chain fatty acids and albumin: determination of free fatty acid levels using the fluorescent probe ADIFAR. Biochemistry 32:7574–7580
15. Olson MK, Hollingworth S, Baylor SM (1988) Myoplasmic binding of fura-2 investigated by steady-state fluorescence and absorbance measurements. Biophys J 54:1089–1104
16. Kuresbayashi N, Harkins AB, Baylor SM (1993) Use of fura red as an intracellular calcium indicator in frog skeletal muscle fibres. Biophys J 64:1934–1960