THE QUENCHING OF THE TRYPTOPHYL AND TYROSYL FLUORESCENCE OF PROTEINS BY CESIUM ION

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1. Introduction

While studying the interactions of neutral salts with proteins in aqueous solutions by fluorescence spectrometry it was observed that CsCl quenched a substantial fraction of protein fluorescence (unpublished data). The results indicated that the quenching action of Cs^+ resembled that due to I^- in that the emission maximum was shifted to a shorter wavelength. Studies on the selective quenching by I^- of tryptophan fluorescence are reported [1–3] and quenching of tryptophyl fluorescence by Cs^+ ions has been shown [4,5]. However, the quenching of fluorescence of tyrosine, free as well as in ribonuclease by Cs^+ is reported here for the first time. Further, to know more about the nature of the quenching caused by Cs^+ the effects of two salts of Cs^+ viz. i) chloride and ii) iodide were studied. The effects of Cs-salts were compared with those of KI and KCl, in terms of their effects on the fluorescence of tryptophan moieties of at least eight different proteins.

2. Materials and methods

The following crystalline proteins of high purity were used: lysozyme, serum albumin, fibrinogen, papain, trypsin, ovalbumin, α-chymotrypsin, aldolase, ribonuclease. L-tryptophan and L-tyrosine, KCl and KI were Analar grade. Optical grade CsCl was used. CsI was prepared from the corresponding carbonate. The fluorescence measurements at 25°C, were made on Aminco-Bowman spectrophotofluorimeter fitted with an x–y recorder. The solutions were in 0.02 M Tris buffer, pH 7.0. The fluorescence spectrum of the tryptophan component of a protein was recorded at wavelength of exciting light = 295 nm. For tyrosine and ribonuclease the excitation wavelength was 275 nm. The effect of each salt was tested separately and varying concentration of salts were used.

3. Results and discussion

3.1. *Effects of salts on free amino acids*

KCl did not have any effect on the emission of L-tyrosine and L-tryptophan but CsCl readily quenched the fluorescence of both (figs. 1A & 2A). It was already moted previously that many other monovalent cation chlorides, at concentrations as high as 3 M, did not quench the emission of tryptophan and tyrosine. Therefore, the loss of fluorescence in CsCl solution must be due to Cs^+ ions. The loss of fluorescence was also seen in CsI solution. KI also quenched fluorescence. The quenching due to CsI was much stronger than that due to CsCl. It was, however, not greater than that caused by KI.

Quenching in the presence of CsI in 0.2 M solution, must be due to iodide ions for two reasons. Firstly, the loss of fluorescence in CsCl was comparatively less at the same concentration. Secondly, the quenching patterns in the presence of CsI or KI were identical at all concentrations upto 0.2 M and KCl was without effect (table 1). Further, CsI did not cause more quenching than KI indicating that the quenching by CsI was not additive with respect to its two constituent ions and was due to the presence

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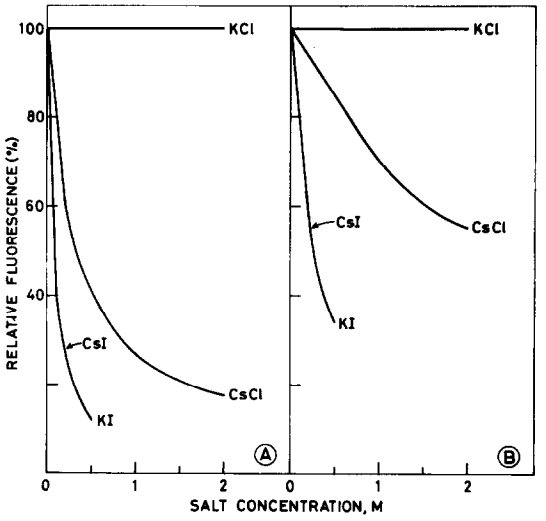

Fig. 1. Effects of varying concentrations of iodides and chlorides of cesium and potassium on fluorescence of L-tyrosine (A) and ribonuclease (B). Concentration; L-tyrosine, 10^{-5} M and ribonuclease 0.01%. λfl = 303 nm. λ excitation = 275 nm. The four salts were used separately. CsI concentration up to 0.2 M only. In 0.02 M Tris buffer pH 7.0, at 25°C. Relative fluorescence in the absence of salt was considered as 100.

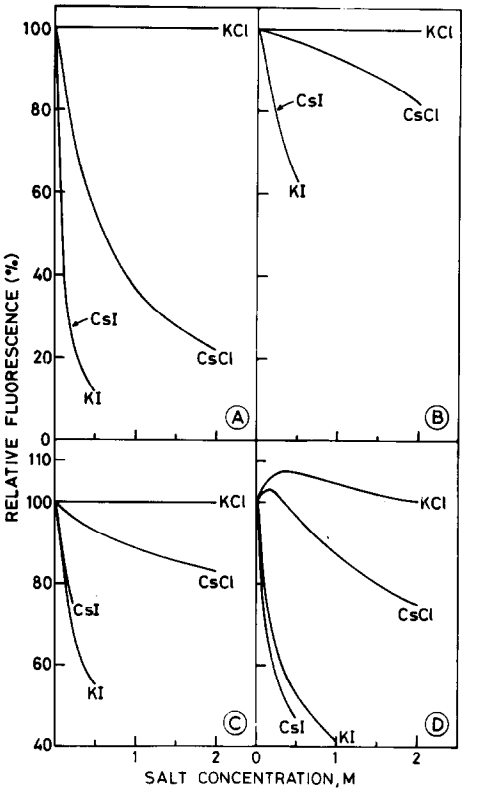

Fig. 2. Effects of varying concentrations of iodides and chlorides of cesium and potassium on fluorescence of L-tryptophan (A); lysozyme (B); fibrinogen (C); serum albumin (D). Concentration and λfl of L-tryptophan, 10^{-5} M and 350 nm; lysozyme, 0.005% and 342 nm; fibrinogen 0.002% and 340 nm; serum albumin, 0.016% and 338 nm. excitation = 295 nm. Rest of the conditions as in fig. 1.

of I^-, at least at the low concentrations. Almost ten-fold more concentration of CsCl as compared to I^- was required to bring about identical quenching indicating that Cs^+ was less effective than I^-.

3.2. *Effect of salts on ribonuclease*

Fig. 1B shows the effect of the four salts on ribonuclease emission. It can be seen that these results were qualitatively similar to those obtained with L-tyrosine. This was not surprising since the emission of ribonuclease is due to its tyrosine residues only.

3.3. *Effects of salts on proteins*

The fluorescence spectra for several proteins showed that the salt effects were qualitatively similar, as illustrated for lysozyme, fibrinogen and serum albumin in figs. 2B, 2C, 2D. The other pertinent data are presented in table 1. Thus in all cases CsCl, CsI and KI quenched the fluorescence while KCl did not. Loss of fluorescence in 0.2 M CsCl was negligible. Quenching in presence of CsI in 0.2 M solution must be due iodide ions for the same reasons as were discussed earlier for tryptophan and tyrosine. These results are also in agreement with Lehrer's, who had used CsI as a source of I^-, that at 0.18 M concentration either CsI or KI caused identical quenching of fluorescence of lysozyme [1].

That Cs^+ ions in CsCl were also capable of quenching protein fluorescence at higher concentration was apparent from the fact that KCl did not quench the emission of free tryptophan. On the contrary it had an enhancing effect on the fluorescence of some proteins e.g. trypsin. The most significant feature of CsCl quenching in case of proteins was that, as with I^-, Cs^+ also caused the emission maximum to shift to

Table 1
Quenching of fluorescence of amino acids and proteins by salts

Compound	Molarity	CsCl Δ λ max nm	CsCl Δ Q%	CsI Δ λ max nm	CsI Δ Q%	KI Δ λ max nm	KI Δ Q%	KCl Δ λ max nm	KCl Δ Q%
a.									
L-Tyrosine	0.2		−39	0	−75	0	−75	0	0
	2.0	0	−82	–	–	–	–	0	0
Ribonuclease	0.2		− 6	0	−45	0	−45	0	0
	2.0	0	−45	–	–	–	–	0	0
b.									
L-Tryptophan	0.2		−25	0	−72	0	−72	0	0
	2.0	0	−78	–	–	–	–	0	0
Lysozyme	0.2		<− 2	−8	−19	−8	−19	0	0
	2.0	−8	−19	–	–	–	–	0	0
Fibrinogen	0.2		− 3	−5	−25	−5	−30	0	0
	2.0	−5	−17	–	–	–	–	0	0
Albumin,	0.2		+ 2	0	−40	0	−32	0	+6
serum	2.0	0	−25	–	–	–	–	0	0
Papain	0.2		+ 2	−8	−38	−8	−25	0	+20
	2.0	−8	−44	–	–	–	–	0	+47
Trypsin	0.2		−12	−10	−34	−10	−40	0	+8
	2.0	−10	−37	–	–	–	–	0	+19
Ovalbumin	0.2		− 1		−16	–	–	0	0
	2.0	0	− 5	–	–	–	–	0	0
α-chymotrypsin	0.2		− 1	− 8	− 9	–	–	0	0
	2.0	−8	− 8	–	–	–	–	0	0
Aldolase	0.2		− 6	− 8	−54	–	–	0	0
	2.0	− 8	−26	–	–	–	–	0	0

Δ λ max = change in emission maximum
Δ Q% = change in height of emission maximum, relative emission of compound alone taken as 100%.

a shorter wavelength by 5 to 10 nm (table 1), without any corresponding change in excitation maximum. Further, much higher concentrations of CsCl (> 10 fold) as compared to CsI and KI were required to bring about quenching of similar magnitude.

In proteins containing both tryptophan and tyrosine the emission is almost entirely due to its tryptophan components [6]. It has been shown that the fluorescence spectrum of the indole chromophore of tryptophan is markedly dependent upon the polarity of its environment [7]. Hence, two types of tryptophan residues are recognised in proteins: those which are 'exposed' to the solvent and considered easily accessible for interactions and those which are 'buried' and shielded from the solvent [3,5,8] and these can be distinguished by the technique of solute perturbation of protein fluorescence by iodide [3]. In this study also the quenching of protein fluorescence due to CsCl resulted in the shift of fluorescence maximum to a shorter wavelength. This can be attributed to quenching of those tryptophan residues that are exposed to the solvent and the remaining fluorescence being of the tryptophan residues that are in the nonpolar environment. Burstein et al. [5] who used a single concentration of 0.5 M CsCl in their work and Ostachevsky et al.

[4] also made similar observations. The order of effectiveness of Cs^+ is between Br^- and I^- in the Hofmeister series (manuscript in preparation).

The quenching effect of Cs^+ is noteworthy since chlorides of many other cations did not quench fluorescence of tryptophan. The enhancement of fluorescence of serum albumin in presence of 0.2 M KCl has been reported by others [9,10] and was attributed to the elimination of quenching effects of charged groups in the vicinity of tryptophan residues. A similar effect was seen on serum albumin and papain in 0.2 M CsCl solution and probably in this case also the ionic interactions are effected. Though KCl enhanced the emission of trypsin and papain, CsCl did not and in 2 M CsCl the magnitude of quenching was similar to that of 0.2 M CsI. Burstein et al. [5] have observed quenching of lysozyme, trypsin and α-chymotrypsin fluorescence in 0.5 M CsCl but at different pHs than used here. The neutral salts can interact with proteins in more than one manner and the molarity of salt solution is also responsible for the effects (unpublished data). More information will also be obtained by additional work on these salt effects on emission at different pHs. This would be particularly interesting because two different types of ions i.e. anion and cation are implicated in a similar kind of effect.

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