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Use of FITC as a Fluorescent Probe for Intracellular pH Measurement

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Fluorescein Isothiocyanate (FITC) is widely used in biology and medicine as a fluorescent marker for labeling various proteins. Particularly fluorescence marking of antibodies could not be imagined without FITC. However, at the same time FITC displays pH-indicative properties. This paper evaluates the limits of the use of FITC as a pH indicator in biological material, namely, for intracellular and intraorganellar pH measurement.

KEY WORDS: Fluorescent probes; ratio imaging; intracellular pH.

INTRODUCTION

Fluorescein derivatives possess pH indicating properties very similar to those of the parent compound fluorescein. In most cases there is only an insignificant change in pK and fluorescence emission, excitation, and lifetime, as can be seen by comparison of fluorescein, carboxyfluorescein, BCECF [2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein], and, to some extent, SNARF (seminaphthorhodafluor) and SNAFL (seminaphthofluorescein),[1] They all seem to be good pH probes, irrespective of whether the pH indication is based on the excitation ratio, the emission ratio, or the lifetime shift. The purpose of this study was to show whether FITC (fluorescein isothiocyanate) can also be considered as a reliable pH-indicating substance, especially after its binding to proteins and sugars.

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MATERIALS AND METHODS

All fluorescent dyes including FITC-dextrans were from Molecular Probes (Eugene, OR). FITC-lactoferrin and FITC-ovalbumin were prepared in the usual way in citrate buffer adjusted to pH 9;[2] lactoferrin and ovalbumin were from Sigma. Fluorescence spectra were recorded in the standard way in a cuvette (Perkin Elmer LS 50B; emission wavelength, 530 nm; slit half-width, 2.5 nm). Fluorescein and FITC concentrations were 2.5 μM; FITC-dextran, lactoferrin, and ovalbumin were used at approximately 100-fold higher concentrations because of their lower quantum yield.

Calibration was done in a series of citrate buffers and 0.2 M triethanolamine–phthalic acid (TEPA) buffers; the latter can be used over the whole range from pH 4 to pH 9 and is routinely applied in this laboratory.[3]

RESULTS AND DISCUSSION

The fluorescence excitation spectra of FITC and its conjugates were almost-identical to those of fluorescein. There was only a red shift accompanied by an additional

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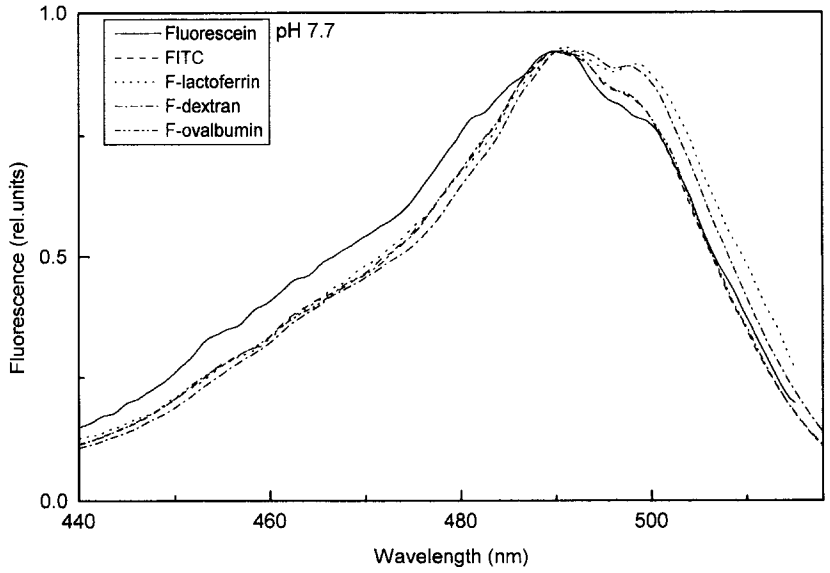

Fig. 1. The effect of protein or dextran binding on fluorescence excitation spectra of FITC.

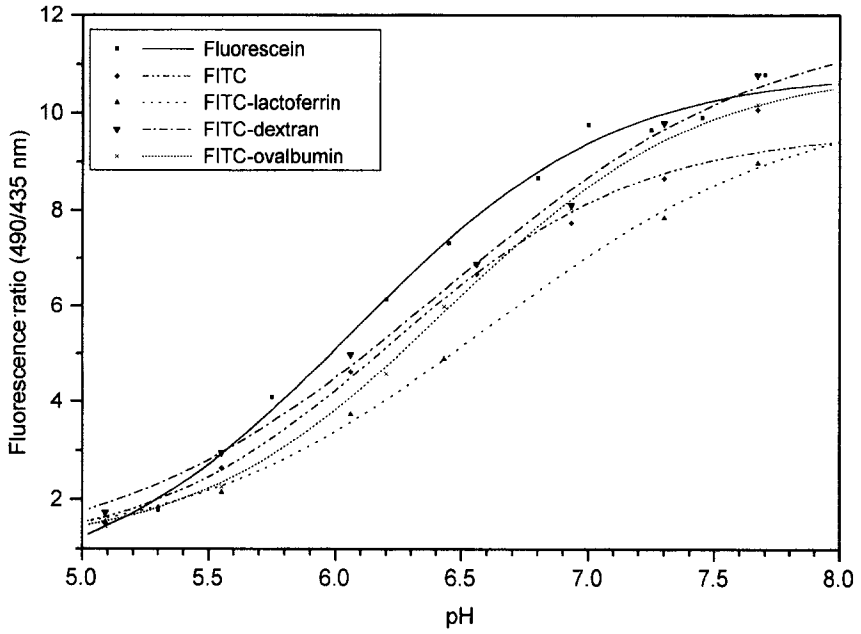

Fig. 2. pH dependence of the fluorescence excitation intensity ratio of fluorescein derivatives measured at 490 versus 435 nm.

small shoulder with a maximum around 498 nm on the red side of FITC excitation, which became more pronounced when FITC was bound either to a protein (in our case, ovalbumin or lactoferrin) or to a sugar (dextran) (Fig. 1).

The time-resolved study of the interaction of FITC with proteins revealed a gradual enhancement of the 498-nm shoulder reaching a stable value some 15 min after mixing of the FITC with the protein under conditions favorable (i.e., pH around 9) for conjugation. The fluorescence then remained stable for at least further 120 min. This happened if lactoferrin or ovalbumin was mixed with FITC in an alkaline buffer (we used either a carbonate buffer of pH 9.5 or a TEPA buffer of pH

9.0). If the mixture of FITC and protein was put into a buffer of a pH lower than 6.5 (which stops the conjugation process), no fluorescence changes were observed. Therefore, we ascribed the enhancement of the 498-nm shoulder to FITC binding to the protein.

Following our previous experiments with fluorescein and BCECF, we chose the excitation ratio 490/435 nm as a basis for pH measurement. The results shown in Fig. 2 show that there is little difference between FITC and its derivatives and fluorescein for pH measurement. Similarly to BCECF or fluorescein the 490/435-nm ratio reflected mainly pH and it was not very sensitive to the medium composition.

In the final stage of this experiment both FITC-dextran and FITC-lactoferrin were used for intracellular measurement. We took advantage of the fact that both compounds are eventually accumulated in the lysosomes of trichomonads.[4] The colocalization of both dyes in the same organelle provided the opportunity to compare the pH-indicating abilities of FITC-dextran with that of FITC-lactoferrin. Using a calibration curve done *in vitro* in a series of buffers (acetate, citrate-phosphate, triethanolamine-phthalate), we determined pH values of 5.2 $\pm$ 0.2, which coincide with each other as well as with the intralysosomal values in the literature.[5–7] Therefore, we may conclude that the conjugation of fluorescein does not impair its good pH-indicating abilities and makes it possible to use FITC derivatives and thus also FITC-labeled antibodies for intracellular pH measurement.

ACKNOWLEDGMENT

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