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New Approach for Fluorescent Peptide Labeling with Pyrylium Cyanine Dyes

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New approach for fluorescent peptide labeling with cyanine dyes utilizing the reaction of pyrylium salts with aliphatic aminogroup is proposed. The reaction of two pyrylium cyanines dye acids was investigated. Lysocyme was used as a model peptide for conjugation. The proposed method can be used as a simple and convenient alternative for the known procedures because it does not require preparation of the unstable amino-reactive intermediates from a carboxyl- or sulfo-derivative of cyanine dyes.

KEY WORDS: Cyanine dyes; peptide labeling; fluorescent detection.

INTRODUCTION

We have reported recently a promising new approach to oligonucleotide labeling using pyrylium dye as alternative amine-specific reactive group. Covalent conjugation of biomolecule with dye was accompanied by simultaneous conversion of low fluorescent pyrylium cyanine into high fluorescent pyridinium dye [1]. Here we report the use of this reaction for labeling amino acids and peptides with two cyanine dyes, monomethine Cyan40 and carbocyanine CCyan40.

EXPERIMENTAL

The reaction of two pyrylium cyanine dyes (Cyan39 and CCyan39) (Fig. 1) was investigated with the 10 amino acids with different functional groups in 0,1M triethylammonium acetate (TEAA) buffers of various pH at 50°C. Equimolar quantities of dye and amino acid were used to monitor the reaction by UV-Vis spectrometry. We considered the reaction to be completed when absorbency maximum shifted from 470 nm to 434 nm for Cyan39→Cyan40 and from 572 nm to 540 nm for CCyan39→CCyan40 conversion (Fig. 2).

The reaction was carried out in 30% DMSO-buffer solution for Cyan39 and 50% DMSO-buffer solution for CCyan39. In 30% DMSO-buffer solution CCyan39 decomposes and does not form Ccyan40, probably because of its lower solubility in water. Reaction times were different depending both from pH and the nature of amino acid (Tables I and II).

Lysocyme was used as a model peptide for conjugation with Cyan39 and CCyan39. 100 μL of peptide solution (2 mg/ml, c.a. $1{,}5\cdot10^{-4}$M) was mixed with 100 μL of 0,1M TEAA (pH 12.1), and 100 μL of $1{,}5\cdot10^{-3}$M DMSO solution of Cyan39 or 200 μL of $0{,}7\cdot10^{-3}$M DMSO solution of CCyan39 was added. The reaction mixtures were incubated for 2 h at 50°C. The absorption spectra of conjugates are shown on Fig. 3 after gel filtration (Sefadex G-25, 10 mm × 300 mm column, 0,01M TEAA buffer).

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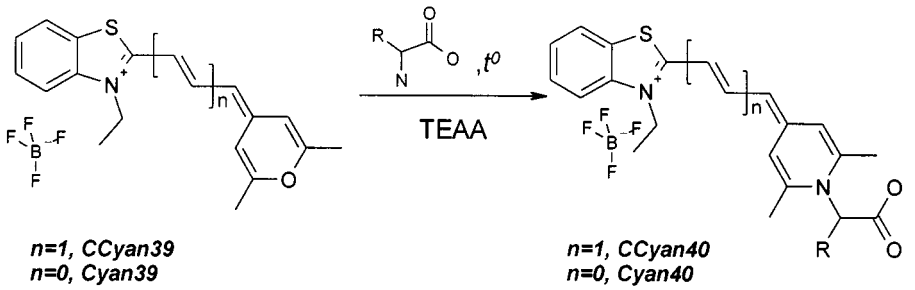

Fig. 1. The reaction of two pyrylium cyanine dyes (Cyan39 and CCyan39) with amino acids.

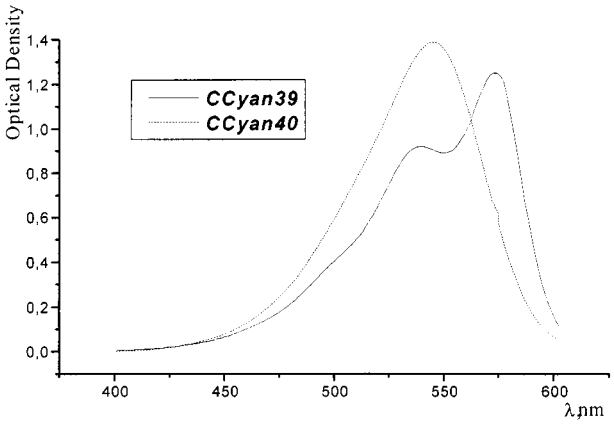

Fig. 2. Absorption spectra of pyrylium dye CCyan39 (λ_{max} = 572 nm) and its pyridinium analogue CCyan40 (λ_{max} = 540 nm) in ethanol.

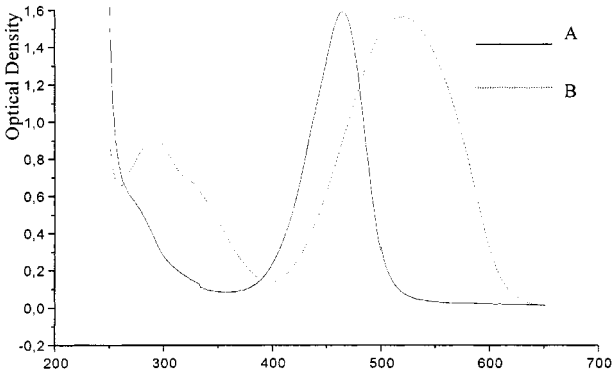

Fig. 3. UV-VIS spectra of lysocyme-Cyan40 (A) and lysocyme-CCyan40 (B) conjugates in water.

Table I. Reaction Time (min) of Cyan39 with Different Amino Acids in TEAA Buffers of Various pH

pH	Acp	Lys	Ser	Arg	Gly	Ala	Val	Trp	His	Asp
11.6	20	20	80	95	9	85	95	100	95	110
11.45	35	37	150	—*	15	—	—	—	—	145
11.3	40	40	>150	—	25	—	—	—	—	>150

Table II. Reaction Time (min) of CCyan39 with Different Amino Acids in TEAA Buffers of Various pH

pH	Acp	Lys	Ser	Arg	Gly	Ala	Val	Trp	His	Asp
12.03	11	13	30	38	8	47	51	56	70	66
11.44	18	20	35	51	16	66	78	106	113	109
11.19	33	26	62	166	22	85	104	130	145	185

After PAAG electrophoresis the bands of dye-peptide conjugate were clearly visible upon UV-illumination as yellow-green for Cyan40 and bright red for CCyan40.

Thus, a new efficient method for peptide labeling with high fluorescent monomethine and carbocyanine dyes is proposed. It can be used as a simple and convenient alternative for known procedures because it does not require any preparation of unstable amino-reactive intermediates from carboxyl- or sulfo-derivative of cyanine dye that are usually used.

ACKNOWLEDGMENTS

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