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Release of catecholamines from pyrenylmethyl urethane conjugates by light

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Abstract- A fluorescent pyrenylmethyl moiety was coupled to the N-*terminus* of a series of catecholamines. The behaviour of the corresponding fluorescent conjugates towards photocleavage was evaluated by irradiation in MeOH/HEPES buffer (80:20) solution, in a photochemical reactor at different wavelengths (254, 300, 350 and 419 nm), followed by HPLC/UV monitoring.

Keywords*:* Pyrene; Catecholamines; Photocleavable protecting groups.

1. Introduction

There is a pratical interest in the fluorescent derivatisation of biologically important analytes to enhance the sensitivity of detection and to quantify and elucidate mechanisms of physiological action [1]. Important examples of such analytes are the biogenic catecholamines epinephrine (adrenaline), norepinephrine and dopamine, which control adrenergic systems in the CNS and are responsible for the awake/asleep cycle, mood swings and act peripherally to modulate blood pressure, among other functions. The catecholamine biosynthetic pathway involves the amino acids phenylalanine, tyrosine and 3,4-dihydroxyphenylalanine (DOPA), as well as the structurally related tyramine [2].

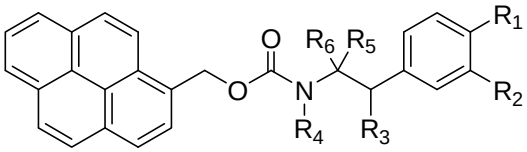
The significance of photorelease applications in neurological sciences for studying the chemical mechanisms and the kinetics of synaptic transmission has grown steadily in recent years [3]. This fact is due to the broad use of caging strategies which make use of photochemically removable protecting groups to mask the activity of biologically relevant molecules and that upon irradiation with light of appropriate wavelength release the bioactive species in a time- and space-resolved manner.

Bearing these facts in mind, as well as our research interests which include the synthesis of new fluorescent heterocyclic compounds, their application on the design of fluorescent conjugates of biologically relevant molecules and studies on their photorelease [4], we now

report the photocleavage study of fluorescent conjugates of a series of catecholamines bearing a pyrenylmethyl moiety as photolabile group, at different wavelengths.

2. Results and discussion

A series of fluorescent conjugates **1a-f** were obtained by linkage of a pyrenylmethyl moiety, through an urethane bond, to the amino group of catecholamines amines dopamine **a**, norepinephrine **b** and their biosynthesis precursors, phenylalanine methyl ester **c**, tyrosine methyl ester **d**, 3,4-dihydroxyphenylalanine (DOPA) methyl ester **e** and tyramine **f** (Figure 1) [5].



1 **a**) $R_1 = R_2 = OH$, $R_3 = R_4 = R_5 = R_6 = H$
b) $R_1 = R_2 = R_3 = OH$, $R_4 = R_5 = R_6 = H$
c) $R_1 = R_2 = R_3 = R_4 = R_5 = H$, $R_6 = CO_2CH_3$
d) $R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = H$, $R_6 = CO_2CH_3$
e)$R_1 = R_2 = OH$, $R_3 = R_4 = R_5 = H$, $R_6 = CO_2CH_3$
f) $R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = R_6 = H$

Figure 1. Structure of catecholamines urethane conjugates **1a-f**.

Photolysis studies of the catecholamines urethane conjugates **1a-f** were carried out and solutions of the mentioned compounds in methanol/HEPES buffer (80:20) solution were irradiated in a Rayonet RPR-100 reactor, at 254, 300 and 350 nm, in order to determine the best cleavage conditions. The course of the photocleavage reaction was followed by reverse phase HPLC with UV detection.

The plots of peak area of the starting material *versus* irradiation time were obtained for each compound, at the considered wavelengths. Peak areas were determined by HPLC, which revealed a gradual decrease with time, and were the average of 3 runs. The determined irradiation time represents the time necessary for the consumption of the starting materials until less than 5% of the initial area was detected (Table 1). The pyrenylmethyl group will be designated by Pym for simplicity of naming the various fluorescent conjugates.

For each compound and based on HPLC data, the plot of ln *A versus* irradiation time showed a linear correlation for the disappearance of the starting material, which suggested a first order

reaction, obtained by the linear least squares methodology for a straight line. The correlation coefficients varied from 0.9880 to 0.9997.

Table 1. Irradiation times (in min) and rate constant (*k*, in min^{-1}) for the photolysis of urethane conjugates **1a-f** at different wavelengths in MeOH/HEPES buffer (80:20) solution.

Compound		254 nm		300 nm		350 nm	
		Irr time	*k*	Irr time	*k*	Irr time	*k*
1a	Pym-dopamine	8.1	0.3861	4.5	0.6906	33.5	0.0898
1b	Pym-norepinephrine	12.0	0.2484	4.8	0.6125	32.7	0.0912
1c	Pym-phenylalanine	39.2	0.0798	23.0	0.1368	229.5	0.0133
1d	Pym-tyrosine	41.0	0.0714	7.1	0.4259	115.3	0.0260
1e	Pym-DOPA	10.5	0.2814	5.0	0.5760	16.5	0.1890
1f	Pym-tyramine	31.5	0.0980	12.9	0.2376	106.3	0.0285

Concerning the influence of the wavelength of irradiation on the rate of the photocleavage reactions of conjugates **1a-f** in methanol/HEPES buffer (80:20) solution, it was found that the most suitable was 300 nm, resulting in shorter irradiation times, accordingly to the lamp power and the wavelength absorption maxima of the conjugates.

Taking into consideration the influence of the conjugate structure on the photocleavage rates, it was found that the presence of hydroxyl groups led to shorter irradiation times as the phenylalanine conjugate **1c** (bearing no OH groups) was the one that cleaved slower at 300 and 350 nm. At 254 nm, conjugate **1c** and tyrosine conjugate **1d** (with one OH group) had similar irradiation times (*ca*. 40 min), and still, larger than those of the other conjugates. These two conjugates, which differ in one hydroxyl group, behaved differently at 300 and 350 nm, with the tyrosine conjugate **1d** cleaving faster than the phenylalanine conjugate **1c**. Regarding the results obtained for the dopamine conjugate **1a** and the norepinephrine conjugate **1b**, it was found that they cleaved readily at 300 nm (less than 5 min). As for the precursors involved in the biosynthetic pathway, tyrosine and DOPA, their conjugates **1d** and **1e**, respectively, also displayed similar irradiation times (between 5 and 7 minutes). These

promising results indicate that the pyrenylmethyl group can be an interesting choice for a photocleavable group, allowing the release of the catecholamines in short time, in this particular case, and used as an alternative to other established photolabile groups.

3. Conclusions

Regarding the photocleavage studies of the fluorescent conjugates, in methanol/ HEPES buffer (80:20) solution, at 254, 300 and 350 nm, it was possible to conclude that the irradiation time depended on the structure of the catecholamine. Considering the short irradiation times obtained at 300 nm, the pyrenylmethyl label can be considered as a suitable photocleavable protecting group for the considered catecholamines.

4. Experimental

General photolysis procedure: A 1×10^{-4} M methanol/HEPES buffer (80:20) solution of conjugates **1a-f** (5 mL) was placed in a quartz tube and irradiated in a Rayonet RPR-100 reactor at the desired wavelength. The lamps used for irradiation were of 254, 300 and 350 ± 10 nm. HEPES buffer solution was prepared in distilled water with HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) (10 mM), NaCl (120 mM), KCl (3 mM), $CaCl_2$ (1 mM) and $MgCl_2$ (1mM) and pH adjusted to 7.2.

Aliquots of 100 µL were taken at regular intervals and analysed by RP-HPLC. The eluent was acetonitrile/water, 3:1, at a flow rate of 0.6 (compound **1e**), 0.8 (compounds **1a-b**, **d**, **f**) and 1.2 (compound **1c**) mL/min, previously filtered through a Millipore, type HN 0.45 µm filter and degassed by ultra-sound for 30 min. The chromatograms were traced by detecting UV absorption at the wavelength of maximum absorption (340 nm)) (retention time: **1a**, 5.9; **1b**, 4.3; **1c**, 8.8; **1d**, 6.9; **1e**, 7.3; **1f**, 9.9 min).

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