# THE PHOTOPHYSICS OF THIAMINE AND PHOTOCHEMISTRY OF PYRUVIC ACID AND PYRUVATE

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The photophysical parameters of thiamine (I) and its pyrophosphate ester, cocarboxylase, are not fully known. The excited state parameters of such systems are of interest in relation to fundamental mechanisms of coenzyme action[1] and also in photochemical studies. A previous study
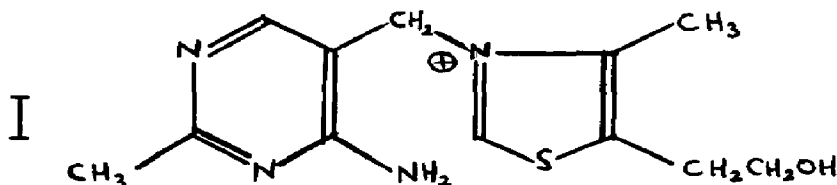
by Razumovich $et\ al$[2] was unable to locate the triplet state of thiamine and it was concluded that triplet-triplet energy transfer from the pyrimidine to the thiazole moiety was the cause of the non-phosphorescent nature of thiamine. Similarly Aaron and Winefordner[3] were unable to detect phosphorescence from thiamine. We have prevously reported the location of the lowest triplet state of thiamine[4], we now present the results of a study of energy transfer to pyruvate and pyruvic acid.

Breslow[5] proposed a mechanism for the decarboxylation of pyruvate by thiamine whereby the substrate forms an adduct at C-2 of the thiazole moiety. Leermakers and Vesley[6] have shown that pyruvic acid and sodium pyruvate in aqueous solution undergo photolysis when excited in the longest wavelength absorption band. We set out to find evidence for such an adduct and also to investigate the possibility of photolysis of pyruvate photosensitised by excited state thiamine.

Addition of pyruvate or pyruvic acid to a solution of thiamine caused quenching of the thiamine fluorescence and enhancement of the phosphorescence of the quencher. No new emission bands were observed, nor was the phosphorescence lifetime of the quencher emission found to be that

of the donor phosphorescence as might be expected in the case of triplet-triplet energy transfer. It is therefore concluded that the quenching mechanism is one of singlet-singlet energy transfer. From Stern-Volmer plots we determined the quenching rate constant, k_q, of thiamine fluorescence by both pyruvate and pyruvic acid in ethanol at 77K. Trivial radiative energy transfer was calculated to account for only 1% of observed fluorescence quenching. From the Förster[7] dipole-dipole energy transfer mechanism the critical transfer distance, R_o, at which energy transfer to the acceptor and the internal deactivator of the donor was equally probable have been calculated as of the order of 1.1 nm which is consistent with dipole-dipole energy transfer. An estimate of the critical transfer distance can be obtained from equation 1[8]:

$$R_o = 0.735 \times (C_A)_o^{-1/3}$$

where $(C_A)_o$ is the concentration of acceptor at which the fluorescence has been quenched to half of its unquenched intensity. The Stern-Volmer constants, K_{sv}, quenching rate constants and critical transfer distances are shown below:

Parameters of thiamine fluorescence quenching

Quencher	K_{sv}/l mole^{-1}	k_q/l mole^{-1} s^{-1}	R_o/nm[a]	R_o/nm[b]
Pyruvate	2500	3.1×10^{11}	1.16	9.9
Pyruvic acid	60	$7.4 \ 10^9$	1.13	2.9

a.   From Förster equation

b.   From equation 1.

The comparison of the critical transfer distances leads us to propose that some association occurs between thiamine and pyruvate, possibly of an ion-pair nature which causes a marked decrease in the distance between thiamine and pyruvate species. The marked difference between the critical transfer distances calculated from equation 1 between pyruvate and pyruvic acid is due to a decrease in the number of ion-pairs formed.

Having demonstrated energy transfer and also the possibility of association between the conenzyme and the substrate an investigation of the possibility of photosensitised decarboxylation of the substrate was undertaken. Although unable to detect any photochemical decarboxylation of substrate in a system sensitised by thiamine we offer the following observation. The phosphorescence quantum yields of pyruvic acid and pyruvate in ethanol at 77K were determined. Leermakers and Vesley determined

the quantum yield of photolysis of pyruvic acid and pyruvate in aqueous
solution. The values are tabulated below:

	ϕ_{phos}^{a}	$\phi_{photolysis}^{b}$
Pyruvic acid	0.18	0.79
Pyruvate	0.53	0.04

a. Determined relative to benzophenone ($\phi_p = 0.74^9$)
b. Taken from reference 6.

Leermakers and Vesley assumed that in pyruvate and pyruvic acid intersystem
crossing is sufficiently efficient for the triplet yield to be unity.
Although no fluorescence is detectable from pyruvate and pyruvic acid this
is not direct evidence for a triplet yield of unity. If we assume that the
phosphorescence quantum yield is proportional to the triplet quantum yield
we can see that the photolysis quantum yields of pyruvic acid and pyruvate
are inversly related to the triplet quantum yield. Therefore we propose
that the photochemistry of pyruvate and pyruvic acid proceeds through the
lowest singlet excited state.

References

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