ACTA CHEMICA SCANDINAVICA 17 (1963) 461–463

Fluorescence of Adenine and Inosine Nucleotides

EVA WALAAS

The Institute of Medical Biochemistry, University of Oslo, Norway

Adenine and inosine nucleotides have been shown to exert native fluorescence, although with low quantum yield. The highest quantum yield was obtained with adenine derivatives in acid solution. An attachment of the ribosyl group to adenine increased the fluorescence intensity, but this increase was somewhat quenched by phosphate groups. At neutrality the fluorescence of adenine derivatives was greatly decreased. Inosine nucleotides showed very low fluorescence intensity at any value of pH.

The substituted purines, adenine and guanine, and their nucleosides and nucleotides have been shown to emit fluorescence in acid solution, although with very low quantum yield (0.2 %) [1].

To evaluate the influence of substituents in the purine ring on the fluorescence intensity, experiments have been performed in the present work, which compare the fluorescence intensity of adenine and inosine derivatives. Differences have been recorded at acid pH where the quantum yield of the fluorescence emission by inosine nucleotides is 1/10 of the fluorescence emitted by adenosine nucleotides. Moreover the observation has been made that among the adenine and adenine derivatives, the highest intensity of fluorescence emission was obtained with adenosine.

EXPERIMENTAL

The spectrofluorometric investigations were made on a "Farrand" spectrofluorometer. All reagents were purchased from the "Sigma" Chemical Co. Before use the adenine nucleotides as well as ITP * and IMP were purified by chromatography on a Dowex-1 column with a formate system according to Hurlbert *et al.*[2] By this procedure any traces of flavin nucleotides were removed from the purine nucleotides. All solutions were made in deionized water (Elgastat deionizer, Elga Ltd., London). FAD and FMN were used as fluorescence references. The readings were performed at concentrations of purine nucleotides of 5×10^{-6} M.

* Abbreviations used: ITP inosine triphosphate, IMP inosine monophosphate, ATP adenosine triphosphate, ADP adenosine diphosphate, AMP adenosine monophosphate, FAD flavin adenine dinucleotide, FMN flavin mononucleotide.

Table 1. Excitation and fluorescence maxima of some nucleotides.

Compound	pH 1		pH 7	
	Excitation mμ	Fluorescence mμ	Excitation mμ	Fluorescence mμ
ATP	285	380	300	390
ADP	290	380	295	390
AMP	290	380	300	390
Adenosine	290	380	300	400
Adenine	290	380	300	390
ITP	300	390	290	380
IMP	300	390	300	390
FMN	340	520	340	520
FAD	350	520	350	520

Table 2. The relative intensity of fluorescence of some nucleotides. The fluorescence of FAD at pH 1.0 used as a standard = 100, equivalent with a quantum yield of 5 %.

Compound	Relative intensity of fluorescence	
	pH 1	pH 7
ATP	8	6
ADP	6	4
AMP	4	2
Adenosine	10	6
Adenine	3	5
ITP	0.31	0.3
IMP	0.25	0.3
FAD	100	90
FMN	90	500

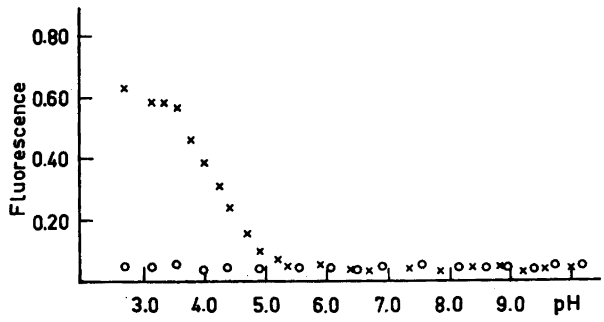

Fig. 1. Fluorescence of ATP and ITP as a function of pH. The following buffer systems were used at 0.05 M concentrations: Citrate-phosphate, phosphate and glycine-NaOH.
×: ATP.
O: ITP.

RESULTS

The uncorrected values for excitation maxima and fluorescence maxima for adenine and adenine derivatives, and for inosine nucleotides are given in Table 1. The excitation maxima for the adenine group were recorded 290 to 300 mμ, *i.e.* at somewhat longer wave-lengths than the values given by Udenfriend [3] on a different apparatus. Agreement was found, however, for the fluorescence maxima at 380 to 390 mμ. Both the excitation maxima and the fluorescence maxima recorded at pH 1. 0, were found to be displaced 5 to 10 mμ towards longer wave-lengths at neutrality. ITP and IMP were excited at 300 mμ at pH 1.0, and at 290 mμ and 300 mμ at neutrality.

While the inosine derivatives showed a low and constant fluorescence emission at any pH values between pH 1.0 to pH 10.0, the adenosine derivatives showed an increase in fluorescence at pH values below 5. In Fig. 1 is presented the difference in fluorescence emission between ATP and ITP as a function of pH. The results indicate that the increased fluorescence at lower pH is related to the protonization of the amino group in 6-position. The pK' of this group in adenine and adenine nucleotides approximates 3.8—4.2. No change in the fluorescence of ITP could be recorded even at high values of pH, and thus the dissociation of the hydroxyl group in 6-position did not seem to affect the fluorescence.

Udenfriend [3] has pointed out that all the adenosine derivatives have similar fluorescence properties, and that the intensity of the emitted fluorescence has some relation to molecular weight, or to adenosine contents. In the present work the observation has been made that the highest quantum yield (0.5 %) was obtained for adenosine (Table 2). Using FAD as a reference, the fluorescence of adenosine was 1/10 of the quantum yield of FAD (5 %). The quantum yield at pH 1.0 decreases in the order: adenosine > ATP > ADP > AMP > adenine. The presence of ribose thus increases the fluorescence emission in acid solution, although this increase is somewhat quenched by phosphate groups. The lowest quantum yield in the adenine group at pH 1.0 was given by adenine itself (0.15 %). The inosine derivatives showed a very low fluorescence intensity (quantum yield 0.015 %), the value for ITP being slightly higher than for IMP.

It is pointed out by Udenfriend [3] that a study of the fluorescence properties of all the purines has both a practical and theoretical significance. The difference in fluorescence intensities can be used for analytical purposes. By the fluorometric method described here determination of the 5′-adenylic acid deaminase activity is possible.

Acknowledgments. This work has been supported by a grant from *Det Videnskapelige Forskningsfond av 1919.*

REFERENCES

1. Duggan, D. E., Bowman, R. L., Brodie, B. B. and Udenfriend, S. *Arch. Biochem. Biophys.* **68** (1957) 1.
2. Hurlbert, R. B., Schmitz, H., Brum, A. F. and Potter, V. R. *J. Biol. Chem.* **209** (1954) 23.
3. Udenfriend, S. *Fluorescence Assay in Biology and Medicine*, Academic Press, New York 1962, pp. 294—296.

Received July 21, 1962.