Acta Chemica Scandinavica B 34 (1980) 603–605

Fluorescence Properties of Alkylated Guanine Derivatives

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The fluorescence excitation spectra of a number of quanine derivatives were characterized at six different pH's. Large differences were observed in the fluorescence intensities between the derivatives, although the fluorescence spectra were largely similar showing maxima at 290–295 nm when emission was at 400 nm. The fluorescence intensities correlated in part with the acid and alkaline dissociations of the molecules. The fluorescence spectra taken at different pH's appears to be helpful in the identification of guanosine alkylation products such as 7-*N*-, 6-*O*- and 2-*N*-alkylguanosine.

Covalent modification of DNA by chemical carcinogens is thought to be one of the underlying mechanisms in the initiation of tumor growth.[1-3] The DNA-adducts of many carcinogens have been characterized using techniques such as UV spectroscopy, mass spectrometry and NMR spectroscopy. In all cases, the isolation of the product is required for the identification. Most known carcinogens alkylate DNA bases, particularly guanine. Some of the established sites of alkylation are *N*7, *O*6, *N*2, *N*1 and *C*8 of guanine,[1-3] depending on the type of carcinogen applied.

Fluorescence spectroscopy has had only a limited application in the study of nucleic acid alkylation products,[4-5] although it can offer a number of advantages. The fluorescence methods are sensitive and specific to certain types of adducts only, thus simplifying or making unnecessary the isolation of the adducts. In the present communication the fluorescent properties of methylated guanine derivatives are analyzed to provide a basis for further application of fluorescence techniques in the identification of fluorescence techniques in the identification of the guanine alkylation products.

EXPERIMENTAL

Methylated guanosine and guanine derivatives were obtained from Sigma Chemical Company, Fluka, Vega Biochemicals and BL Biochemicals. 6-*o*-Methylguanosine was a generous gift from Dr. P. L. Lawley. Solutions (5 mM) of the compounds were prepared and 50 μl of each were mixed with 2.5 ml of 20 % methanol containing 0.1 M phosphate buffer, NaOH or HCl to give the appropriate pH.

The fluorescence excitation spectra (220–360 nm) were taken in a cuvette with 1 cm light path with the emission at 400 nm[6] in a Perkin-Elmer Double Beam Fluorescence Spectrometer Model 512. The reference cuvette contained the solution in which the samples were dissolved. The fluorecence spectra are presented without corrections. Fluorescence intensities were measured at the maximal intensity at 290–295 nm. It is possible that the high concentrations of the nucleic and derivatives used caused somewhat distorted excitation spectra. Yet high concentrations were necessary for the detection of low fluorescence of some derivatives. Moreover the study emphasized qualitative aspects of analysis.

RESULTS

The fluorescence excitation spectra of guanine and several of its derivatives were investigated at a constant emission (400 nm). The fluorescence intensity of the guanine derivatives varied extensively, although the shape of the spectra appeared largely similar. Fig. 1 shows the fluorescence spectra of 6-*o*-methylguanosine at pH 7 and of guanine at pH 1. The fluorescence maxima are about 290 to 295 nm, in analogy to the other fluorescent derivatives studied, with the exception of 2-*N*-methylguanine showing a different spectrum at the neutral pH.

The fluorescence intensity of the guanine derivatives is dependent on pH, and excitation spectra

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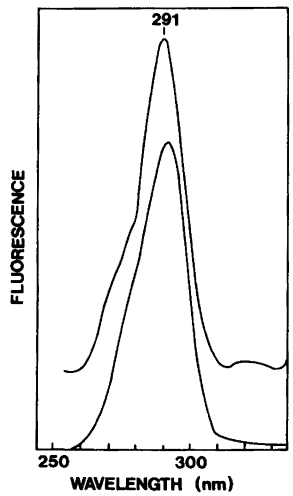

Fig. 1. Fluorescence excitation spectra of 6-*O*-methylguanosine at pH 7 (top) and guanine at pH 1 (bottom). The emission is at 400 nm.

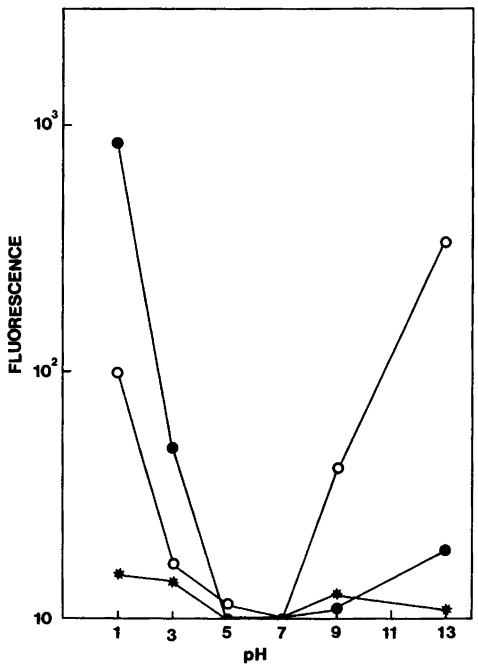

Fig. 2. pH-dependent fluorescence intensity of guanine (○), guanosine (●) and xanthine (∗). The fluorescence intensity is given in arbitrary units measured at the excitation maximum (290–295 nm) using emission at 400 nm.

were recorded at 6 different pH's. The excitation spectra of guanine, guanosine and xanthine, at the maximal intensity, are shown in Fig. 2 as a function of pH. Guanine is fluorescent in the acid and in the alkaline region. Guanosine is strongly fluorescent only in the acid region. Xanthine shows hardly any fluorescence at all.

The fluorescence spectra of methylated guanosine derivatives are shown in Fig. 3 as a function of pH. 6-*O*-Methylguanosine has a very strong fluorescence in the acid but it is fluorescent even in the alkaline. 7-Methylguanosine has a strong fluorescence in the acid region declining to a very low intensity at pH 13. 1-*N*-Methylguanosine is only fluorescent in the acid while 2-*N*-methylguanosine is fluorescent in the acid and in the alkaline but non-fluorescent at pH's 3 to 7.

The fluorescent intensities of the different methylated derivatives of guanine follow a rather uniform

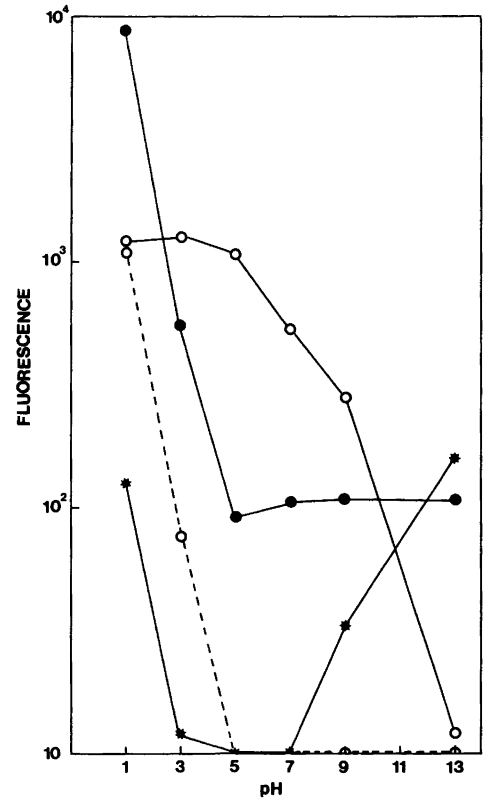

Fig. 3. pH-dependent fluorescence intensity of 1-*N*-methylguanosine (– – –), 2-*N*-methylguanosine (∗), 6-*O*-methylguanosine (●) and 7-*N*-methylguanosine (○).

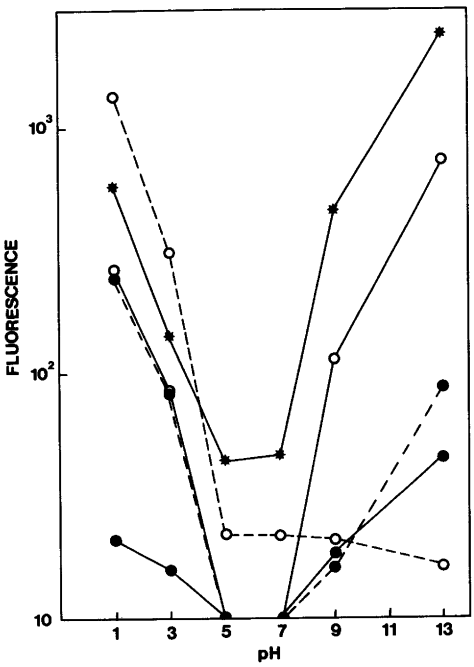

Fig. 4. pH-dependent fluoresence intensity of 1-*N*-methylguanine (●---), 2-*N*-methylguanine (*), 3-*N*-methylguanine ((● —), 7-*N*-methylguanine (○ —), and 9-*N*-methylguanine ((○ ---).

pattern as a function of pH (Fig. 4). The fluorescence is high in acid, low or nil at neutrality, and high again in alkaline. The only exception is 9-*N*-methylguanine exhibiting a low fluorescence at pH 13.

DISCUSSION

Previously the fluorescence techniques have been used mainly inthe detection of alkylated guanine derivatives[6-7] or of any types of adducts formed by fluorescent carcinogens.[8-9] Fluorescence measurements have been applied only to a limited extent in the identification of carcinogen adducts. With polycyclic aromatic hydrocarbons and with other fluorescent carcinogens structural information has been obtained in fluorescence studies.[8] Fluorescence spectra of some simple adenine[10] and guanine[11] derivatives have been characterized. The present paper surveyed the pH-dependent fluorescence properties of the most common guanine derivatives formed by chemical carcinogens. The application of fluorescence techniques in the identification of carcinogen-guanine adducts would appear informative.

The changes in the fluorescence intensity correlated for most compounds with their acidic and alkaline dissociation.[1,4] For example, all the methylated guanosine derivatives studied (*N*1, *N*2, *O*6, *N*7, Fig. 3) dissociate in the acid region, while only 2-*N*-methyl- and 7-*N*-methylguanosine dissociate in the alkaline region. The reason for the low fluorescence of 7-*N*-methylguanosine in the alkaline is the destruction of the imidazole ring in the alkaline.[4]

The application of the fluorescence measurements in the identification of guanosine alkylation products would require pH-conditions, where the unreacted nucleoside would not be fluorescent (pH 5–13, Fig. 2). Any fluorescence in this pH region would suggest alkylation at *O*6 or *N*7. Alkaline treatment would destroy the fluorescence of *N*7-products, and at pH 13 *O*6- and *N*2-derivatives would be detected. Upon reneutralization, only 6-*O*-alkyl products could be fluorescent.

Acknowledgement. The excellent technical assistance of Ritva Suni is gratefully acknowledged.

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Received April 14, 1980.