STUDY OF NUCLEIC ACID BASES BY THE DADI METHOD

R. Razakov and A. K. Kasimov

UDC 547.963.3

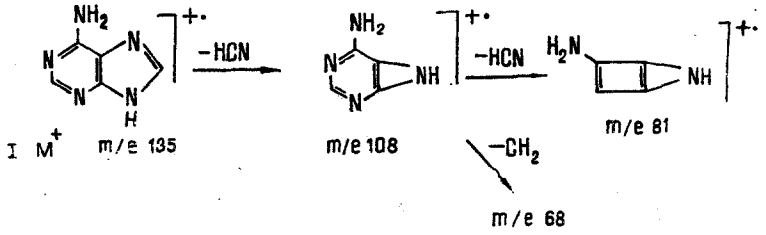
The DADI spectra of the nucleic acid bases adenine (I), guanine (II), Uracil (III), thymine (IV), cytosine (V), and 5-methylcytosine (VI) have been studied. It has been established that the samples of nucleic acid bases investigated are characterized by a specific pattern of the DADI spectra which reflects the nature of the molecular ion due to the structure of the given base and, in the case of M^+ for (IV) and (VI) also the tautomeric state. The possibility has been shown of using the information obtained for the quantitative determination of (VI) in a mixture of bases.

Mass spectrometry is widely used to study the components of nucleic acids — oligonucleotides [1, 2], nucleosides [3], and bases [4]. The method of the direct analysis of daughter ions (DADI) has come into use comparatively recently in structural-analytical investigations of natural compounds [5, 6]. The advantage of this method is that it permits the detection and recording of a fragmentation spectrum accompanied by the metastable transition of a given ion in the presence of many others. Each concrete molecular ion (M^+) or fragmentary ion has an individually specific decomposition pathway which is reflected in its DADI spectrum. The degree and extent of the occurrence of fragmentation with a metastable transition are shown in DADI spectra both in the number and in the form (intensity and width) of the peaks of the daughter ions. Thus, if the DADI spectra of M^+ and some key fragments of the sample under investigation are available it is possible to identify it in a mixture with other substances. There is no doubt that the use of the DADI method for studying the components of nucleic acids will expand the possibilities of mass spectrometry in structural investigations in this field.

We have considered the DADI spectra of the nucleotide bases adenine (I), guanine (II), uracil (III), thymine (IV), cytosine (V), and 5-methylcytosine (VI).

The DADI spectrum of M^+ of (I) with m/e 135 is characterized by one very strong peak at m/e 108 which accompanies the splitting out of a molecule of hydrocyanic acid (Fig. 1a). The daughter ion with m/e 108 is, in its turn, capable of losing a HCN molecule and a CN_2 group, giving transitions to ions with m/e 81 and 68, respectively (Scheme 1).

The ejection of a molecule of hydrocyanic acid from M^+ is also observed for (II), but the peak corresponding to this transition has a low intensity. The other peak in the DADI spectrum of (II), which has a triplet form with m/e values of 110, 109, and 108, shows the splitting out of the fragments HCN_2 and $H_2N{-}C{\equiv}N$ from the six-membered ring and of HNCO from the five-membered ring, and masses of 41, 42, and 43, respectively (Fig. 1b). The spectra of (III) and (IV) are very similar to one another and are characterized by an intense peak of the $(M - 43)^+$ ion which then loses a CO group (Scheme 2; Fig. 1c, d).



Scheme 1

Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR, Tashkent.
Translated from Khimiya Prirodynkh Soedinenii, No. 6, pp. 838-844, November-December, 1979. Original article submitted June 27, 1979.

0009-3130/79/1506-0743$07.50

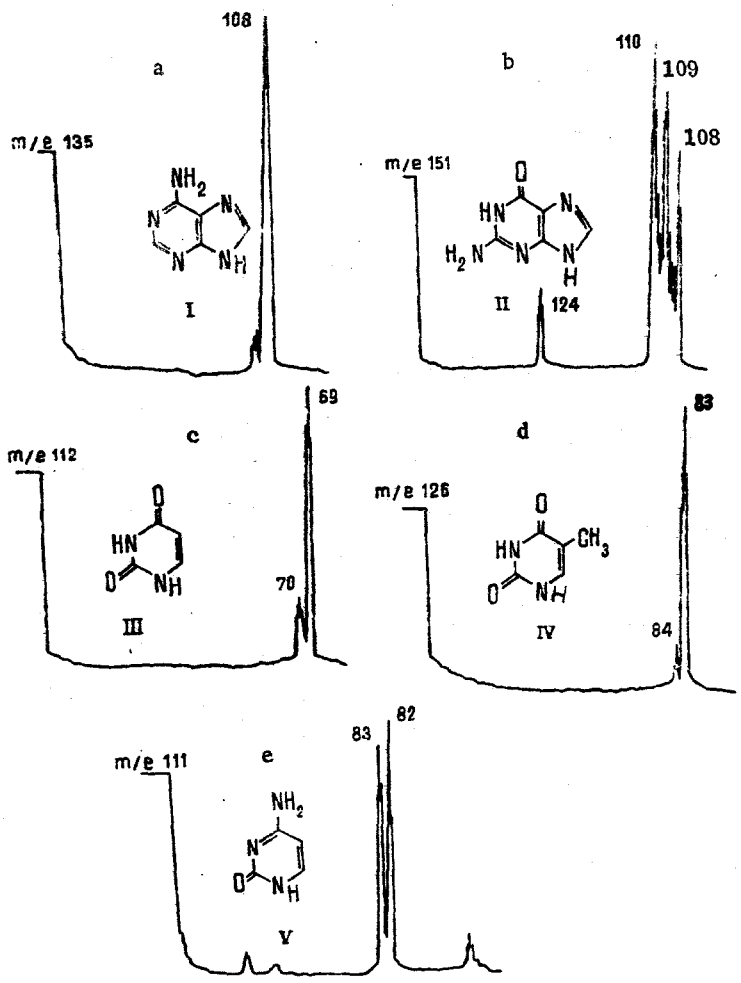
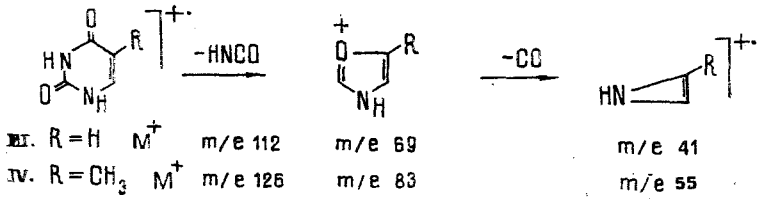
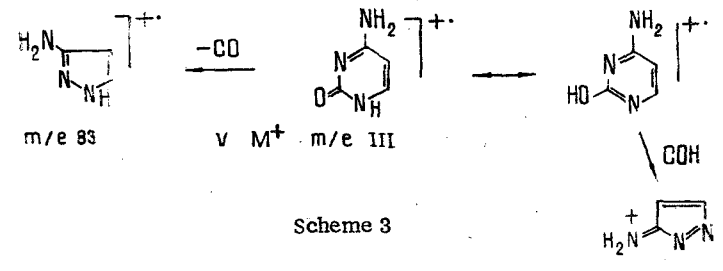


Fig. 1. DADI spectra of M^+ for adenine (a), guanine (b), uracil (c), thymine (d), and cytosine (e).



Scheme 2



Scheme 3

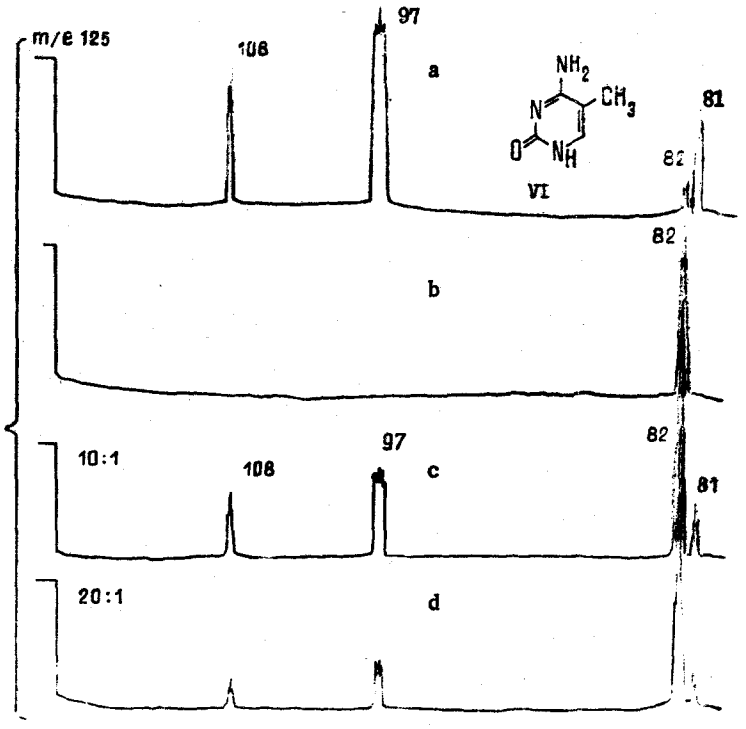
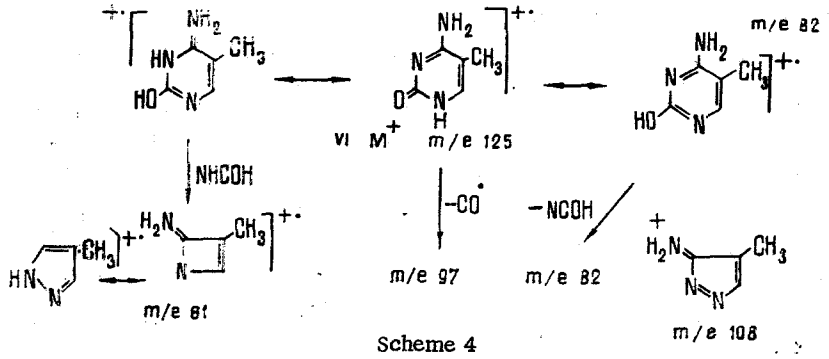


Fig. 2. DADI spectra of 5-methylcytosine (a), of $(M-1)^+$ of thymine (b), and of the ions M^+ for (VI) and $(M-1)^+$ for (IV) with m/e 125 in their mixture (c, d).



Scheme 4

However, the structural similiarity of cytosine (V) and of 5-methylcytosine (VI) is not shown in their DADI spectra to the same degree as was observed in the case of (III) and (IV). In the spectrum of (V) a broadened peak split into a doublet with mass values of 83 and 82 corresponds to the ejection of CO and CHO from M^+ with m/e 111 (Fig. 2). The splitting out of the latter (a formyl radical) is due to the fact that M^+ for (V) isomerizes to some degree from the lactam form to the lactim form (Scheme 3).

The existence of lactam–lactim tautomerism in the molecular ion of (VI) is well shown in its DADI spectrum by an intense peak of an ion with m/e 108 corresponding to the ejection of the hydroxy group from the lactam form of M^+. The lactim form of this ion loses CO and HNCO molecules, which leads to the appearance of peaks of ions with m/e 97 and 82, respectively. The existence of yet another possible tautomeric form of M^+ for (VI), namely an imine form is shown in the spectrum by the intense peak of an ion with m/e 81. In these circumstances, a group with a mass of 44 is eliminated (Fig. 2a and Scheme 4).

Thus, the samples of nucleic acid bases that we have studied are characterized by a specific pattern of spectra reflecting the nature of the molecular ion, which is due to the structure of the given base, and in some cases also reflecting the tautomeric state of the molecular ion.

The results obtained can be used for structural-analytical purposes in the study of nucleic acids and, in particular, for studying the degree of modification of DNA and RNA. As is well known, in DNA molecules under the action of methylases cytosine is converted into 5-methylcytosine. The amount of the latter ranges from 0.005 to 2%, depending on the source of DNA.

It stands to reason that the qualitative detection and qualitative determination of 5-methylcytosine when it is present in such a small proportion leads to the necessity for an accurate and sensitive method, such as mass spectrometry. However, in the mass-spectrometric determination certain difficulties arise. For example, the peak of the molecular ion of 5-methylcytosine with m/e 125 is superposed on the peak of the $(M-1)^+$ ion of thymine, although the accurate values of the masses of these ions are different (125.0589 and 125.0350, respectively). The difference in the accurate masses of these ions has been used by Razin et al. to determine 5-methylcytosine in DNA by high-resolution mass spectrometry [7].

In order to overcome this difficulty, we have measured the relative intensities of $(M-1)^+$ of thymine at various temperatures and the ratios of the intensities of its peaks and of the peak of the metastable ion with m/e 82 formed from it. The results of the measurements showed that the intensity of the $(M-1)^+$ ion of thymine amounts to 0.67% of M^+, and the ratio of the intensities of the peaks of the $(M-1)^+$ ion and the metastable ion with m/e 82 is 0.615, these values remaining constant in the range of temperatures measured.

The amount of thymine in the molecules of animal DNAs is about 30% of the total amount of bases. In the mass spectra of the total bases the slightest change in the ratios of the intensities of the M^+ and $(M-1)^+$ peaks of thymine shows an inhomogeneity of these ions. Since in extracts of the total bases of a homogeneous fraction of DNA the presence of other components with the same m/e value is unlikely, a disturbance of the ratio of the intensities of the peaks of these ions will be caused by the superposition of the peak of M^+ for 5-methylcytosine on the peak of the $(M-1)^+$ ion of thymine, which can be checked by recording the DADI spectrum of the ion with m/e 125.

When the composition of the ion with the nominal value m/e 125 is not homogeneous, i.e., it consists of $(M-1)^+$ of thymine and M^+ of 5-methylcytosine, which have different compositions and structures, all the peaks of the metastable daughter ions arising from these two types of ions appear in its DADI spectrum. In these circumstances, the peaks of the ions with m/e 108, 97, and 81 formed from M^+ of 5-methylcytosine have the same relative intensities as in the DADI spectrum of M^+ for (VI) taken in the individual state, and the intensity of the peak of the ion with m/e 82 rises because of the superposition on it of the peak of the ion with the same nominal value of m/e arising from $(M-1)^+$ for (IV) (Fig. 2b). For the quantitative characterization of such a change we recorded the DADI spectra of mixtures of (IV) and (VI) prepared in molar ratios of 1:1;, 10:1, 20:1 (Fig. 2c, d), and 30:1. The results of the measurements of the absolute intensities (millivolts) of the peaks of the daughter ions with m/e 82 and 97, and also the ratios of their intensities in the DADI spectra of the mixture of (IV) and (VI) are given below:

Thymine, mg	5-Methyl-cytosine, mg	Thymine:5-methyl-cytosine	m/e 82	m/e 97	$I_{82}:I_{97}$	$I_{82}:I_{97}$
1	1	1:1	7	23	1:3.3	0.33
0.40	0.041	10:1	68	20	3:4:1	3.4
0.60	0.03	20:1	121	18	6.6:1	6.6
0.91	0.030	30:1	98	10	9.8:1	9.8

With a change in the molar ratios in the mixtures of (IV) and (VI) in the direction of a decrease in the amount of (IV), the intensity of the peak with m/e 82 increases, which, in its turn, leads to a change in the ratio of the intensities of the peaks with m/e 82 and 97. With a change in the molar ratios of (IV) and (VI) from 1:1 to 30:1 the ratio of the intensities of the peaks of the ions measured with m/e 82 and 97 rises from 0.33 to 9.8, giving a proportionality coefficient C = 3.1.

Thus, knowing the ratio of the intensities of the ions with m/e 82 and 97 in a mixture of bases with an unknown content of (VI) it is possible to determine its molar ratio in relation to the (IV). This can be seen clearly from the formula $N_T = XC$, where N_T is the

number of moles of thymine corresponding to one mole of 5-methylcytosine, X is the measured ratio of the intensities of the ions with m/e 82 and 97, and C = 3.1 is a proportionality coefficient.

Since thymine is universally present in DNA hydrolysates and, for example, for animals it amounts to 30% of the total bases, from the value found it is possible to calculate the percentage of (VI) in the total bases from the formula

$$M = 30\frac{1}{N_T} = \frac{30}{XC},$$

where M is the percentage of 5-methylcytosine in the total bases. The minimum amount of a sample for obtaining a reproducible spectrum averaged $2\text{-}8\cdot10^{-6}$ g, which corresponds to $4\cdot10^{-8}$ mole.

EXPERIMENTAL

The spectra were taken on a Varian MAT-311 mass spectrometer with a MS-100 data-processing system at an energy of the ionizing electrons of 70 eV, a temperature for the evaporation of the sample of 180°C, and a temperature of the ionization chamber of 200°C. In the DADI spectra the mass of each daughter ion was calculated from the formula:

$$m_2 = m_1\frac{E_1}{E_0}$$

where m_2 is the mass of the daughter ion; m_1 mass of the mother ion; E_1, voltage of the energy analyzer at which m_2 is recorded; and E_0, initial value of the voltage of the energy analyzer at which m_1 is recorded.

The values of E_0 and E_1 were measured with a digital voltmeter.

Academician A. S. Sadykov, Prof. Kh. A. Aslanov, and V. B. Leont'ev took part in the discussion of the experimental results.

SUMMARY

1. The DADI spectra of adenine, guanine, uracil, thymine, cytosine, and the minor base of DNA 5-methylcytosine have been studied. It has been shown that the samples of nucleic acid bases investigated are characterized by a specific pattern of DADI spectra reflecting the nature of the molecular ion which is due to the structure of the given base and, in some cases, even the tautomeric state of the molecular ion.

2. The possibility has been shown of using the results obtained for the quantitative determination of 5-methylcytosine in a mixture of DNA bases.

LITERATURE CITED

1. J. L. Wilbbers, Anal. Biochem., 51, 542 (1973).
2. A. M. Lawson, R. N. Stillwell, M. M. Tacker, K. Tsubojama, and J. A. McCloskey, J. Am. Chem. Soc., 94, 1014 (1971).
3. S. J. Show, D. M. Desiderio, T. Tsubojama, and J. A. McCloskey, J. Am. Chem. Soc., 92, 2510 (1970).
4. J. M. Rise and G. O. Dudec, J. Am. Chem. Soc., 89, 2719 (1967).
5. D. H. Smith, C. Djerassi, K. H. Maurer, and U. Rapp, J. Am. Chem. Soc., 96, 3482 (1974).
6. R. Razakov, Kh. A. Aslanov, A. S. Sadykov, and B. V. Rozynov, Bioorgan. Khim., 3, 600 (1977).
7. J. Deutch, A. Razin, and J. Sedat, Anal. Biochem., 72, 586 (1976).