PMR STUDY OF N^6,N^6-DIMETHYLADENOSINE CONFORMATIONS UNDER HIGH PRESSURE

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1. Introduction

High hydrostatic pressure affects in various ways the reactivity and the stability of biogenic macromolecules [1]. Pressures of several hundred bars reduce or increase enzymic activity [2–4]. Proteins are usually irreversibly denaturated by pressures in excess of 1 kbar. On the other hand, calf thymus DNA is stabilized by increased pressure [5].

In general, an increase in pressure shifts an equilibrium in the direction reducing the sum of the partial molar volumes. In a macromolecule, such volume changes result from several interactions acting toward or again each other. It is, therefore, necessary to study the influence of pressure on the conformational equilibria of smaller molecules. In continuation of our studies on the conformations of the common purine(β)-nucleosides [6–8] and their analogs [9–11], we have analysed the pressure dependence of the conformational equilibria of N^6,N^6-dimethyladenosine.

2. Materials and methods

N^6,N^6-dimethyladenosine was purchased from Pharma Waldhof (Mannheim). The deuterated solvents are commercial products from Sharp and Dohme (München). In order to lower the freezing point of the aqueous solutions 20% w/w methanol-d_4 were added to the deuterium oxide solutions of N^6,N^6-dimethyladenosine. Also, 10% w/w deuterium oxide and 20% w/w methanol-d_4 were added to acetone-d_6 to increase the solubility of N^6,N^6-dimethyladenosine in acetone-d_6. The acetone solutions contained 2% tetramethylsilane and the aqueous solutions an equal concentration of 2,2-dimethyl-2-silapentane-5-sulfonic acid sodium salt as internal standards. The spectra were taken off 0.25 molal solutions with a Varian XL-100-15-FT-Spectrometer connected to a Varian 620-1-100 16 K computer.

The spectra were taken in a high pressure cell described earlier [12]. Temperatures were monitored to ± 0.5°C. By variation of the temperature, the coalescence of the two *N*-methyl signals was determined in 500 bar intervals. The inversion rate k_c at the coalescence temperature and ΔG^* the free activation enthalpy, were calculated by standard procedures [13]. The inversion rates, given in fig.3 were calculated assuming Arrhenius behaviour. From the slope of the lines the activation volume ΔV^* was determined:

$$\frac{d \ln k}{dp} = -\frac{\Delta V^*}{RT}$$

3. Results

3.1. *Chemical shifts*

The effect of hydrostatic pressure on the chemical shifts of the two base protons H(C2) and H(C8) is shown in fig.1. In aqueous solutions, one observes with increasing pressure an upfield shift of both protons, especially marked for H(C2). It is known that, in aqueous solutions of purine-nucleosides, the proton resonances of the base are shifted to higher fields as the solute concentration increases [14]. This behaviour is due to the self-association of the nucleoside by vertical stacking of the bases [14].

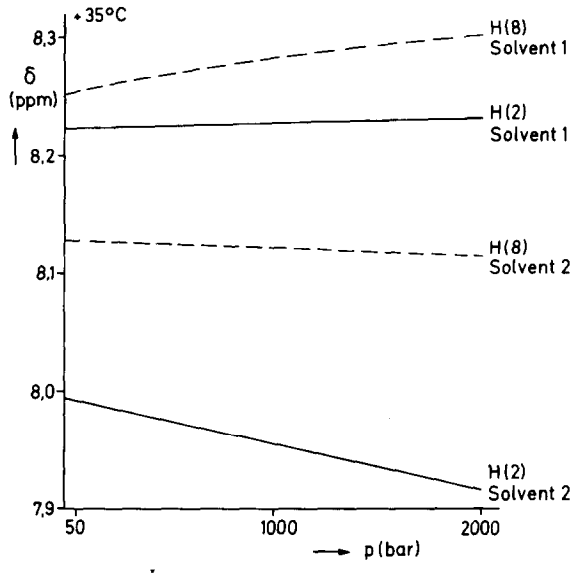

Fig.1. Pressure dependence of the chemical shifts of the two purine protons of N^6,N^6-dimethyladenosine at +35°C. Solvent 1: 70% w/w acetone-d_6, 20% w/w methanol-d_4, and 10% w/w deuterium oxide. Solvent 2: 80% w/w deuterium oxide and 20% w/w methanol-d_4.

Thus, application of pressure favours self-association of the bases by vertical stacking. Similar, but less pronounced, pressure effects were found for aqueous solutions of 9-methyl-purine [12]. This is because methylation of amino groups of purine-nucleosides enhances the self-association in aqueous solutions [14,15]. In acetone solutions, where self-association is absent, pressure has no effect on the chemical shift of H(C2) and increases slightly that of H(C8).

3.2. *Ribose conformations*

An approximate analysis of the vicinal proton–proton coupling constants shows that pressure has no influence on the populations of the three rotamers of the exocyclic-group CH_2-OH bonded to C5′ in both solutions. From the vicinal proton–proton coupling constant between the ribose protons H(C1′) and H(C2′) ($J^{1'2'}$), it is possible to obtain a good approximation of the position of the $N \rightleftarrows S$ equilibrium of the ribose [16]. In acetone solutions, there is no pressure dependence of $J^{1'2'}$ which is equal to 6.4 Hz. However, in aqueous solutions, a pressure dependence appears. The coupling constant $J^{1'2'}$ decreases from 6.1 Hz at atmospheric pressure to 5.7 Hz at 2000 bar. This behaviour of $J^{1'2'}$ shows that the population of the *S* state decreases in favour of the *N* state under application of pressure. A similar behaviour is observed in the concentration dependence of adenosine-5′-monophosphate [17] and in the influence of dimerization of dinucleoside phosphates [18]. This behaviour is taken to mean that, in the stacked conformations of ribonucleotides, the ribose part favours the *N* state [18]. Since it is known that pressure enhances self-association, it can be concluded that in the stacked molecules the *N* state is favoured because the stacked molecule in *N* possesses a lower partial molar volume than the *S* state.

3.3. *Hindered internal rotation of the dimethylamino group*

NMR evidence for hindered rotation about the exocyclic C(6)N(6) bond has been presented for dimethyladenine in nonaqueous solvents [19] and aqueous solutions [20]. This was attributed to the partial double bond character of the exocyclic C–N bond [19]. In fig.2 are shown the methyl resonances

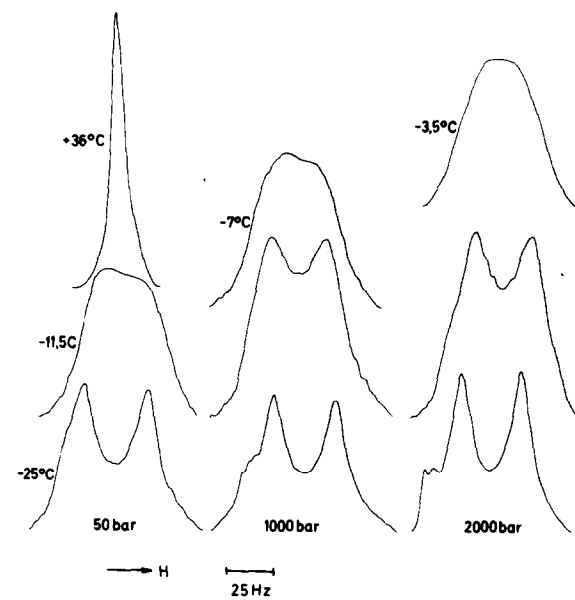

Fig.2. Pressure and temperature dependence of the PMR spectra of the *N*-methyl groups in M_2^6Ado (solvent: 80% w/w deuterium oxide and 20% w/w methanol-d_4).

Table 1
Experimental results (T_c and Δ_ν) for the two solvents used (see fig.1)

	P (bar)	T_c (°C)	Δ_ν (Hz)
Solvent 1	50	−14.5	49.5
	1000	−11.0	48.8
	2000	− 7.5	47.5
Solvent 2	50	−11.5	32.5
	1000	− 7.0	32.5
	2000	− 3.5	32.0

of dimethyladenosine as a function of pressure and temperature. It can be observed that the temperature has to be increased by about 4°C per 1000 bar pressure increase in order to obtain the coalescence temperature. This means that the internal rotation of the dimethylamino group is slowed down by the pressure. In table 1 the coalescence temperatures, T_c, are given and the difference in resonance frequency of the two methyl groups under very slow rotation, Δ_ν. The dependence on pressure of the inversion rate of the dimethylamino group in the two solvents used is shown in fig.3 for two temperatures. The influence of the solvent is clearly seen in this figure. The rates of internal rotation are 2.5 times lower in aqueous than in acetone solutions. One likely explanation might be self-association, since it has already been remarked that vertical stacking interferes with internal rotation of the dimethylamino group in N^6,N^6-dimethyladenosine [20]. Also, it shows that a pressure of 2 kbar reduces the inversion rate to half of its value at atmospheric pressure in both solvents. The activation volumes obtained from these curves are (7.7 ± 0.8) $cm^3 \cdot mol^{-1}$ in acetone and (8.9 ± 0.9) $cm^3 \cdot mol^{-1}$ in D_2O.

These values are surprisingly similar to those obtained for several amides, which range between 8.5 and 10.3 $cm^3 \cdot mol^{-1}$ [21]. However, the variation in ΔG^* is much stronger, up to 30% [21]. The variation in ΔG^* can be explained by the influence of the various substituents on the double bond character of the C–N bond. We tried therefore to explain the constancy of the activation volume by geometric or steric arguments. In order to rotate

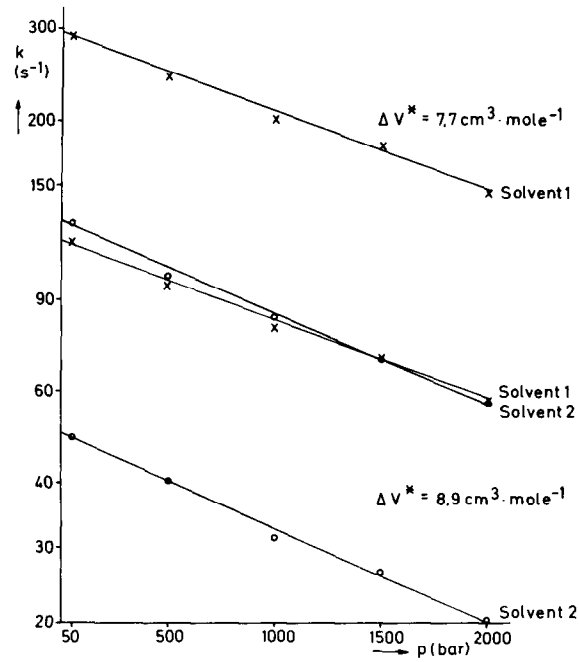

Fig.3. Inversion rate of the dimethylamino group as a function of pressure in the two solvent mixtures (see fig.1).

around the C–N bond, the dimethylamino group requires that a toroidal volume be kept free of other molecules. The difference between the volume of the torus and the volume of the dimethylamino group is 60 Å^3 per molecule, which is more than the 14–17 Å^3 per molecule observed. For a second model, we consider a molecule where the methyl groups are coplanar with the purine ring. To allow rotation of the methyl groups, the solvent molecules located on either side of the purine ring in the vicinity of the dimethylamino group have to be dislodged of a certain volume. This volume is equal to 16 Å^3 per molecule if one assumes a closed-packed solvent consisting of spheres with the van der Waals radius of methyl groups. The agreement between this calculated value and the experimental one might be artificial. However, if this model does describe the real process, it predicts that ΔV^* should be rather insensitive to the size of the surrounding molecules. Hence, the activation volume should vary little with solvent, as is observed experimentally.

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