BASE STACKING INTERACTIONS OF L-HISTIDINE AND THYROTROPIN-RELEASING HORMONE WITH ADENOSINE-5′-MONOPHOSPHATE

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Received 11 November 1977

1. Introduction

The selective recognition of particular mononucleotides by certain proteins or enzymes must involve direct interactions between the nucleotides and individual amino acyl residues. The participation of amino acyl side-chains in localized electrostatic or hydrogen bonding interactions with nucleic acid components is much better understood [1,2] than their involvement in delocalized phenomena such as hydrophobic or base stacking interactions. Tryptophan and related indole derivatives [3–9] as well as tyrosine and related phenol derivatives [10–12] have been shown to interact with purine nucleotides in aqueous solution. However, there is no experimental evidence that histidine is able to undergo such stacking, although the complex involvement of histidine in proton transfer reactions is well investigated [13].

We report here a ^{1}H NMR study which confirms the involvement of histidine and of the histidine-containing tripeptide TRH in a specific complex with the base moiety of adenine nucleotides.

2. Materials and methods

All biochemicals were commercial products of the highest available purity. Adenosine-5′-monophosphate (disodium salt) was from Boehringer Mannheim and L-histidine and TRH from Sigma GmbH München.

The ^{1}H NMR spectra were taken with a Varian XL-100-12 spectrometer operating at 100 MHz in the pulsed Fourier transform mode at a probe temperature of 35°C. The digital resolution using a Varian 620 C computer was 0.24 Hz. Samples were prepared in 99.8% D_2O at p*D* 7.4 (meter reading), containing 10 mM potassium phosphate buffer, 1 mM NaCl, 50 μM EDTA and 1 mM *t*-butanol. Individual 5′-AMP concentrations were determined from the ultraviolet A_{260}, using ϵ_{max} 15 400. Between 10 and 100 transients were accumulated depending on the dilution of individual samples. Chemical shifts were measured and are reported relative to *t*-butanol as internal reference.

3. Results and discussion

In order to investigate the binding of histidine to excess 5′-AMP, the self-association of the latter had to be evaluated first under identical conditions. Over the concentration range investigated, up to 500 mM, the resonances of the non-exchangeable protons A(8), A(2) and A(1′) show a large upfield shift with increasing concentration. These concentration-dependent chemical shift data were analysed by the isodesmic model [5] to yield the following microscopic association constants for the self-association of 5′-AMP through base stacking:

Abbreviations: TRH, thyrotropin-releasing hormone; H, histidine (H) protons, A, adenosine (A) protons, e.g., H(2) versus A(2)

$K^{35°C} = 1.75\ M^{-1}$ from the A(8) proton;
$K^{35°C} = 1.69\ M^{-1}$ from the A(2) proton;
$K^{35°C} = 1.72\ M^{-1}$ from the A(1') proton.

The corresponding average apparent equilibrium constant is $\bar{K}^{35°C} = 1.72\ M^{-1}$, a value slightly lower than that obtained at 30.5°C [14] from the data [15] ($\bar{K}^{30.5°C} = 2.19\ M^{-1}$). The derived standard Gibbs energy change for the 5'-AMP self-association is $-1.4\ kJ.mol^{-1}$. The calculated association shifts for a number of 5'-AMP oligomers are given in table 1.

In contrast to 5'-AMP, histidine alone shows no tendency for self-association through base stacking in aqueous solutions at neutral pH. However, if the ^1H NMR chemical shifts of 50 mM L-histidine are monitored in the presence of an increasing amount of 5'-AMP, an upfield shift is observed for the H(2) and H(4) protons of the imidazole moiety. While most intermolecular interactions lead to downfield shifts, this upfield shift is indicative for a stacking type interaction with the base moiety of 5'-AMP [16].

Table 1
Association shifts for different 5'-AMP oligomers in aqueous solutions

	$\delta\sigma_{A_2}$	$\delta\sigma_{A_3}$	$\delta\sigma_{A_4}$	$\delta\sigma_{A_6}$
H(1')	14.2	17.3	18.9	20.4
H(8)	18.5	22.2	24.1	25.9
H(2)	43.1	55.1	61.1	67.1

The shifts are in Hz upfield from the corresponding monomer shifts at infinite dilution

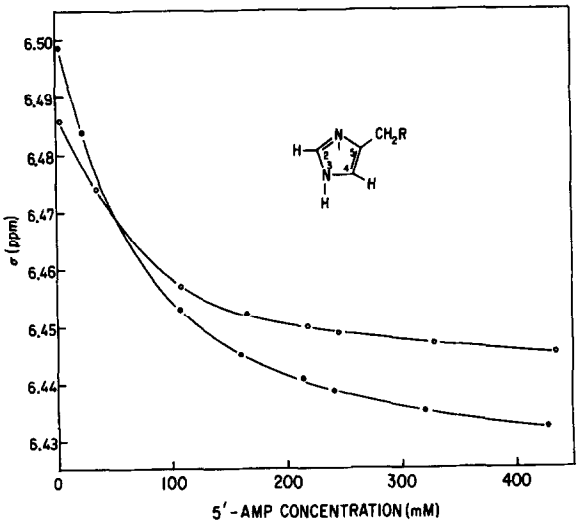

Fig.1. Variation of the H(2) chemical shift of 50 mM histidine (○) and 25 mM TRH (●) as induced by complex formation with 5'-AMP. The chemical shifts are in ppm relative to internal *t*-butanol.

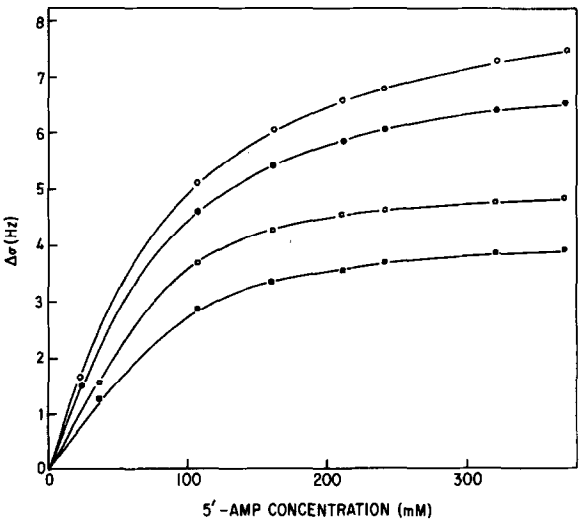

Fig.2. Profile of the chemical shift differences between 'free' and 5'-AMP 'complexed' histidine: (■) H(2); (□) H(4); TRH (●) H(2); (○) H(4).

Figure 1 shows the dependence on 5'-AMP concentration of the H(2) chemical shifts in histidine and TRH. The dependence is quite steep up to 100 mM and begins to level off at 5'-AMP concentrations above 400 mM. A similar concentration dependence is shown by the H(4) ring protons, but not by the methylene protons, showing that the imidazole ring of histidine is primarily implicated in this interaction.

Figure 2 displays the chemical shift differences between 'free' and 5'-AMP 'complexed' histidine or the histidyl residue of TRH for both ring protons as a function of the 5'-AMP concentration. The similarities between the behaviour of histidine and the histidine-containing tripeptide TRH show that it is the imidazole moiety of the latter which is involved in this interaction.

Since the rate of exchange between complexed and free histidine is rapid on the ^1H NMR time scale, only a single resonance is observed for each proton and the measured chemical shifts are the weighted

average of chemical shifts for the free and complexed molecules. A simple Scatchard plot of these data proved unsatisfactory; however, the ^{1}H NMR data could be analysed by the mathematical model derived [17] and extended [5] to the case where the compound in excess self-associates strongly. This is illustrated in fig.3 for the complex between TRH and 5′-AMP. According to this model, a plot of $\Delta\sigma/\delta\sigma$ versus/ $\Delta\sigma/\delta\sigma_{A_2}$ ($\Delta\sigma$ being the difference between the individual histidine proton chemical shifts in the presence and absence of 5′-AMP, $\delta\sigma$ the difference between the 5′-AMP proton chemical shifts at a given concentration and at infinite dilution and $\delta\sigma_{A_2}$ a complex chemical shift difference accounting for the 5′-AMP self-association) yielded straight lines for all the six binary combinations considered, from which the microscopic association constants in table 2 were calculated.

Table 2
Microscopic equilibrium constants (K_c) and standard Gibbs energy changes (ΔG°) for the complex formation of L-histidine and TRH with excess 5′-AMP

	K_c		$\bar{K}_c$	ΔG°
	H(2)	H(4)	(M^{-1})	(kJ.mol^{-1})
L-Histidine	14.4	18.5	16.4	−7.2
TRH	9.7	10.8	10.2	−6.0

Both the equilibrium constants and the standard Gibbs energy changes reported here are based on concentrations as recommended [18] by the ICSU Interunion Commission on Biothermodynamics

The data in table 2 show that the degree of self-association of 5′-AMP is actually small compared to that of complex formation with histidine; the apparent association constant for the former ($\bar{K}$ = 16.4 M^{-1}) is about an order of magnitude larger than that of 5′-AMP self-association ($\bar{K}$ = 1.72 M^{-1}). This is also revealed by the corresponding difference in binding strength as shown by the standard Gibbs energy changes of −1.4 kJ.mol^{-1} for self-association versus that of −7.2 kJ.mol^{-1} for complex formation.

An upfield shift of the A(2) proton resonances of 5′-AMP in the complex with pancreatic ribonuclease A, along with a concomitant upfield shift of the well separated H(2) signal of the histidyl-119 residue is reported [19]. In view of the results of our present investigation this observation could well be explained by a stacking interaction indicating that even the histidine residue in a large protein is able to engage in complex formation with individual adenine nucleotides.

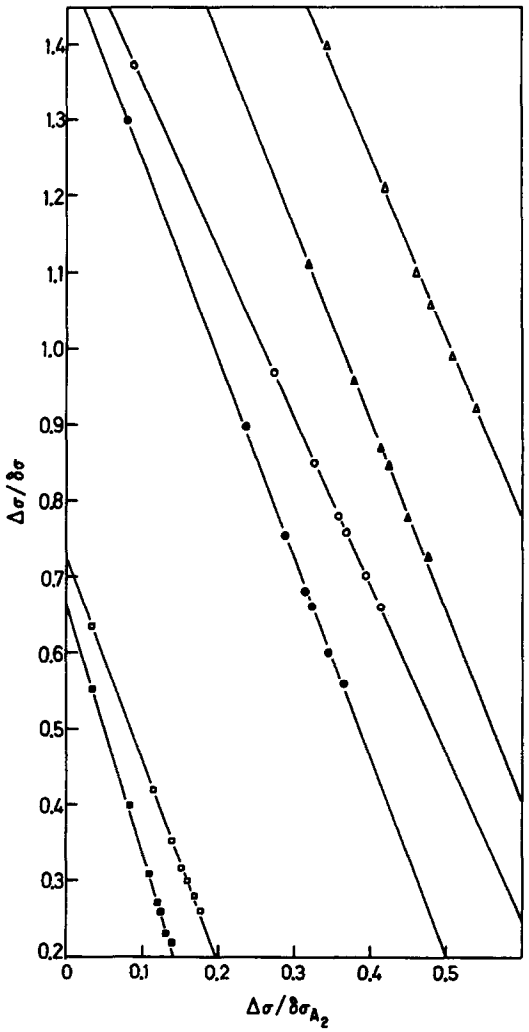

Fig.3. Evaluation of the constants for complex formation between 25 mM TRH and excess 5′-AMP according to the formalism [5]. Shown is the interaction of the two histidine protons H(2) and H(4) with three different adenosine protons A(2), A(8) and A(1′). The individual combinations are: (■) A(2) H(2); (□) A(2) H(4); (●) A(8) H(2); (○) A(8) H(4); (▲) A(1′) H(2); (△) A(1′) H(4).

Acknowledgements

The authors gratefully acknowledge support by the Deutsche Forschungsgemeinschaft through grant Ma 726/1 and stimulating discussions with Dr H. O. Kalinowski (Giessen) and Dr I. C. P. Smith (Ottawa).

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