

# Complex Formation of Carnosine with Purine Nucleotides in Aqueous Solution

Klaus J. Neurohr and Henry H. Mantsch

Division of Chemistry, National Research Council of Canada, Ottawa, Canada K1A 0R6

Z. Naturforsch. **35 c**, 557–561 (1980); received December 10, 1979

Carnosine, Purine Nucleotides, Proton NMR, Base Stacking, Equilibrium Constants

This  $^1\text{H}$ -NMR study provides experimental evidence for an intermolecular interaction between the dipeptide carnosine ( $\beta$ -alanyl-L-histidine) and the purine nucleoside 5'-monophosphates 5'-AMP, 5'-IMP and 5'-GMP. From the observed upfield shifts of the purine nucleotide and imidazole proton resonances it is concluded that the interaction is of the stacking type and that it involves the purine base of the nucleotide and the histidine moiety of carnosine. Apparent microscopic equilibrium constants and complex shifts are obtained with a microscopic model, which considers the formation of both 1:1 and 1:2 complexes. The stacking pattern for complex formation between the histidine moiety of carnosine and the adenine moiety of 5'-AMP is constructed by fitting the experimental 5'-AMP complex shifts to the calculated isoshielding contours for histidine.

## Introduction

The specific recognition of particular base sequences in nucleic acids by proteins is one of the central problems in molecular biology. It has been suggested that aromatic amino acid side chains of proteins may play a special role in this recognition phenomenon through stacking interaction with nucleic acid bases [1]. Indeed, it has been shown that the simple tripeptide Lys-Trp-Lys is able to discriminate between double- and single stranded nucleic acids by a stronger binding to the latter [2] and moreover, that the binding is sequence dependent [3]. CD studies have suggested that the binding of gene 5 protein of phage fd and of gene 32 protein of phage T4 to single stranded nucleic acids involves an intercalation of tyrosine residues between nucleic acid bases [4, 5]. With stacking interactions favoured in single stranded over double stranded DNA, this type of interaction could be important for anchoring proteins to single stranded regions in a nucleic acid.

While the stacking association of tryptophan and tyrosine, or their derivatives, with nucleotides or nucleic acids has been extensively studied by different experimental techniques [6–10], we have recently shown that the aromatic amino acid histidine itself, as well as the histidine moiety in thyrotropin-releasing hormone (TRH), is also able to interact specifically via base stacking with nucleic acid bases [11]. In order to investigate the stacking

properties of histidine further, we have studied the intermolecular interaction of the dipeptide carnosine ( $\beta$ -alanyl-L-histidine) with purine nucleotides in aqueous solution.

## Experimental procedures

### Materials and sample preparation

All biochemicals were commercial products of the highest available purity. L-Carnosine was from Sigma, adenosine 5'-monophosphate, inosine 5'-monophosphate and guanosine 5'-monophosphate (disodium salts), were from Boehringer, Mannheim. Nuclear magnetic resonance samples were prepared in 99.8%  $\text{D}_2\text{O}$  at pH 7.4 (meter reading), containing 10 mM phosphate buffer, 1 mM NaCl, 50  $\mu\text{M}$  EDTA and 1 mM *tert*-butanol. The carnosine concentration varied from about 50 to 500 mM, while the concentration of the nucleotide component was kept constant at 50 mM.

### Nuclear magnetic resonance measurements

The  $^1\text{H}$  NMR spectra were obtained in the Fourier transform mode at 100 MHz on a Varian XL-100/12 spectrometer, digital resolution 0.0025 ppm, 90° pulse time 20  $\mu\text{s}$ . The probe temperature was 30 °C. All samples were examined in 5 mm tubes, the sample volume was 0.4 ml. Typically three, but at least two independent measurements were made for each sample. All proton chemical shifts were measured with respect to *tert*-butanol as internal reference. For the evaluation of binding parameters a PDP8/I computer was used.

Reprint requests to Prof. Dr. H. H. Mantsch. Issued as NRCC No. 18180.

0341-0382/80/0700-0557 \$ 01.00/0



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

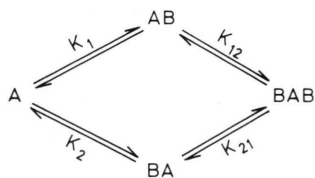
This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

## Theory

The interaction between the aromatic purine base of a nucleotide and the side chain of an aromatic amino acid can be conveniently studied by  $^1\text{H}$  NMR. The aromatic protons of molecules engaged in a base stacked complex experience an increased shielding due to the large magnetic anisotropy of aromatic ring systems and therefore show upfield shifts in the  $^1\text{H}$  NMR spectrum. If the rate of exchange between free and complexed molecules is rapid on the proton NMR time scale, only a single resonance is observed for each proton and the measured chemical shift is the weighted average of chemical shifts for the free and complexed molecules. The concentration dependence of the magnitude of these upfield shifts for different protons of the same molecule, in principle, allows the determination of association constants, and the stereochemistry of the complexes.

$^1\text{H}$  NMR chemical shift data have usually been analyzed according to relationships derived for 1:1 complex formation, however, complexes with a stoichiometry different from 1:1 should be considered as well. For the analysis of chemical shift data in the present work, we have therefore used the microscopic model of Deranleau [12], which takes into account the formation of both 1:1 and 1:2 complexes, as represented by the following scheme:



where A represents the dilute component, to which two molecules of the excess component B can bind. In our case, A stands for the purine nucleotide, *i. e.* 5'-AMP, 5'-IMP or 5'-GMP, and B for carnosine. The individual *K*'s represent the microscopic equilibrium constants for the association processes shown. It is implied that there are two microscopically distinct species AB and BA, each of which can bind another molecule B. It is further assumed that the two binding sides on molecule A are independent, with  $K_{12} = K_2$  and  $K_{21} = K_1$  and that they are characterized by the same microscopic constant, *i. e.*,  $K_1 = K_2 = K$  and the same complex shift  $\Delta\sigma_{AB} = \Delta\sigma_{BA}$ . Thus, for the overall equilibrium

$A \rightleftharpoons AB \rightleftharpoons AB_2$ , one obtains the following equation:

$$\Delta\sigma/C = -K(\Delta\sigma - \Delta\sigma_{BAB}) \quad (1)$$

where  $\Delta\sigma = \sigma - \sigma_0$ , and represents the difference in chemical shift of a proton of the dilute component A in the presence ( $\sigma$ ) and absence ( $\sigma_0$ ) of the excess component B. *K* is the microscopic equilibrium constant and *C* the total concentration of the excess component B. A plot of  $\Delta\sigma/C$  versus  $\Delta\sigma$  will then give straight lines, from the slope of which the apparent microscopic equilibrium constant *K* can be obtained, while the *x*-intercept yields the complex shift  $\Delta\sigma_{BAB} = 2\Delta\sigma_{AB}$ .

## Results

### Complex formation between carnosine and adenosine 5'-monophosphate

We were particularly interested in the interaction of carnosine with 5'-AMP under physiological conditions and have therefore carried out such studies at neutral pH and 30 °C. To probe this interaction, we have used the two adenine proton resonances H2 and H8, as well as the ribose proton resonance H1', which have been unequivocally assigned in the literature [13].

We have monitored these three proton resonances in the presence of an increasing amount of carnosine. As shown in Fig. 1., upfield shifts were observed for all three resonances. Upfield shifts (*i. e.*, increased shielding) were also observed for the H2 and H4 imidazole proton resonances of carnosine in the presence of 5'-AMP. The association therefore must be due to base stacking between the purine base of the nucleotide and the histidine moiety of the dipeptide. Carnosine alone did not show any concentration-dependent variation of the chemical shift for the imidazole proton resonances H2 and H4. Thus, under the experimental conditions used in the present study, the dipeptide carnosine does not self-associate via base stacking.

The  $^1\text{H}$  NMR chemical shift data were then analyzed according to equation (1). Fig. 2 shows a plot of  $\Delta\sigma/C$  versus  $\Delta\sigma$  for the three 5'-AMP protons H2, H8 and H1'. The apparent microscopic equilibrium constants and complex shifts characterizing the interaction of carnosine with 5'-AMP were obtained from the slopes and the *x*-axis intercepts of the linear traces in Fig. 1 and are listed in Table I. Note that the complex shifts in this table are for 1:2

Table I. Apparent microscopic equilibrium constants (in  $M^{-1}$ ), standard Gibbs energy changes (in  $kJ\ mol^{-1}$ ) and complex shifts (in Hz) for the association of carnosine with purine nucleotides <sup>a</sup>.

	Nucleotide		
	5'-AMP	5'-IMP	5'-GMP
$K(H2)$	3.3	3.5	—
$K(H8)$	5.2	4.2	6.8
$K(H1')$	4.7	5.6	4.5
$\bar{K}$	4.4	4.4	5.7
$\Delta\bar{G}^0$	-3.7	-3.7	-4.4
$\Delta\sigma_{BAB}(H2)$	7.5	5.5	—
$\Delta\sigma_{BAB}(H8)$	7.5	3.9	3.9
$\Delta\sigma_{BAB}(H1')$	4.6	2.2	2.3

<sup>a</sup> All data refer to 30 °C and pH 7.4. Estimated errors in  $K$  are  $\pm 10\%$ , barred values indicate proton averaged constants.

complexes with  $\Delta\sigma_{BAB} = 2\Delta\sigma_{AB}$ , and that the complex shifts in a 1:1 complex are half those in a 1:2 complex. The equilibrium constants obtained from the individual 5'-AMP protons are slightly different, a phenomenon observed in most NMR studies dealing with stacking interactions. This may arise from the fact that equilibrium constants derived from NMR data are determined for different positions within the molecule, rather than for the molecule as a whole.

#### Complex formation of carnosine with inosine 5'-monophosphate and guanosine 5'-monophosphate

We have also investigated the association of carnosine with these two purine nucleotides, and have carried out such studies under conditions identical to those used in the experiment with 5'-AMP. As a probe of the intermolecular interaction, we have used the H8, H2 and H1' proton resonances of 5'-IMP and the H8 and H1' proton resonances of 5'-GMP, respectively, which also have been assigned unequivocally in the literature [14]. These proton resonances were monitored in 50 mM 5'-IMP and 50 mM 5'-GMP in the presence of an increasing amount of carnosine. Upfield shifts were observed in all cases, indicating that these two purine nucleotides are also able to engage in base stacking associations with the histidine moiety of carnosine. The <sup>1</sup>H NMR chemical shift data were analyzed according to equation (1). The apparent microscopic equilibrium

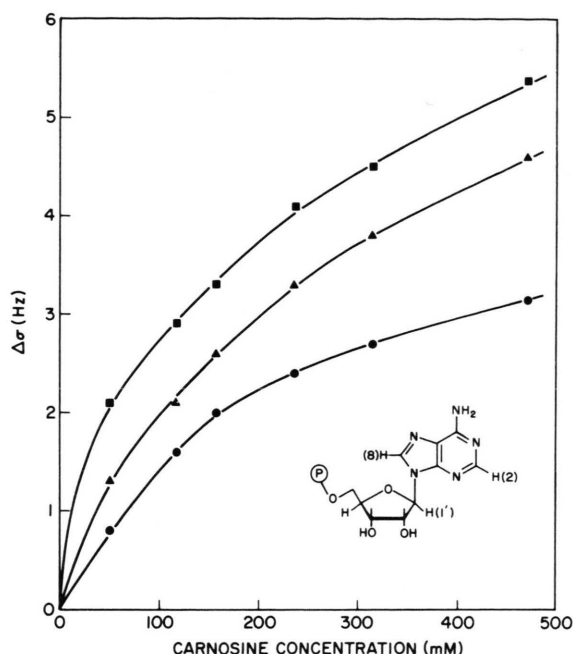


Fig. 1. <sup>1</sup>H NMR chemical shift differences ( $\Delta\sigma$ ) for the H2 (■), H8 (▲) and H1' (●) protons of 50 mM 5'-AMP (see inset) as a function of carnosine concentration, at 30 °C and pH 7.4.

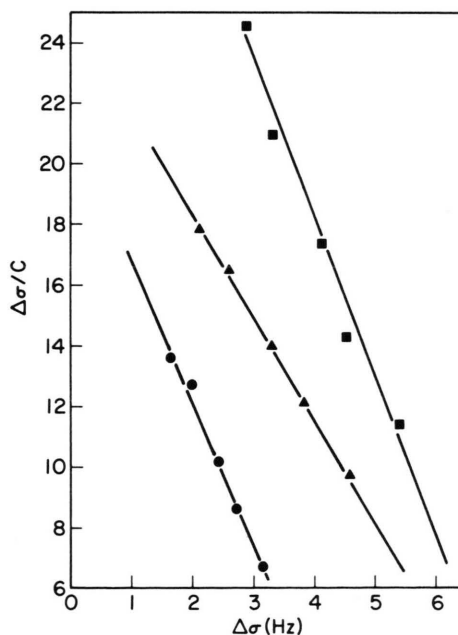


Fig. 2. Graphical evaluation, according to Eqn (1), of the stacking association between carnosine and 5'-AMP at 30 °C and pH 7.4. Shown are the plots obtained for the three 5'-AMP protons H2 (■), H8 (▲) and H1' (●). Apparent microscopic equilibrium constants are obtained from the slopes of the linear traces, while the x-intercepts yield the corresponding complex shifts.

constants and complex shifts, obtained from linear plots similar to those in Fig. 2, are listed in Table I along with the corresponding Gibbs energy changes.

## Discussion

The results of the present investigation show that the dipeptide carnosine interacts with purine nucleotides in aqueous solution. The upfield shifts observed for the nucleotide and imidazole proton resonances indicate that the association is due to base stacking, and that it involves the aromatic purine base of the nucleotide and the histidine moiety of carnosine. The data in Table I reveal that although the individual microscopic equilibrium constants are slightly different, if averaged over all the observed protons, the complex formation of carnosine with 5'-AMP and 5'-IMP is characterized by the same equilibrium constant and Gibbs energy change, while the association with 5'-GMP is found to be slightly stronger. In all three purine nucleotides the coupling constant  $^3J_{1'2'}$  was found to decrease with increasing carnosine concentration. This phenomenon has also been observed for the complex formation of tryptamine with 5'-AMP and 5'-GMP [15] and for the self-association of nucleotides [16, 17], and is due to a shift in the  $^2E$ - $^3E$  sugar pucker equilibrium towards the  $^3E$  form with increasing base stacking [18].

The  $^1H$  NMR complex shifts, as listed in Table I, for the individual nucleotide protons allow, in principle, the determination of the stereochemistry of the complexes, if these values are fitted to theoretically computed isoshielding contours. Isoshielding contours for the imidazole moiety of histidine, based on the ring current effect, have been computed by Giessner-Prettre and Pullman [19]. The shielding effects of the imidazole moiety are rather small due to the low intensity of the imidazole ring current and the small size of the ring. Fig. 3 shows a model of the stacking pattern for the interaction of the histidine moiety of carnosine with the adenine moiety of 5'-AMP, as obtained from fitting the complex shifts of the protons H2 and H8 in Table I to the isoshielding contours of Giessner-Prettre and Pullman [19]. Because of the different sizes of the overlapping imidazole and adenine rings, the adenine ring protons are located far from the imidazole shielding centre, that is they lie within the third isoshielding contour, which explains the rather small upfield

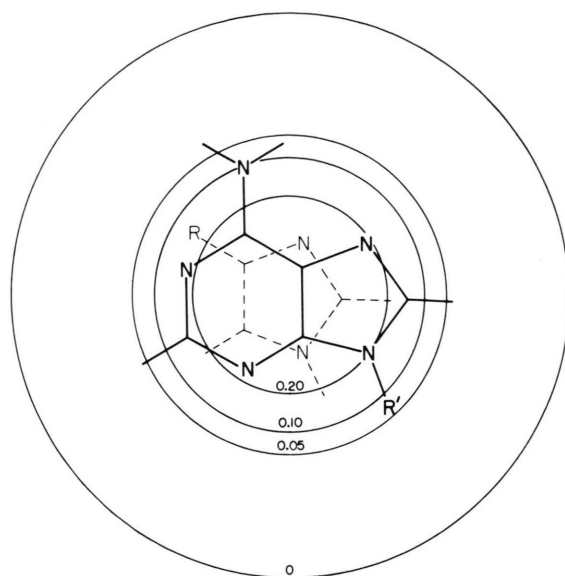


Fig. 3. Model for the stacking pattern of 5'-AMP (solid line, R' = phosphoribosyl) with carnosine (broken line, R =  $\beta$ -alanine), as obtained from fitting the experimental complex shifts for 5'-AMP to the isoshielding contours of the histidine ring in carnosine. The isoshielding contours were computed by Giessner-Prettre and Pullman [19] and represent the shielding (in ppm) experienced by the base protons of the adenine moiety of 5'-AMP due to the ring current effect of the histidine moiety of carnosine in a plane 0.34 nm above and below the histidine ring.

shifts observed for the nucleotide protons in the presence of carnosine. Fig. 3 displays a 1:1 complex, however, a 1:2 complex is obtained if a further carnosine molecule is added to the stack onto the other side of the adenine moiety.

The amino acid histidine is known to be involved in a variety of biological functions, including such functions as a hydrogen bonding agent, a ligand in histidine-metal complexes in certain metalloproteins, or an acid base catalyst in proton transfer reactions in the active site of many enzymes [20]. The present study shows that the histidine moiety in a peptide, such as carnosine, is also able to engage in vertical base stacking interactions with nucleic acid bases, emphasizing the remarkable versatility of this amino acid.

Carnosine is present in the olfactory epithelium and olfactory bulb in higher concentrations than anywhere else in the mammalian central nervous system [21]. Despite extensive studies [22, 23], its function in the olfactory pathway is still unknown. However, there is strong biochemical evidence, suggesting that carnosine may be the principal neuro-

transmitter released by olfactory nerve terminals [24]. The binding of carnosine to bovine serum albumin, as studied by  $^1\text{H}$  NMR spectroscopy [23], revealed the histidinyl side chain of the dipeptide to be responsible for primary recognition by the binding site. Thus, besides other interactions, a stacking interaction of the histidine moiety of carnosine may

be involved in its specific recognition by the receptor.

#### *Acknowledgement*

The authors are grateful to the Deutsche Akademische Austauschdienst for a fellowship to K. J. N.

- [1] C. Hélène, *Stud. Biophys.* **57**, 211–222 (1976).
- [2] J. J. Toulmé and C. Hélène, *J. Biol. Chem.* **252**, 244–249 (1977).
- [3] J. C. Maurizot, G. Boubault, and C. Hélène, *Biochemistry* **17**, 2096–2101 (1978).
- [4] R. A. Anderson and J. E. Coleman, *Biochemistry* **14**, 5485–5491 (1975).
- [5] R. A. Anderson, Y. Nakashima, and J. E. Coleman, *Biochemistry* **14**, 907–917 (1975).
- [6] J.-L. Dimicoli and C. Hélène, *Biochemistry* **13**, 713–723; 724–730 (1974).
- [7] V. Wray and K. G. Wagner, *Z. Naturforsch.* **32 c**, 315–320 (1977).
- [8] N. H. Kolodny, A. C. Neville, D. L. Coleman, and P. C. Zamecnik, *Biopolymers* **16**, 259–275 (1977).
- [9] J. Reuben, *FEBS Lett.* **94**, 20–24 (1978).
- [10] R. Mayer, F. Toulmé, Th. Montenay-Garestier, and C. Hélène, *J. Biol. Chem.* **254**, 75–82 (1979).
- [11] H. H. Mantsch and K. J. Neurohr, *FEBS Lett.* **86**, 57–60 (1978).
- [12] D. A. Deranleau, *J. Am. Chem. Soc.* **91**, 4044–4049; 4050–4054 (1969).
- [13] I. Feldman and R. P. Agarwal, *J. Am. Chem. Soc.* **90**, 7329–7333 (1968).
- [14] C. D. Jardetzky and O. Jardetzky, *J. Am. Chem. Soc.* **82**, 222–229 (1960).
- [15] K. G. Wagner and R. Lawaczeck, *J. Magnetic Res.* **8**, 164–174 (1972).
- [16] F. E. Evans and R. H. Sarma, *Biopolymers* **13**, 2117–2132 (1974).
- [17] F. E. Evans and R. H. Sarma, *Nature* **263**, 567–572 (1976).
- [18] R. H. Sarma and S. S. Danyluk, *Int. J. Quantum Chem.* **4**, 269–276 (1977).
- [19] C. Giessner-Prettre and B. Pullman, *J. Theoret. Biol.* **31**, 287–294 (1971).
- [20] F. Schneider, *Angew. Chemie* **90**, 616–625 (1978).
- [21] D. Ferriero and F. L. Margolis, *Brain Res.* **94**, 75–86 (1975).
- [22] C. E. Brown, F. L. Margolis, T. H. Williams, R. G. Pitcher, and G. Elgar, *Neurochem. Res.* **2**, 555–579 (1977).
- [23] C. E. Brown, F. L. Margolis, T. H. Williams, R. G. Pitcher, and G. J. Elgar, *Arch. Biochem. Biophys.* **193**, 529–542 (1979).
- [24] F. L. Margolis, *Trends in Neurosciences*, **1**, 42–44 (1978).