CHEMICALLY-SELECTIVE NUCLEOTIDE–AMINO ACID INTERACTIONS IN AQUEOUS SOLUTION. A PMR STUDY

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1. Introduction

The Watson-Crick scheme of base pairing by hydrogen bonding provides, inter alia, an acceptable basis not only for rationalization of the fact that nucleic acids code for their own replication but also for understanding the origin and evolution of the coding process. Nucleic acids are also intimately involved in protein synthesis and, by means of the genetic code, determine the amino acid sequence of proteins. However, the basic physico-chemical interaction due to which nucleotides could carry information regarding amino acids, thereby originating the processes that led to the evolution of the presently known elaborate system of coding and translation, has remained elusive [1]. The most attractive among several hypotheses involves chemically selective association between nucleotides and amino acids. It has been formulated and discussed in detail, e.g., by Woese et al., [2] and by Crick [3]. Recent simulation studies of models of protein synthesis systems have led to the conclusion that even small preferences in the interaction of nucleotides with different amino acids could be sufficient to lead eventually to an accurate translation system [4]. However, attempts to detect specificity in such interactions have been inconclusive [2,5–7]. A probable reason for this seems to be the relatively weak association in aqueous solution. Thus, equilibrium constants (at pH 7, 25°C) of 8.7 M^{-1} and 5.6 M^{-1} have been reported for the association of L-tryptophan methyl ester with AMP obtained, respectively, by ultraviolet spectroscopy [8] and by NMR [9]. A stronger interaction (45 M^{-1} at pH 9.9, 28°C) has been reported for the system AMP–tyrosine methyl ether [10].

The NMR approach used in [9,10] is based on the fact that aromatic amino acids as well as nucleotides give rise (upon complex formation) to ring-current-induced chemical shifts. However, these shifts are greatly attenuated when the observed nucleus is away from the effective center of the ring system. Thus, e.g., the maximal effect of AMP on the chemical shifts of H_2 and H_4 of histidine is only 5 Hz at 100 MHz [11]. The effects of aromatic amino acids on nucleotide protons are expected to be larger [9,12]. While NMR spectroscopy has repeatedly been shown to be among the best-suited methods for studying weak interactions in solution, there seem to be two main obstacles on the way to a systematic study of the interaction between nucleotides and amino acids in aqueous solution.

(i) Only aromatic amino acids give rise to measurable effects, but there are only three amino acids (of interest) of this kind. One way to circumvent this problem is to use the interaction of an aromatic amino acid as a probe for the measurement of that of the non-aromatic ones by competition.

(ii) Because the association is relatively weak, relatively large concentrations are required for its proper measurement [13], while on the other hand the solubility of the aromatic amino acids is relatively low.

It becomes imperative, therefore, to employ a highly soluble derivative of the amino acid, e.g., the methyl ester (ME) or the amide. Reported in this article are the results of an exploratory study along these lines.

The methyl ester of tryptophan was selected as the aromatic amino acid derivative. It has been shown that L-TrpME forms a 1:1 complex with AMP in aqueous solution and that the shifts induced in the proton spectrum of AMP, particularly those in the H_2 resonance, are large enough to be measured accurately with an iron-core NMR spectrometer [9].

2. Materials and methods

Nucleoside monophosphates and amino acid methyl esters were products of Sigma Chemical Co., St Louis, MO, and were used without further treatment. Samples were made up in 50 mM phosphate buffer, pH (meter reading) 7.2, in D_2O containing 0.025% v/v *tert*-butanol, the methyl resonance of which served as an internal standard for chemical shift measurements. PMR spectra were recorded at 100 MHz and an ambient probe temperature of 30 ± 1°C with a Varian XL-100 spectrometer equipped with Nicolet Fourier transform accessories. Titrations were carried out at constant nucleotide concentrations of 5 mM for AMP and GMP, 10 mM for CMP and UMP. Amino acid ME concentrations varied between 40 and 500 mM. Chemical shift differences are expressed in Hz relative to a solution containing the nucleotide alone.

2.1. *Methods of data analysis*

The dissociation constant for the equilibrium:

$$A + N \rightleftarrows AN \tag{1}$$

with A denoting the amino acid methyl ester and N the nucleotide is:

$$K_A = [A][N]/[AN] \tag{2}$$

If experiments are carried out in a large excess of A the assumption $[A] \approx A_t$ can be made, where the subscript t denotes total concentration. Making the substitution:

$$[N] = N_t - [AN] \tag{3}$$

and rearranging one obtains the Scatchard equation:

$$\frac{[AN]}{A_t N_t} = \frac{1}{K_A} - \frac{[AN]}{K_A N_t} \tag{4}$$

Upon complex formation and under conditions of rapid chemical exchange between the complexed and uncomplexed forms, the chemical shift observed in the resonance of N (relative to the uncomplexed state) is given by:

$$\delta = [AN]\ \Delta/N_t \tag{5}$$

where Δ is the shift of the complex (relative to the uncomplexed state). Combining eq. (4) and eq. (5) one obtains:

$$\delta/A_t = \Delta/K_A - \delta/K_A \tag{6}$$

Thus a plot of δ/A_t versus δ should be linear with a slope of $1/K_A$ and an intercept on the abscissa of Δ.

With a competing equilibrium of the type:

$$B + N \rightleftarrows BN \tag{7}$$

$$K_B = [B][N]/[BN] \tag{8}$$

one has now:

$$[N] = N_t - [AN] - [BN]$$

$$= N_t - [AN] - B_t[N]/K_B$$

and therefore:

$$[N] = \frac{N_t - [AN]}{1 + B_t/K_B} \tag{9}$$

where the assumption $[B] \approx B_t$ has been made. Upon combining eq. (2), eq. (9) and eq. (5) one obtains:

$$\delta = \frac{\Delta}{1 + K_A\,(1 + B_t/K_B)/A_t} \tag{10}$$

or the more suitable for analysis form:

$$\delta B_t = A_t\, K_B\, \Delta/K_A - \delta K_B\,(1 + A_t/K_A) \tag{11}$$

The measurement of δ at fixed A_t and N_t and varying

B_t will permit the evaluation of K_B from a plot of δB_t versus δ, provided K_A is known. It is instructive to consider also eq. (10), which shows that δ will decrease with increasing B_t and more so for smaller values of K_B, provided the ratio B_t/K_B is not much smaller than unity. This is the basis of the approach for measuring dissociation constants by competition. It requires of course that the chemical shift of the BN complex is the same as that of pure N.

3. Results

Upfield shifts of the AMP H_2, H_8, and H_1 resonances were observed as anticipated [9] upon titrating a 5 mM AMP solution with L-TrpME. The chemical shift change of the H_2 resonance is plotted according to eq. (6) in fig.1. A very good linear relationship between δ/A_t and δ is obtained indicating that the assumptions made in deriving eq. (6) hold. The values of the parameters obtained from the analysis of the plot are summarized in table 1. The uncertainties were estimated from the standard deviation and the range of the saturation factor $[AN]/N_t$ using the relative errors computed by Deranleau (cf. fig.1 in [13]). The results thus obtained are in good agreement with values in the literature [8,9]. Both a smaller slope and a smaller intercept on the abscissa are obtained with D-TrpME (cf. fig.1). The value of Δ as compared to that obtained with L-TrpME is barely outside the calculated uncertainty (see table 1), however, the difference in the dissociation constants appears to be more significant.

Table 1
Dissociation constants and chemical shift changes for nucleotide–amino acid methyl ester complexes

AA ME	Nucl.	K (mM)	Proton	Δ (Hz)
L-Trp	AMP	120 ± 7	H_2	65 ± 2
D-Trp	AMP	154 ± 13	H_2	60 ± 2.5
L-Trp	GMP	191 ± 10	H_8	49 ± 1.5
L-Trp	CMP	408 ± 18	H_6	62 ± 3
L-Trp	UMP	439 ± 24	H_6	76 ± 4
L-Met	AMP	325 ± 60	–	–
L-Ser	AMP	850 ± 110	–	–

The results obtained with 5 mM GMP, 10 mM CMP, and 10 mM UMP are plotted in fig.2. Again good linear relationships between δ/A_t and δ are obtained. The constants obtained from the data

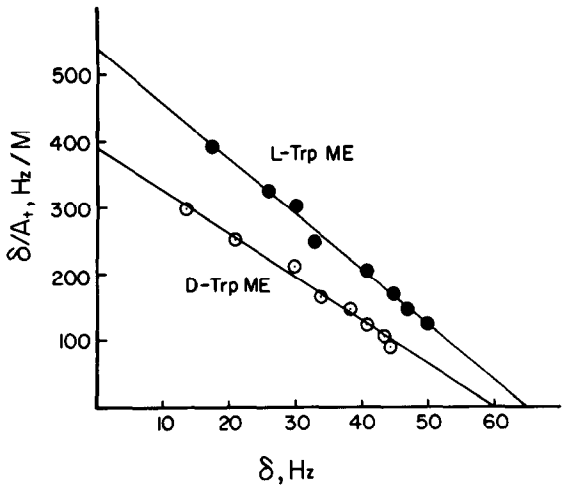

Fig.1. Plots according to eq. (6) of the chemical shift changes induced by L-TrpME (filled symbols) and D-TrpME (open symbols) in the H_2 resonance of AMP.

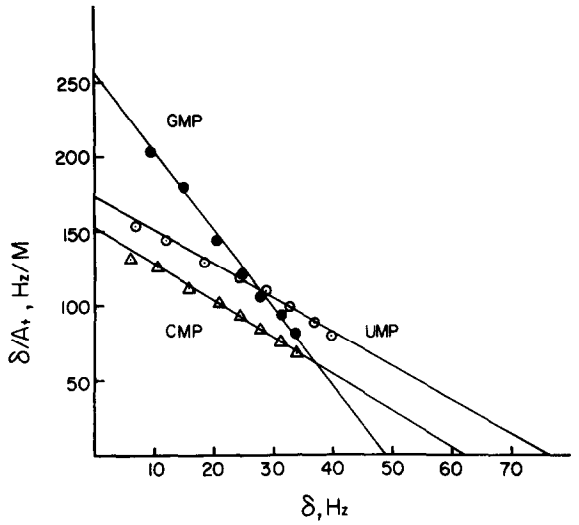

Fig.2. Plots according to eq. (6) of the chemical shift changes induced by L-TrpME in the H_8 resonance of GMP (filled circles), the H_6 resonance of UMP (open circles), and the H_6 resonance of CMP (triangles).

analysis are summarized in table 1. A clear trend of the dissociation constants of nucleotide–L-TrpME complexes is observed:

$$AMP < GMP < CMP \approx UMP$$

Values of similar magnitude have been reported for the interaction of nucleotides and tryptamine [14–16].

Competition experiments were carried out with L-MetME and L-SerME. Solutions containing 5 mM AMP and 100 mM L-TrpME were titrated with each of the methyl esters and the change in the chemical shift of H_2 of AMP monitored. No chemical shift changes were observed in the absence of L-TrpME. The results are presented in fig.3. It is seen that L-MetME produces larger changes than L-SerME indicating (cf. eq. (10)) stronger interaction. The results were analyzed according to eq. (11). The resulting parameters (see table 1) were used to calculate the curves drawn in fig.3. While the standard deviations are 1.02 Hz for L-MetME and 0.58 Hz for L-SerME, the larger relative errors given are due to the fact that the saturation factor $[BN]/N_t$ spans a relatively narrow range: 0.15–0.43 for L-MetME and 0.06–0.25 for L-SerME. Yet the differences in dissociation constants are well outside the experimental uncertainties and the trend:

$$L\text{-}TrpME < L\text{-}MetME < L\text{-}SerME$$

is established.

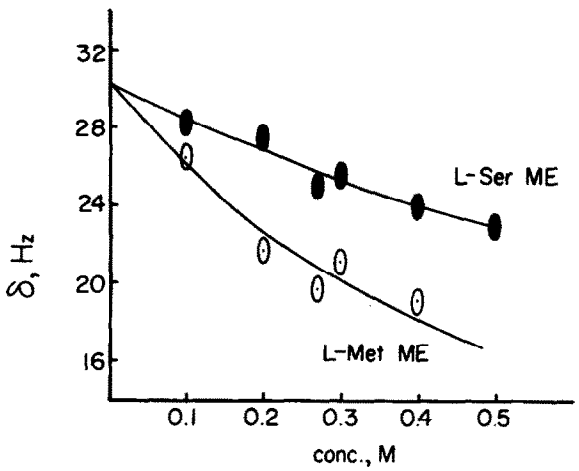

Fig.3. The chemical shift changes induced by increasing concentrations of L-MetME (open symbols) and L-SerME (filled symbols) in the H_2 resonance of AMP in solutions containing 5 mM AMP and 100 mM L-TrpME. The curves are calculated with eq. (10) and the corresponding parameters given in table 1.

4. Discussion

The chemical selectivity in the association of L-TrpME with nucleoside monophosphates is clearly demonstrated by the present results. The main interaction responsible for this selectivity is likely to be the ring–ring stacking as has been discussed in detail by Lawaczeck and Wagner [12]. Hydrophobic interactions are probably also important particularly in determining the selectivity in the association of a nucleotide with different amino acids and with a different enantiomer of the same amino acid.

The suitability of the approach whereby the association of an aromatic amino acid derivative with a nucleotide is used to monitor by competition the interaction of a non-aromatic acid has been established. Using this approach it is shown that nucleotide–amino acid interactions are chemically selective. The differences observed here span a range of almost an order of magnitude. It remains to be determined by a more detailed simulation study whether differences of this magnitude are sufficient to have served as the origin for a coding and translation system whereby nucleotides could carry information on amino acids, at the beginning in the form of nucleotide–amino acid complexes. Correlations with the present genetic code may be attempted only when data for a larger number of amino acids become available. It may be noteworthy at this time that L-Trp and L-Met, which interact strongly with AMP are coded for by one codon, whereas the weakly interacting L-Ser has a degenerate code composed of 6 triplets.

The difference observed here between L- and D-Trp requires further experimental verification. The implication of such a difference is, inter alia, that in the course of chemical evolution it would have been sufficient to have an enantiomeric preference for

either an amino acid or for the ribose moiety of nucleotides, which could then propagate via the stereochemically selective nucleotide–amino acid interactions.

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