

# Selective Binding of Amino Acid Residues to tRNA Molecules Detected by Anticodon-Anticodon Interactions

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Anticodon-anticodon pairing of complementary tRNA's has been studied by fluorescence temperature jump measurements in the presence of different ligands as an approach for the evaluation of ligand binding to tRNA. This procedure is particularly useful for ligands which do not show spectroscopic changes upon binding, but affect the pairing potential of anticodons. Addition of phenylalanine-, tyrosine- and tryptophan-amide leads to a substantial decrease of the  $\text{tRNA}^{\text{Phe}} \cdot \text{tRNA}^{\text{Glu}}$  pairing constant  $K_p$ , whereas  $K_p$  remains almost unaffected by addition of leucine amide and increases upon addition of glycine amide. The effects observed for the aromatic amino acid amides can be described quantitatively by a site binding model with preferential binding of the amides to  $\text{tRNA}^{\text{Phe}}$ . The binding constants evaluated according to this model (Phe-amide  $120 \text{ M}^{-1}$ , Tyr-amide  $160 \text{ M}^{-1}$  and Trp-amide  $580 \text{ M}^{-1}$ ) are consistent with values obtained independently by fluorescence titrations with  $\text{tRNA}^{\text{Phe}}$ . Selective binding of these amino acid residues to  $\text{tRNA}^{\text{Phe}}$  is deduced from the observed concentration dependence, which is not compatible with a corresponding binding process to  $\text{tRNA}^{\text{Glu}}$ . Addition of glutamic acid diamide induces an increase of the  $\text{tRNA}^{\text{Phe}} \cdot \text{tRNA}^{\text{Glu}}$  pairing constant, which is however equivalent to that observed for  $\text{tRNA}^{\text{Phe}} \cdot \text{tRNA}^{\text{Lys}}$  pairing and thus does not demonstrate a selective binding to  $\text{tRNA}^{\text{Glu}}$ . The pairing of  $\text{tRNA}^{\text{Phe}}$  with  $\text{tRNA}^{\text{Glu}}$  is strongly enhanced by addition of  $\text{Mg}^{2+}$  or spermine. Evaluation of the  $\text{Mg}^{2+}$  data by a site model leads to constants of  $360 \text{ M}^{-1}$  for the binding of  $\text{Mg}^{2+}$  to monomer tRNA and  $3000 \text{ M}^{-1}$  for the binding of  $\text{Mg}^{2+}$  to the  $\text{tRNA}^{\text{Phe}} \cdot \text{tRNA}^{\text{Glu}}$  dimer. A comparison of the enhanced pairing observed in the presence of  $\text{Mg}^{2+}$ , which is known to induce a 3'-stack conformation of the anticodon loop, with the reduced pairing affinity observed in the presence of aromatic amino acid amides suggests induction of a 5'-stack conformation by the latter ligands.

## Introduction

An essential step in the processing of the genetic information is the assignment of amino acids to codon triplets. The mechanism used *in vivo* for an accurate assignment is very complex and involves many components. Due to this complexity it is hardly possible to decide from the mechanism, whether the assignment originated from some stereochemical relationship or from a frozen accident [1–2]. A stereochemical relationship would certainly enhance the probability for self-organization of a simple living system [3]. Furthermore stereochemical relationships are of great interest for an understanding of the specificity of protein nucleic acid interactions.

From a large number of investigations it is well known that the affinity of amino acids and their simple derivatives to various oligo- and polynucleotides is relatively low [4–8]. Nevertheless, it has been possible to demonstrate a characteristic dependence of the affinity between amino acid residues and polynucleotides upon the amino acid side chain [8, 9], which can be related to the structure of the genetic code. These results have been encouraging for further investigations using tRNA molecules, since it is at least conceivable that tRNA adaptors have binding sites for selection of amino acid residues. Our first experiments using  $\text{tRNA}^{\text{Phe}}$  proved to be very successful and demonstrated a selective interaction of aromatic amino acid residues to this adaptor [10]. The selective interaction could be demonstrated relatively easily owing to the strong fluorescence of the Wye base located at the anticodon of  $\text{tRNA}^{\text{Phe}}$  and its sensitivity to changes resulting from ligand binding. Unfortunately other tRNA molecules do not have any spectroscopic label of similar sensitivity, and thus a convincing demonstration of another case of a selective interaction proves to be much more dif-

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ficult. Since the introduction of artificial spectroscopic labels may induce unpredictable changes, we prefer to exploit some inherent property of tRNA molecules. A unique property of tRNA molecules is the strong base pairing affinity of their anticodons, which has been demonstrated most convincingly for complexes between complementary anticodons [11, 12]. These interactions can be characterized relatively easily by temperature jump experiments [12, 13], which provide both thermodynamic and kinetic parameters with high accuracy and do not require as large amounts of tRNA as other conventional methods. We have used this procedure to determine equilibrium parameters of complementary anticodon-anticodon complexes in the presence of various amino acid residues and evaluated from the dependence of this equilibrium the coupled binding equilibrium of amino acid residues to tRNA molecules. In our previous investigation [10] we did not detect any substantial binding of free aromatic amino acids to tRNA<sup>Phe</sup>, whereas binding of the corresponding amide derivatives could be detected relatively easily. The amide derivatives were used for various reasons. First of all, the amide function does not introduce any major modification of the molecular character. The positive charge at the amino group resulting from the amide function supports interactions with polynucleotides, but does not dominate these interactions to the extent which is typical for peptide models favored in the past. Finally, the charged amino group is equivalent to that of activated esters serving as precursor for loading of tRNA adaptors. Thus, amino acid amides are appropriate models to test the affinity of amino acid side chains to tRNA adaptors.

## Materials and Methods

tRNA<sup>Phe</sup> from yeast and tRNA<sub>2</sub><sup>Glu</sup> from *E. coli* were from Boehringer-Mannheim. A partially purified preparation of tRNA<sup>Lys</sup> from *E. coli* was generously presented by H. Sternbach; the final purification of this sample was achieved by high performance liquid chromatography on ODS Hypersil coated with trioctylmethylammonium chloride according to the procedure of Bischoff *et al.* [14] with extensive advice given by L. W. McLaughlin. The tRNA's were dialyzed first against 100 mM NaClO<sub>4</sub>, 20 mM EDTA pH 7, then against water and finally against several changes of a standard buffer contain-

ing 50 mM NaClO<sub>4</sub>, 80 mM Tris-cacodylate pH 6.5, 0.1 mM EDTA.

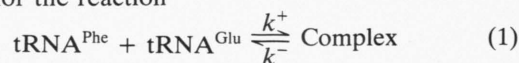
The amides of L-phenylalanine, L-tyrosine, L-tryptophan, L-leucine and glycine as well as the diamide of L-glutamic acid were from Bachem and were purified as described. The concentrations of the aromatic amides were determined by absorbance measurements using the following absorbance coefficients given in units of [cm<sup>-1</sup> M<sup>-1</sup>]: Phe-amide 206 at 258 nm; Tyr-amide 1400 at 274 nm and Trp-amide 5330 at 280 nm. The concentrations of Gly-amide, Leu-amide and of Glu-diamide were determined by ninhydrine according to the procedure of Moore and Stein [15].

Chemical relaxation was measured by a fluorescence temperature jump instrument first described by Rigler *et al.* [16] and then further developed by Rabl [17]. The fluorescence was excited at 313 nm and the emitted light was selected by Schott GG385 cut off filters. The temperature of the samples was controlled by a Pt100 inserted in the upper electrode of the cell. The relaxation curves were stored on a Biomation 1010 transient recorder and transmitted to the Univac 1108 of the Gesellschaft für wissenschaftliche Datenverarbeitung, Göttingen, for evaluation by an exponential fitting procedure.

## Results

### *Aromatic amino acid amides affect the interaction between tRNA<sup>Phe</sup> and tRNA<sup>Glu</sup>*

As first demonstrated by Eisinger [11], tRNA<sup>Phe</sup> forms a very strong complex with the complementary tRNA<sup>Glu</sup>. The formation of this complex is accompanied by an almost complete quenching of the fluorescence emitted by the Wye base, located at the anticodon of tRNA<sup>Phe</sup>. When a temperature jump is applied to a solution containing the tRNA<sup>Phe</sup>-tRNA<sup>Glu</sup> complex, dissociation of this complex is indicated by an increase of the fluorescence intensity associated with a relaxation time constant in the msec-time range (under usual conditions, *cf.* Fig. 1). Measurements of the relaxation time constant  $\tau$  as a function of the reactant concentrations provide the equilibrium constant together with the rate constants [13] for the reaction



according to

$$1/\tau = k^+ (\bar{c}_{\text{tRNA}^{\text{Phe}}} + \bar{c}_{\text{tRNA}^{\text{Glu}}}) + k^- \quad (2)$$

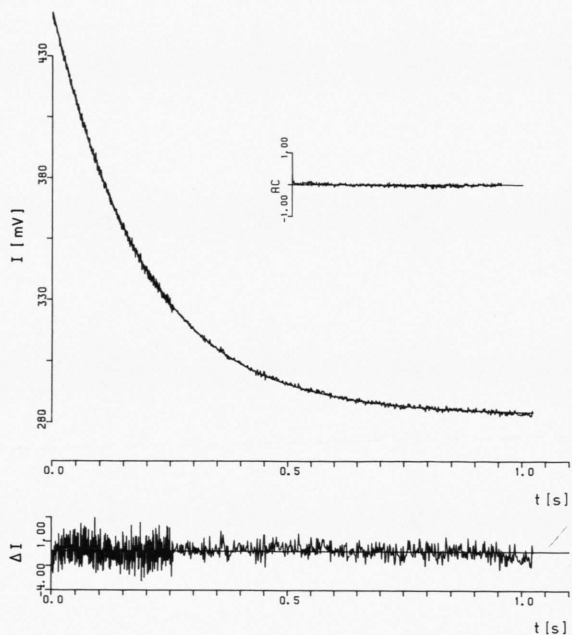


Fig. 1. Chemical relaxation of anticodon-anticodon interactions between tRNA<sup>Phe</sup> and tRNA<sup>Glu</sup> by a temperature jump experiment with fluorescence detection (standard buffer with addition of 5 mM glutamic acid diamide; temperature jump from 6.8 to 9.2 °C; total concentrations of tRNA<sup>Phe</sup>: 8.72 μM and of tRNA<sup>Glu</sup>: 4.25 μM). The lower part of the diagram shows the difference ΔI between the experimental light intensity I and a least squares fit by a single exponential as a function of time t; the insert shows the autocorrelation of the deviation ΔI.

where  $\bar{c}_{\text{tRNA}}^{\text{Phe}}$  and  $\bar{c}_{\text{tRNA}}^{\text{Glu}}$  are the equilibrium concentrations of the reactants;  $k^+$  and  $k^-$  are the rate constants of association and dissociation, respectively. We have determined the  $k^+$  and  $k^-$ -values according to Eqn. (2) by a least squares fit procedure with calculation of the equilibrium concentrations from the  $k^+/k^-$  ratio. In order to reduce potential errors in the evaluation, the total concentration of (the less expensive) tRNA<sup>Phe</sup> was usually higher by a factor of 2 than that of tRNA<sup>Glu</sup>. As shown in Fig. 2, the reaction parameters can be determined by this procedure with a relatively high accuracy.

When the pairing of tRNA<sup>Phe</sup> and tRNA<sup>Glu</sup> is analyzed by this procedure in the presence of a constant excess of *e.g.* 5 mM phenylalanine-amide, the results demonstrate a clear change in the pairing parameters. As shown in Fig. 2, addition of phenylalanine-amide leads to an increase of the dissociation rate and a decrease of the association rate; both effects contribute to a clear decrease of the equilibrium constant. Similar changes are observed upon addition of tyrosine amide and of tryptophan amide. The results are compiled in Table I.

#### *Binding parameters evaluated by a competition model are consistent with results from independent titrations*

The change of the pairing parameters demonstrates a competition of amide binding with the anti-

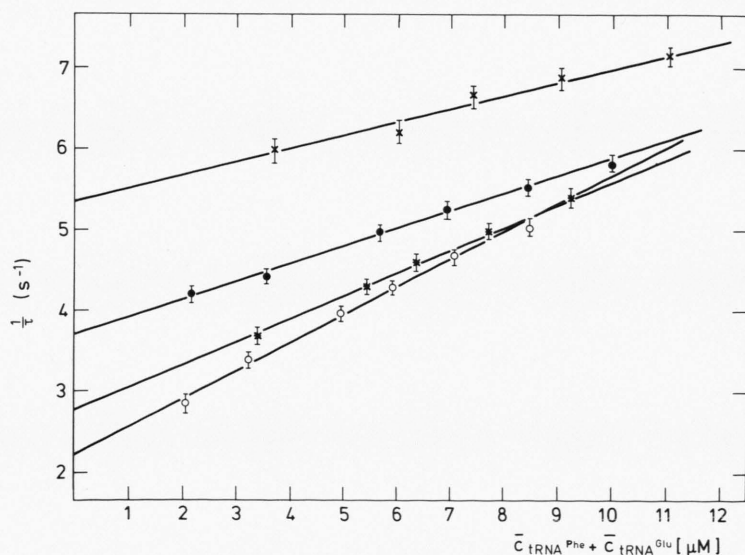
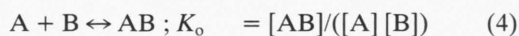


Fig. 2. Reciprocal relaxation time  $1/\tau$  for the tRNA<sup>Phe</sup>-tRNA<sup>Glu</sup> complex as a function of the sum of free tRNA<sup>Phe</sup> and tRNA<sup>Glu</sup> concentrations at phenylalanine-amide concentrations of 0 mM (○), 5 mM (\*), 15 mM (●) and 30 mM (x) (standard buffer, 9.2 °C).

Table I. Thermodynamic and kinetic parameters for the pairing of tRNA<sup>Phe</sup> and tRNA<sup>Glu</sup> in the standard buffer at 9.2 °C in the presence of different ligands of different concentrations.

| Ligand           | Concentration | $K_p$ [M <sup>-1</sup> ] | $k^+$ [M <sup>-1</sup> s <sup>-1</sup> ] | $k^-$ [s <sup>-1</sup> ] |
|------------------|---------------|--------------------------|------------------------------------------|--------------------------|
| Phe-amide        | 0             | $1.56 \times 10^5$       | $3.46 \times 10^5$                       | 2.21                     |
| Phe-amide        | 5             | $1.01 \times 10^5$       | $2.83 \times 10^5$                       | 2.8                      |
| Phe-amide        | 15            | $5.7 \times 10^4$        | $2.1 \times 10^5$                        | 3.8                      |
| Phe-amide        | 30            | $3.0 \times 10^4$        | $1.61 \times 10^5$                       | 5.37                     |
| Tyr-amide        | 5             | $8.33 \times 10^4$       | $2.99 \times 10^5$                       | 3.6                      |
| Tyr-amide        | 15            | $4.28 \times 10^4$       | $1.99 \times 10^5$                       | 4.62                     |
| Tyr-amide        | 30            | $2.91 \times 10^4$       | $2.16 \times 10^5$                       | 7.45                     |
| Tryp-amide       | 2             | $7.22 \times 10^4$       | $2.41 \times 10^5$                       | 3.34                     |
| Tryp-amide       | 5             | $3.04 \times 10^4$       | $1.71 \times 10^5$                       | 5.61                     |
| Gly-amide        | 5             | $1.8 \times 10^5$        | $3.8 \times 10^5$                        | 2.15                     |
| Gly-amide        | 15            | $1.93 \times 10^5$       | $3.21 \times 10^5$                       | 1.66                     |
| Leu-amide        | 5             | $1.46 \times 10^5$       | $3.41 \times 10^5$                       | 2.05                     |
| Leu-amide        | 15            | $1.24 \times 10^5$       | $3.37 \times 10^5$                       | 2.71                     |
| Glu-diamide      | 0             | $1.56 \times 10^5$       | $3.46 \times 10^5$                       | 2.21                     |
| Glu-diamide      | 2             | $1.84 \times 10^5$       | $3.81 \times 10^5$                       | 2.06                     |
| Glu-diamide      | 5             | $1.92 \times 10^5$       | $3.97 \times 10^5$                       | 2.14                     |
| Glu-diamide      | 5             | $1.86 \times 10^5$       | $3.86 \times 10^5$                       | 2.07                     |
| Glu-diamide      | 10            | $2.30 \times 10^5$       | $4.54 \times 10^5$                       | 1.97                     |
| Glu-diamide      | 15            | $2.36 \times 10^5$       | $4.70 \times 10^5$                       | 1.99                     |
| Glu-diamide      | 22            | $2.73 \times 10^5$       | $4.82 \times 10^5$                       | 1.76                     |
| Glu-diamide      | 22            | $2.94 \times 10^5$       | $4.68 \times 10^5$                       | 1.59                     |
| Glu-diamide      | 37            | $2.97 \times 10^5$       | $5.22 \times 10^5$                       | 1.76                     |
| Glu-diamide      | 45.4          | $4.6 \times 10^5$        | $6.14 \times 10^5$                       | 1.33                     |
| Mg <sup>2+</sup> | 0.236         | $4.02 \times 10^5$       | $5.77 \times 10^5$                       | 1.44                     |
| Mg <sup>2+</sup> | 0.9           | $1.11 \times 10^6$       | $9.8 \times 10^5$                        | 0.88                     |
| Mg <sup>2+</sup> | 2.9           | $3.86 \times 10^6$       | $1.7 \times 10^6$                        | 0.44                     |
| Mg <sup>2+</sup> | 5.9           | $5.9 \times 10^6$        | $2.11 \times 10^6$                       | 0.36                     |
| Mg <sup>2+</sup> | 9.9           | $6.6 \times 10^6$        | $2.26 \times 10^6$                       | 0.34                     |
| Spermine         | 0.1           | $2.55 \times 10^6$       | $1.31 \times 10^6$                       | 0.514                    |
| Spermine         | 0.33          | $5.33 \times 10^6$       | $1.99 \times 10^6$                       | 0.372                    |
| Spermine         | 1.00          | $1.80 \times 10^7$       | $2.9 \times 10^6$                        | 0.160                    |
| Spermine         | 3.00          | $1.9 \times 10^7$        | $3.1 \times 10^6$                        | 0.166                    |

codon-anticodon interactions. For a quantitative description of this competition it may be assumed in a first approximation that the amide ligands L preferentially bind to tRNA<sup>Phe</sup>(≡A) resulting in a complex AL, which is characterized by a strongly reduced affinity to tRNA<sup>Glu</sup>(≡B). In this case the system can be described by the reactions



where AB represents the complex between tRNA<sup>Phe</sup> and tRNA<sup>Glu</sup>. Since the ligand L is always in large excess with respect to A and B, the dependence of the apparent pairing constant

$$K_p = \frac{[AB]}{[A + AL][B]} \quad (5)$$

upon the ligand concentration [L] is simply given by

$$\frac{1}{K_p} = \frac{1}{K_o} + \frac{K_{AL}}{K_o} \cdot [L] \quad (6)$$

As shown in Fig. 3, the reciprocal apparent pairing constant  $1/K_p$  is indeed a linear function of the ligand concentration [L] and the binding constant of the ligand  $K_{AL}$  can be conveniently evaluated from the slope. As shown in Table II, the  $K_{AL}$ -values are in satisfactory agreement to those obtained independently by fluorescence titrations of tRNA<sup>Phe</sup> and thus show that the simple model is valid.

A further examination demonstrates that the assumption of ligand binding to only one tRNA species, which was introduced for the derivation of Eqn (6), is already justified by our present data set,

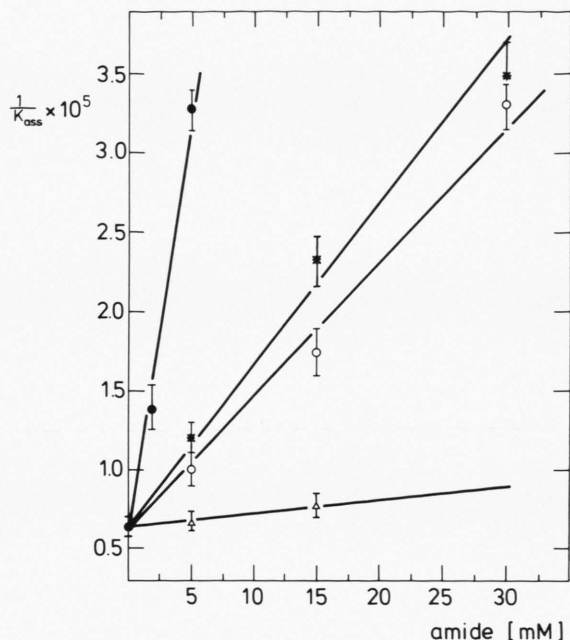


Fig. 3. Reciprocal association constant  $1/K_p$  for the  $\text{tRNA}^{\text{Phe}}\text{--tRNA}^{\text{Glu}}$  complex as a function of amino acid amide concentrations: tryptophan–amide (●), tyrosine–amide (x), phenylalanine–amide (○) and leucine–amide (Δ) in standard buffer at 9.2 °C. The amide binding constants evaluated from the slopes of the straight lines are given in Table I.

without support required from independent measurements. An extended reaction scheme with consideration of ligand binding to species B described by a binding constant  $K_{\text{BL}}$  leads to an equation

$$\frac{1}{K_p} = \frac{1}{K_o} + \frac{K_{\text{AL}} + K_{\text{BL}}}{K_o} \cdot [\text{L}] + \frac{K_{\text{AL}} \cdot K_{\text{BL}}}{K_o} \cdot [\text{L}]^2 \quad (7)$$

If  $K_{\text{BL}}$  would be of similar magnitude as  $K_{\text{AL}}$ , a plot of  $1/K_p$  against  $[\text{L}]$  should not be linear, but should show a deviation resulting from the quadratic term in Eqn (7). Thus our present results demonstrate pref-

erential binding of the aromatic amino acid amides to either  $\text{tRNA}^{\text{Phe}}$  or  $\text{tRNA}^{\text{Glu}}$ . A comparison with previous results (*cf.* Table II) shows that the preferential binding is to  $\text{tRNA}^{\text{Phe}}$ . Our present approach has a clear advantage, since it can be applied to the analysis of ligands, which bind to tRNA molecules without a detectable spectrophotometric response. However, the present approach can only be used when the ligand has some influence on anticodon interactions.

#### *Enhancement of anticodon–anticodon interactions by glutamic acid diamide*

Since selective binding of aromatic amino acids to  $\text{tRNA}^{\text{Phe}}$  can be demonstrated by analysis of anticodon–anticodon interactions, the same procedure may be applied to test for preferential interactions between  $\text{tRNA}^{\text{Glu}}$  and simple derivatives of glutamic acid. Because the negative charges at the carboxyl groups of glutamic acid may decrease the affinity to the negatively charged tRNA, we have used the diamide of glutamic acid, which is positively charged at pH 6.5 of our standard buffer due to protonation of the amino group.

Addition of Glu-diamide induces a clear increase of the  $\text{tRNA}^{\text{Phe}}\text{--tRNA}^{\text{Glu}}$  pairing constant. However, a similar increase of the pairing constant is observed when other comparable cationic ligands – for example glycine amide – are added. Thus, the increase of the pairing constant induced by addition of Glu-diamide seems to be simply due to non-specific shielding of repulsive interactions between  $\text{tRNA}^{\text{Phe}}$  and  $\text{tRNA}^{\text{Glu}}$ . This interpretation is also supported by reference measurements using another pair of tRNA's. The anticodon–anticodon interactions between  $\text{tRNA}^{\text{Phe}}$  and  $\text{tRNA}^{\text{Lys}}$  are also enhanced by addition of Glu-diamide.

A quantitative interpretation of these effects is relatively difficult. Since the increase of the pairing constants upon addition of amide ligands appears to be mainly due to shielding of phosphate repulsions, this increase may be described by some relation from polyelectrolyte theory. According to polyelectrolyte theory [18] the free energy of base pairing increases with the logarithm of the ion concentration. Thus we have plotted the logarithm of the pairing constants as a function of the logarithm of the Glu-diamide concentration (*cf.* Fig. 4). Due to the contribution of the standard buffer and due to the complex structure of the reactant, the slope  $d \log(K)/d \log(c)$  cannot be

Table II. Comparison of ligand binding constants obtained by the anticodon pairing procedure  $K_L$  and by fluorescence titrations with  $\text{tRNA}^{\text{Phe}}$   $K$ . (Standard buffer;  $K$  has been measured at 7.2 °C and  $K_L$  at 9.2 °C.)

|           | $K_L$ [ $\text{M}^{-1}$ ] | $K$ [ $\text{M}^{-1}$ ] |
|-----------|---------------------------|-------------------------|
| Phe-amide | 120                       | 100                     |
| Tyr-amide | 160                       | 110                     |
| Trp-amide | 580                       | 300                     |

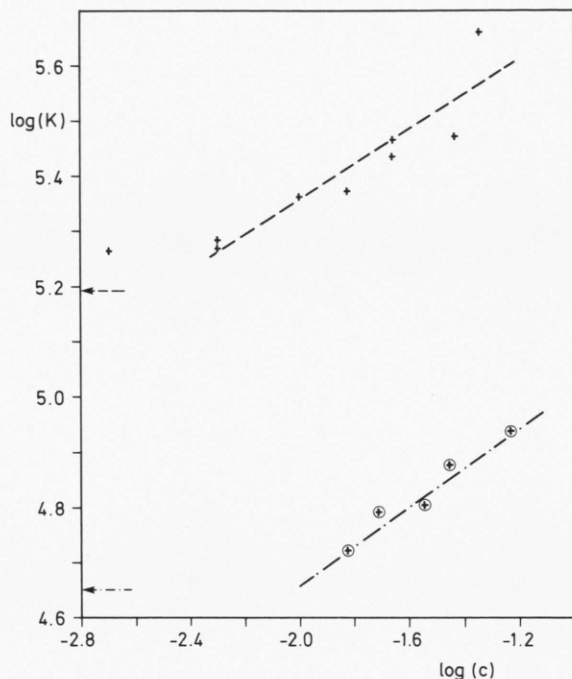


Fig. 4. Logarithm of the pairing constants for  $\text{tRNA}^{\text{Phe}} \cdot \text{tRNA}^{\text{Glu}}$  (+, ---) and for  $\text{tRNA}^{\text{Phe}} \cdot \text{tRNA}^{\text{Lys}}$  (O, -.-) as a function of the logarithm of the Glu-diamide concentration. The arrows show the  $\log(K)$  values observed in the absence of Glu-diamide. The straight lines are evaluated by linear regression of the data obtained at Glu-diamide concentrations  $\geq 5$  mM.

interpreted quantitatively. Nevertheless, it may be concluded that the slopes are very similar for the pairs  $\text{tRNA}^{\text{Phe}} \cdot \text{tRNA}^{\text{Glu}}$  (0.32) and  $\text{tRNA}^{\text{Phe}} \cdot \text{tRNA}^{\text{Lys}}$  (0.35). Thus, our measurements do not provide any evidence for preferential association of Glu-diamide to  $\text{tRNA}^{\text{Glu}}$ .

#### Effect of $\text{Mg}^{2+}$ and spermine on the $\text{tRNA}^{\text{Phe}} \cdot \text{tRNA}^{\text{Glu}}$ pairing constant

The anticodon–anticodon pairing reaction of  $\text{tRNA}^{\text{Phe}} \cdot \text{tRNA}^{\text{Glu}}$  has been measured at different  $\text{Mg}^{2+}$  and spermine concentrations. As should be expected, the pairing constant increases with the concentration of these ions (cf. Fig. 5). Both  $\text{Mg}^{2+}$  and spermine ions have been identified by X-ray analysis at specific sites of tRNA crystals [19–22]. Thus, it is justified to describe our experimental data by a site binding model. For simplicity we assume that a single  $\text{Mg}^{2+}$  or spermine ion is bound to each tRNA molecule. Furthermore we assume that the binding

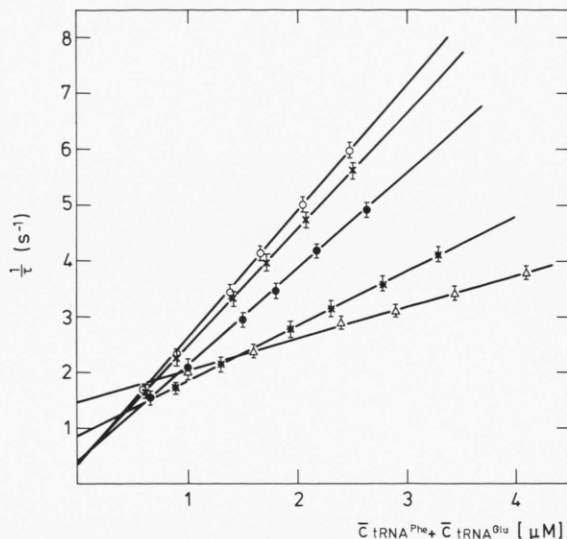


Fig. 5. Reciprocal relaxation time  $1/\tau$  for  $\text{tRNA}^{\text{Phe}} \cdot \text{tRNA}^{\text{Glu}}$  pairing as a function of the sum of free  $\text{tRNA}^{\text{Phe}}$  and  $\text{tRNA}^{\text{Glu}}$  concentrations at  $\text{Mg}(\text{ClO}_4)_2$  concentrations of 0.236 mM ( $\Delta$ ), 0.9 mM (\*), 2.9 mM ( $\bullet$ ), 5.9 mM (x) and 9.9 mM ( $\circ$ ) (standard buffer without EDTA, 9.2 °C).

constant to tRNA in the monomer state  $\alpha$  is equivalent for  $\text{tRNA}^{\text{Phe}}$  and  $\text{tRNA}^{\text{Glu}}$ , whereas a different binding constant  $\beta$  is assigned to these sites in the  $\text{tRNA}^{\text{Phe}} \cdot \text{tRNA}^{\text{Glu}}$  dimer. Although the complete reaction scheme (Fig. 6) looks rather complex, the apparent pairing constant evaluated from our experimental data

$$K_p = \frac{D + DL_1 + DL_2 + DLL}{(A + AL) \cdot (B + BL)} \quad (8)$$



Fig. 6. Formation of dimer D from two different monomers A and B. Binding of ligand L to both monomers is described by a constant  $\alpha$ , whereas ligand binding to the dimer is described by a constant  $\beta$ .

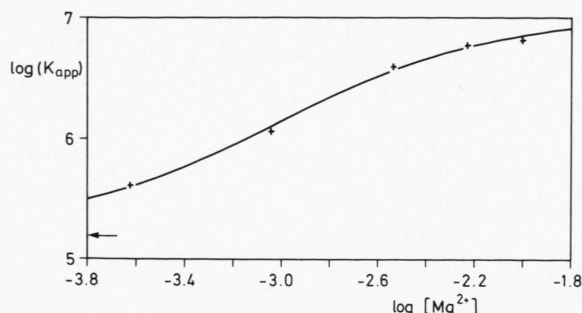


Fig. 7. Pairing constants  $K$  for  $\text{tRNA}^{\text{Phe}} \cdot \text{tRNA}^{\text{Glu}}$  as a function of the  $\text{Mg}(\text{ClO}_4)_2$  concentration  $c$ . The line shows a least squares fit according to Eqn (9):  $K_0 = 1.57 \cdot 10^5 \text{ M}^{-1}$ ,  $\alpha = 360 \text{ M}^{-1}$  and  $\beta = 3000 \text{ M}^{-1}$ .

can be expressed as a relatively simple function of the individual binding constants and the free ligand concentration  $L$

$$K_p = K_0 \cdot \frac{(1 + \beta \cdot L)^2}{(1 + \alpha \cdot L)^2} \quad (9)$$

In the case of  $\text{Mg}^{2+}$  the ligand concentration is much higher than the concentration of tRNA molecules and thus the total  $\text{Mg}^{2+}$  is equivalent to the free ligand concentration. Least squares fitting of the binding constants  $\alpha$  and  $\beta$  to the experimental  $K_p$ -values provided the following parameters:  $\alpha = 360 \text{ M}^{-1}$  and  $\beta = 3000 \text{ M}^{-1}$  (cf. Fig. 7).

A corresponding analysis of the data obtained in the presence of spermine provided the spermine binding constants  $\alpha = 3.3 \times 10^3 \text{ M}^{-1}$  and  $\beta = 4.1 \times 10^4 \text{ M}^{-1}$ .

## Discussion

The potential existence of a stereochemical relationship between amino acids and nucleotides is a fundamental problem in the domain of protein nucleic acid interactions. Experimental investigations are difficult because of low affinities between simple components of proteins and nucleic acids and in many cases also due to the absence of sufficiently large spectroscopic changes upon interaction. Thus, for a serious attempt to solve this problem we have to test also less conventional procedures. The procedure applied in the present investigation uses anticodon-anticodon interactions as a probe for ligand binding to tRNA. These interactions can be analyzed quantitatively at a relatively high accuracy and at a relatively low demand on tRNA quantities [12]. Of course, the procedure can only be successful, when ligand binding affects the pairing potential of the

anticodon. Thus, the absence of changes in the pairing constant does not prove the absence of ligand binding. However, if a ligand induced change of the pairing constant can be demonstrated, a quantitative evaluation of this change in terms of a ligand binding constant is relatively simple and direct, because the binding constants are coupled to each other directly without interference by any spectroscopic parameter.

The binding of amino acids and amino acid amides to tRNA has been analyzed previously [23] by the anticodon-pairing approach. However, the authors of the previous investigation measured their relaxation time constants only at a single tRNA reactant concentration; due to compensation effects these time constants may remain unaffected in spite of clear changes in the rate constants (cf. examples in both Fig. 2 and 5). Furthermore, the experimental conditions used previously [23] do not favour binding of simple ligands like amino acid amides. Our present experiments clearly demonstrate that the amides of phenylalanine, tyrosine and tryptophan strongly bind to native  $\text{tRNA}^{\text{Phe}}$  molecules. These results confirm our previous conclusions on binding of aromatic amino acid residues to  $\text{tRNA}^{\text{Phe}}$  obtained by fluorescence titrations [10]. Our present results provide further evidence on the selective nature of this binding process: according to the concentration dependence of the pairing constants the amides of the aromatic amino acids bind more strongly to  $\text{tRNA}^{\text{Phe}}$  than to  $\text{tRNA}^{\text{Glu}}$ .

Selective binding of aromatic acid residues to  $\text{tRNA}^{\text{Phe}}$  suggests that there may be a corresponding selective affinity of glutamic acid derivatives to  $\text{tRNA}^{\text{Glu}}$ . However, our present experiments do not show any effect to support this hypothesis. Due to the special nature of our approach it cannot be excluded that preferential affinity exists but does not influence anticodon pairing and thus could not be detected. It is also possible that the conditions of our experiments did not favour binding. Some experimental data on specific interactions between  $\text{tRNA}^{\text{Glu}}$  and glutamic acid have been reported by Watanabe and Miura [24]. However, the change of the circular dichroism used as a basis of their evaluation appears to be at the limit of experimental accuracy. Further investigations by other techniques and also under different buffer conditions are required to settle this problem.

Finally we have used the anticodon pairing procedure for another look on binding of  $\text{Mg}^{2+}$  (and sper-

mine) to tRNA. Since the binding of these ions to tRNA has been studied by many other techniques [25, 26], our present results should be useful for comparison. It is well-known that tRNA molecules bind a large number of  $Mg^{2+}$  ions, which have been classified into 3 groups according to the binding affinity [25–27]. The anticodon pairing technique detects  $Mg^{2+}$  sites of a rather low affinity in the monomer state and  $Mg^{2+}$  sites with intermediate affinities in the dimer state. It is remarkable that the binding constant of the site found in the monomer state ( $360\text{ M}^{-1}$ ) is much lower than that observed previously for the  $Mg^{2+}$  site located in the anticodon loop of tRNA<sup>Phe</sup> ( $\sim 1900\text{ M}^{-1}$ , cf. ref. [26]). Since the anticodon loop site is very close to the anticodon and its occupation by  $Mg^{2+}$  is accompanied by a conformation change of the loop, a strong coupling to anticodon pairing should be expected. Probably, our present result is distorted by a difference in the  $Mg^{2+}$  binding constant to tRNA<sup>Phe</sup> and tRNA<sup>Glu</sup>, which has not been considered in our evaluation. If this simplification would be the only reason for the

deviation, however, the difference of these binding constants would be surprisingly large.

The contrast between the decrease of the pairing constant observed upon addition of aromatic amino acid amides and the increase of the same constant upon addition of  $Mg^{2+}$  or spermine indicates clearly opposite effects of these ligands on the conformation of the anticodon loop. Since it is known from independent measurements that  $Mg^{2+}$  ions stabilize the 3'-stacked conformation [26], it is likely that the aromatic amino acid amides favor the 5'-stacked conformation. This conclusion is also supported by the special coupling between the binding processes of  $Mg^{2+}$  and the aromatic amides, which leads to cooperative binding phenomena [10].

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