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Contributions to selective binding of aromatic amino acid residues to $tRNA^{Phe}$

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The binding of aromatic amino acid amides to two fragments of $tRNA^{Phe}$ has been measured, in order to explore contributions to selective interactions of amino acid residues with $tRNA^{Phe}$. Measurements of the Wye base fluorescence demonstrate that the isolated anticodon loop of $tRNA^{Phe}$ (15 nucleotide residues) has a binding constant $K = 75\ M^{-1}$ for Phe-amide, which is only slightly lower than that observed previously for the complete $tRNA^{Phe}$ ($K = 100\ M^{-1}$). The hexamer $G_mAAYA\psi$ – possessing most residues of the loop, but without loop structure – shows a further reduced binding constant of $42\ M^{-1}$. According to these data, part of the selective interaction results from the special loop structure and another part probably from the nature of the Wye base. The particular influence of the loop structure is also demonstrated by binding experiments performed in the presence of Mg^{2+}. As expected, addition of Mg^{2+} decreases the binding affinity of the aromatic amino acid amides. Moreover, Mg^{2+} at concentrations ≥ 0.3 mM induces cooperative binding of the amides to the anticodon loop similar to that found previously for the complete $tRNA^{Phe}$, whereas the hexamer does not show any indication for cooperativity. A special coupling of Mg^{2+} and amide binding to the anticodon loop is also indicated by inhibition of Mg^{2+} binding in the presence of amides, which is much stronger than expected for a simple salt effect. These results demonstrate a complex coupling of Mg^{2+} and amide binding to different conformational states of the anticodon loop, which resembles an allosteric type of reaction mechanism.

1. Introduction

The specificity of protein-nucleic acid interactions has been studied at various levels of complexity. Contributions of individual amino acids to interactions with nucleic acids have been investigated mainly with oligopeptide and homopolynucleotide model compounds [1–3]. Individual contributions were also studied at the level of monomer components, with oligopeptide-oligonucleotide complexes and with other combinations of model compounds [4–6]. However, very few results have been reported for interactions of amino acids (or their simple derivatives) with tRNA molecules [8–11], although these interactions are of great interest for various reasons. Because the structure of tRNAs with their many loops and tertiary interactions is rather complex, these molecules may have specific binding sites for amino acid residues. Since tRNA molecules are believed to be conserved from a very early period of evolutionary history [12], tRNAs may show some relic of a primitive adaptor function. Interactions between tRNAs and amino acid residues may also reflect specific recognition between tRNAs and proteins like aminoacyl-tRNA synthetases.

In a recent investigation [9] we have been able to demonstrate the existence of selective interactions between $tRNA^{Phe}$ and aromatic amino acid residues. Since the interactions could be analysed simply by measurements of the Wye base fluo-

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rescence, it is likely that the binding site is located close to the Wye base in the anticodon loop. For an examination of this assignment, we have measured the binding of aromatic amino acid amides to a pentadecamer, which includes all the residues of the anticodon loop and stem domain of $tRNA^{Phe}$. We have also analysed binding of aromatic amino acid amides to a hexamer, which contains most residues of the anticodon loop, but cannot form a hairpin loop. Furthermore, we have used the hexamer and the pentadecamer to study the nature of the cooperativity, which has been observed for the binding of aromatic amino acid amides to $tRNA^{Phe}$ in the presence of Mg^{2+}. Our model experiments serve to identify individual contributions of tRNA domains to the reactions of the whole adaptor molecule.

2. Materials and methods

$tRNA^{Phe}$ from yeast with an amino acid acceptor activity of 1.4 pmol/A_{260} was obtained from Boehringer-Mannheim. The pentadecamer including residues 28–42 $tRNA^{Phe}$ (yeast) and the hexamer $G_mAAYA\psi$ (residues 34–39 of $tRNA^{Phe}$ (yeast) were kindly provided by Dr. L.W. McLaughlin and E. Graeser [13]. The amides of L-phenylalanine, L-tyrosine and L-tryptophan were purchased from Bachem (Bubendorf, Switzerland). All measurements have been carried out in a standard buffer containing 80 mM Tris-cacodylate (pH 6.5) and 50 mM $NaClO_4$.

Fluorescence intensities were measured using an SLM 8000 spectrofluorimeter with excitation at 325 nm and selection of the emitted light by a KV 399 cut-off filter (Schott & Gen, Mainz, F.R.G.). The output from the control unit was transmitted to a Commodore Pet computer, averaged and corrected for inner filter effects when necessary.

3. Results

3.1. Binding of aromatic amino acid amides to pentadecamer and hexamer from Wye-base fluorescence quenching

The Wye base located in the anticodon loop of $tRNA^{Phe}$ is well known for the strong sensitivity of its fluorescence upon changes in its environment [14,15]. We have used this property to analyse the interactions of aromatic amino acid amides with the pentadecamer and the hexamer. The experimental conditions were selected to guarantee a stable hairpin loop structure of the pentadecamer. From melting experiments [13] we know that the hairpin loop is stable up to 25 °C at 0.13 M monovalent ion concentration of our standard buffer. The hexamer cannot form any loop structure and should be in a partly stacked single-stranded conformation. Addition of Phe-, Tyr- and Trp-amide to both the pentadecamer and hexamer induces a decrease in fluorescence of the Wye base (cf. figs. 1 and 2). This effect corresponds to that observed previously [9] for the complete $tRNA^{Phe}$, but is the reverse of that found for simple monovalent ions such as Na^+, which induces an increase of the Wye base fluorescence [16]. Our experimental data obtained in the standard buffer could be fitted to a reasonable degree of accuracy by a single-step binding model. The binding constants, which have been evaluated by a least-squares fitting procedure, are compiled in table 1 together with those obtained previously for the complete $tRNA^{Phe}$.

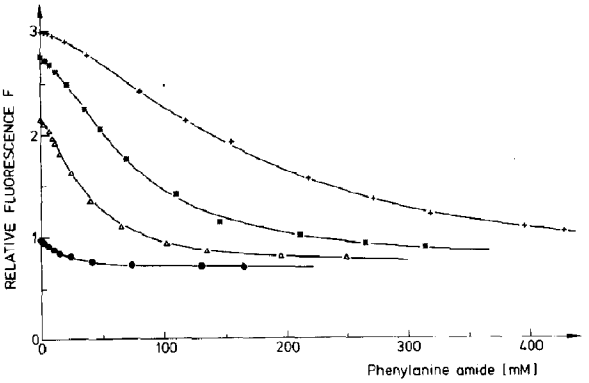

Fig. 1. Relative fluorescence intensity of the Wye base in the pentadecamer fragment of $tRNA^{Phe}$ as a function of Phe-amide concentration at different concentrations of Mg^{2+}; 0 mM (●), 0.3 mM (△), 1 mM (*) and 3 mM (+) in 80 mM Tris-cacodylate (pH 6.5), 50 mM $NaClO_4$ at 7.2 °C. Continuous lines represent least-squares fits according to the two-step model (eqs. 3 and 4).

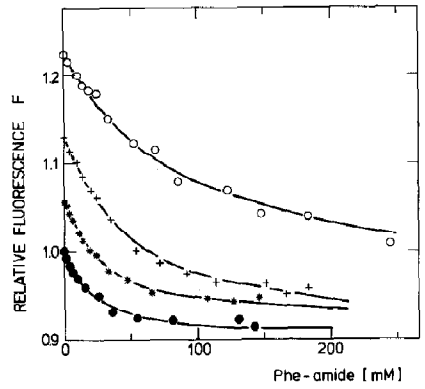

Fig. 2. Relative fluorescence intensity of the hexamer as a function of Phe-amide concentration at different concentrations of Mg^{2+}: 0 mM (●), 1 mM (*), 3 mM (+) and 9 mM (○) in 80 mM Tris-cacodylate (pH 6.5), 50 mM $NaClO_4$ at 7.2 °C. Continuous lines represent least-squares fits according to a single-step binding model.

3.2. Cooperativity of binding to the pentadecamer in the presence of Mg^{2+}

It is known that the conformation of the anticodon loop of tRNA^{Phe} is strongly affected by the presence of Mg^{2+} [13,17]. Thus, Mg^{2+} may influence the binding equilibrium not only by electrostatic shielding, but also by its special effect on the loop conformation. In fact, such a phenomenon has been demonstrated for the binding of aromatic amino acid amides to tRNA^{Phe}. The expected decrease in binding affinity with increasing Mg^{2+} concentration, resulting from shielding of the negative phosphate charges, was accompanied by a shift from a non-cooperative to a cooperative binding equilibrium [9]. To explore the nature of this cooperativity found for complete tRNA^{Phe} molecules, we have studied the binding of aromatic amino acid amides to the pentadecamer and hexamer in the presence of Mg^{2+}.

The results of fluorescence titrations given in fig. 1 demonstrate that the shift of the binding reaction from a non-cooperative to a cooperative one upon addition of Mg^{2+} is fully conserved for the isolated anticodon hairpin loop. As for the case of the complete tRNA^{Phe} we represent our experimental data by a model with two consecutive binding steps [9]

$$L + A \rightleftharpoons LA \tag{1}$$

$$LA + A \rightleftharpoons LA_2 \tag{2}$$

and with two equilibrium constants

$$K_1 = \frac{[LA]}{[A][L]} \tag{3}$$

$$K_2 = \frac{[LA_2]}{[LA][A]} \tag{4}$$

where L denotes the anticodon loop and A the amino acid amides. We assume that the degree of fluorescence quenching is identical for the two binding steps. By this simple model, we could fit our experimental data to a remarkably high degree

Table 1

Binding constants of amino acid amides to tRNA^{Phe}, the pentadecamer and the hexamer in 80 mM Tris-cacodylate (pH 6.5), 50 mM $NaClO_4$ at 7.2 ° C from fluorescence titrations

Binding constants are expressed in M^{-1}; numbers in parentheses denote percent reduction of the fluorescence intensity upon complex formation (estimated accuracy of the data cf. heading of table 2).

	tRNA^{Phe}	Pentadecamer	Hexamer
Phe	100 (35)	75 (36)	40 (10)
Tyr	110 (42)	90 (43)	
Trp	310 (81)	270 (67)	200 (55)

Table 2

Equilibrium constants K_1 and K_2 (in M^{-1}) for the interaction of Phe- and Trp-amide with the pentadecamer as a function of the Mg^{2+} concentration according to a two-step model (eqs. 3 and 4) from fluorescence titrations in 80 mM Tris-cacodylate + 50 mM $NaClO_4$ at 7.2 ° C.

The accuracy is estimated to be ±20% for binding constants above 100 M^{-1} and ±50% for those below 10 M^{-1} (linear interpolation in the intermediate range); quenching parameters, which are given in parentheses and refer to the percent reduction of the fluorescence intensity upon saturation of the sites, are accurate to ±3.

[Mg^{2+}] (mM)	Phe		Trp	
	K_1	K_2	K_1	K_2
0	100	40 (36)	460	190 (67)
0.3	19	46 (67)	–	–
1.0	8	26 (75)	45	170 (85)
3.0	3	10 (82)	16	140 (85)

of accuracy (cf. fig. 1). As discussed previously [9], fitting of individual fluorescence parameters does not improve the quality of the fits to any considerable extent nor does it lead to any large change of the binding constants. The binding constants evaluated for the pentadecamer (cf. table 2) are very similar to those obtained previously for the complete tRNA^{Phe}.

The binding constants evaluated using our simple reaction model consider the competition of amide binding by Mg^{2+} implicitly but not explicitly. An explicit treatment would require the evaluation of binding constants for all the reaction steps, which do not only include the steps of ligand binding but also a conformational change of the loop. Although such a treatment is of course possible in principle, we have not pursued this approach partly because of the danger of overinterpretation of our limited data set and also because of problems associated with the polyelectrolyte nature of tRNA. In spite of these limitations of our approach, we can be sure that our conclusions with respect to cooperativity are justified. A corresponding comment applies to our data analysis given in section 3.4, whereas the case described in section 3.3 has been treated via a competition model due to the absence of cooperativity.

3.3. Absence of cooperativity for amide binding to hexamer

The nature of the cooperativity observed for binding of aromatic amino acid amides to tRNA^{Phe} has been explored in more detail through fluorescence titrations using the hexamer. The hexamer fragment of the anticodon loop contains all residues required for contact with Mg^{2+} at its site in the anticodon loop [18,19] but cannot form a loop structure. Our results obtained for Phe-amide binding to the hexamer (cf. fig. 2) demonstrate as expected that the affinity decreases with increasing Mg^{2+} concentration. However, in contrast to the complete tRNA^{Phe} and the pentadecamer, the hexamer does not show any indication of cooperativity. All the binding curves could be fitted by a single-step binding model to a high degree of accuracy.

Similar data have been obtained for the binding of Trp-amide to the hexamer. The dependence of the equilibrium constants on the Mg^{2+} concentration obtained for this case does not follow a simple model for a competition between Mg^{2+} and Trp-amide. Using the binding constant of Mg^{2+} to the hexamer determined previously [13], a simple competition for the same binding site should lead to a greater decrease in the binding constant than that observed for Trp-amide at high Mg^{2+} concentrations (cf. fig. 3). For a reasonable description of our data we had to use a more complex reaction scheme with three different binding reactions:

$$H + Mg^{2+} \rightleftharpoons H \cdot Mg^{2+} \tag{5}$$

$$H + A \rightleftharpoons H \cdot A \tag{6}$$

$$H \cdot Mg^{2+} + A \rightleftharpoons H \cdot Mg^{2+} \cdot A \tag{7}$$

where H and A denote hexamer and amide, respectively. We define an apparent binding constant

$$K_{app} = \frac{[HA] + [HMg^{2+} \cdot A]}{[A] \cdot ([H] + [HMg])} = \frac{K_a + K_m K_{am} [Mg^{2+}]}{1 + K_m \cdot [Mg^{2+}]} \tag{8}$$

The parameters K_m, K_a and K_{am} denote the

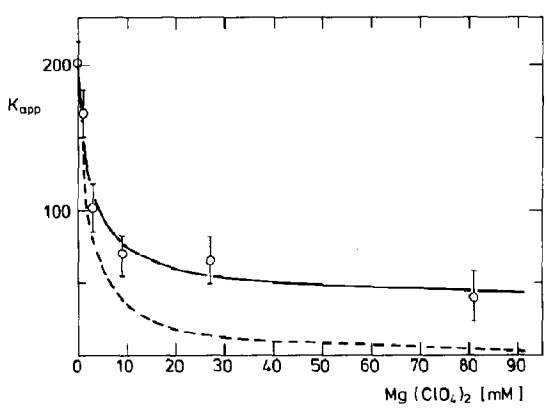

Fig. 3. Equilibrium constant K_{app} (cf. eq. 8) for the binding of Trp-amide to the hexamer as a function of Mg^{2+} concentration. The continuous line represents a least squares fit according to eq. 8. The dashed line represents a model with competition of Trp-amide and Mg^{2+} for the same site on the hexamer using a Mg^{2+}-binding constant of 500 M^{-1} (cf. text).

association constants for reactions 5, 6 and 7, respectively. The association constant K_a is known from the fluorescence titration performed in the absence of Mg^{2+}. Least-squares fitting of the data obtained for Trp-amide (cf. fig. 3) according to eq. 8 provides the values $K_m = 415\ M^{-1}$ and $K_{am} = 40\ M^{-1}$. The K_m value is in satisfactory agreement with the association constant of $500\ M^{-1}$ evaluated from a fluorescence titration of the hexamer with Mg^{2+} in the absence of amides [13]. Another set of data obtained for the binding of Phe-amide to the hexamer in the presence of Mg^{2+} yielded a Mg^{2+}-binding constant $K_m \approx 700\ M^{-1}$. All the binding constants refer to simple site binding without consideration of nonspecific shielding effects.

3.4. Inhibition of Mg^{2+} binding to the anticodon loop of tRNA^{Phe} by aromatic amino acid amides

The experiments on the binding of aromatic amino acid amides to tRNA^{Phe} and the pentadecamer indicate that Mg^{2+} affects this binding equilibrium by more than simple competition. Apparently Mg^{2+} induces a change in the loop to a conformation with a lower affinity for aromatic amino acid amides. Since coupling of the ligands should affect both equilibria, we may expect an unusual influence of amides on the binding of Mg^{2+} to the anticodon loop. The binding of Mg^{2+} to tRNA^{Phe} is reflected by a strong increase in Wye base fluorescence, which can be fitted by a one-step binding model to a high degree of accuracy in both the absence and presence of aromatic amino acid amides (cf. fig. 4). The binding constants K obtained from these titrations are a linear function of the logarithm of the added ligand concentration, for both simple monovalent salt and amino acid amides (cf. fig. 5). As expected the K values decrease with increasing concentration of all ligands used in our experiments. However, the slope $s = d(\log K)/d(\log c)$ is quite different for the various ligands. While the slope $s = -1.6$ obtained from experiments with Na^+ is close to that expected according to polyelectrolyte theory [20,21], the corresponding slopes obtained for Trp-amide (-7.5) and Phe-amide (-4) are much greater than expected. These results indicate

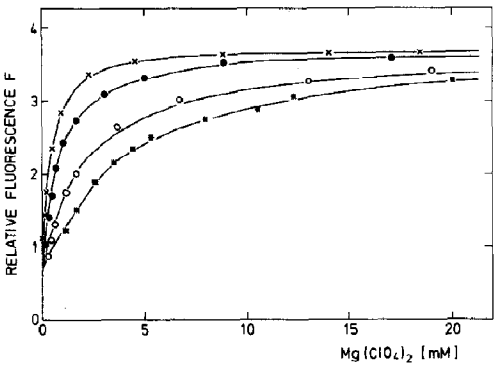

Fig. 4. Relative fluorescence intensity of tRNA^{Phe} as a function of Mg^{2+} concentration at different concentrations of Phe-amide: 10 mM (×), 30 mM (●), 90 mM (○) and 150 mM (*) in 80 mM Tris-cacodylate (pH 6.5), 50 mM $NaClO_4$ at 7.2 °C. Continuous lines represent least-squares fits according to a single-step model.

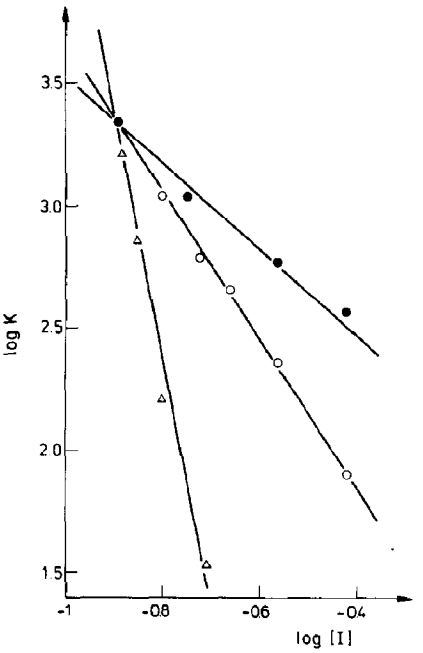

Fig. 5. Logarithm of the equilibrium constant K for the binding of Mg^{2+} to tRNA^{Phe} as a function of the logarithm of the cation concentration [I], which is calculated by addition of the contributions from various components. All the solutions contained the standard buffer with 80 mM Tris-cacodylate and 50 mM $NaClO_4$; the ion concentration was increased by addition of $NaClO_4$ (●), Phe-amide (○) or Trp-amide (△) (7.2 °C).

again a conformational change of the anticodon loop induced by binding of aromatic amino acid amides.

4. Discussion

The biological function of macromolecules in general and of nucleic acids likewise depends to a large extent on the complexity of their structure and the potential to undergo conformational transitions. Among the various nucleic acid species, tRNA molecules are particularly useful examples for complex folding of a polynucleotide chain. Obviously, a complex combination of structure elements is required for optimal adaptor function. Dissection of the tRNA adaptor into separate elements should help to identify the relation between these elements and their contribution to the function.

In previous investigations we have demonstrated that the isolated anticodon loop of $tRNA^{Phe}$ is very similar to the complete $tRNA^{Phe}$ with respect to binding of Mg^{2+} and the cognate codon UUC [13,17,22–24]. Furthermore, the conformational transitions induced by these ligands are very similar for the anticodon loop and the complete $tRNA^{Phe}$. In the current study we have analysed the binding of amino acid residues, in order to obtain information about the elements of specificity contributing to protein binding, representing the other important function of tRNA adaptors. These experiments should also be useful for establishing potential mechanisms of adaptor function at an early stage of molecular self-organization.

Our results demonstrate that the isolated anticodon loop is again very similar to the complete tRNA – with respect to both the binding affinity of aromatic amino acid amides and the cooperativity of their binding induced by addition of Mg^{2+}. The loop structure is essential for the binding process, because the binding affinity is reduced and the cooperativity is lost in the case of the hexamer, which contains most important residues of the loop, but does not have a loop structure. The cooperativity of amide binding to the loop observed in the presence of Mg^{2+} can be explained easily in the context of already established properties of the loop structure. It is known that the anticodon loop exists in two major conformations: the 3′ stacked and 5′ stacked structures. The former has been found in crystals by X-ray analysis [18,19] and is dominant in the presence of Mg^{2+}, whereas the latter appears to be favoured [13,17] in the absence of Mg^{2+}. Our results can be explained by preferential binding of amino acid amides to the 5′ stacked form and the existence of (at least) two binding sites. According to this model, binding follows the standard law of mass action in the absence of Mg^{2+}, whereas a transition from a 3′ stack to a 5′ stack conformation must be induced by the first binding step in the presence of Mg^{2+}, which is the source of the observed cooperativity. This model also explains the unusually strong sensitivity of Mg^{2+} binding with respect to addition of amino acid amides.

A relatively large contribution to the affinity of aromatic amino acid amides to $tRNA^{Phe}$ and the isolated anticodon loop apparently results from the presence of the Wye base. Owing to its tricyclic aromatic system the Wye base appears to be particularly hydrophobic and thus contributes to an increased affinity towards aromatic amino acid residues. This is indicated by the binding constants observed for the hexamer (cf. table 1). Apparently, the contributions of base modification and loop structure to selective binding of aromatic amino acid residues to $tRNA^{Phe}$ are of similar magnitude.

The interactions characterized in the present investigation may be involved in the binding of the cognate aminoacyl-tRNA synthetase to $tRNA^{Phe}$. Studies of Wye base fluorescence upon binding of the synthetase and of energy transfer from tryptophan residues to the Wye base indicate a particular function of the anticodon loop in the interaction with the protein [11,25,26]. Furthermore, our results have general implications for models of molecular self-organization at an early stage of biological evolution. Selective binding of amino acid residues to simple loop structures and their high affinity to the cognate codon together with codon-induced loop association [22] suggest that these loops are optimal candidates for a primitive adaptor function.

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References

1 C. Helene and Y.C. Maurizot, Crit. Rev. Biochem. 19 (1981) 213.
2 C. Helene and G. Lancelot, Prog. Biophys. Mol. Biol. 39 (1982) 1.
3 E.J. Gabbay, in: Bioorganic chemistry, vol. 3, Macromolecular and multimolecular systems, ed. E.E. Van Tamelen (Academic Press, New York, 1977) p. 33.
4 J.C. Lacey and C.W. Mullins, Jr, Origins Life 13 (1983) 3.
5 D. Porschke, Eur. J. Biochem. 86 (1978) 291.
6 D. Porschke and J. Ronnenberg, Biopolymers 22 (1983) 2549.
7 D. Porschke, J. Mol. Evol. 21 (1985) 192.
8 C. Helene, J.L. Dimicoli, H.H. Borazan, M. Durand, J.C. Maurizot and J.J. Toulme, Jerusalem Symp. Quant. Chem. Biochem., eds. E.D. Bergmann and B. Pullman (Academic Press, New York, 1973) vol. 5, p. 361.
9 W. Bujalowski and D. Porschke, Nucleic Acids Res. 12 (1984) 7519.
10 K. Watanabe and K. Miura, Biochem. Biophys. Res. Commun. 129 (1985) 679.
11 J.F. Lefevre, R. Ehrlich, M.C. Kilhoffer and P. Remy, FEBS Lett. 114 (1980) 219.
12 M. Eigen and R. Winkler-Oswatitsch, Naturwissenschaften 68 (1981) 217.
13 W. Bujalowski, E. Graeser, L.W. McLaughlin and D. Porschke, Biochemistry 25 (1986) 6365.
14 K. Beardsley, T. Tao and C.R. Cantor, Biochemistry 9 (1970) 3525.
15 J. Eisinger, B. Feuer and T. Yamane, Proc. Natl. Acad. Sci. U.S.A. 65 (1970) 638.
16 D. Labuda, T. Haertle and J. Augustyniak, Eur. J. Biochem. 79 (1977) 293.
17 D. Labuda and D. Porschke, Biochemistry 21 (1982) 49.
18 S.R. Holbrook, J.L. Sussman, R.W. Warrant, G.M. Church and S.H. Kim, Nucleic Acids Res. 4 (1977) 2811.
19 G.J. Quigley, M.M. Teeter and A. Rich, Proc. Natl. Acad. Sci. U.S.A. 75 (1978) 64.
20 G.S. Manning, Q. Rev. Biophys. 11 (1978) 179.
21 M.T. Record, Jr, C.F. Anderson and T.M. Lohman, Q. Rev. Biophys. 11 (1978) 103.
22 W. Bujalowski, M. Jung, L.W. McLaughlin and D. Porschke, Biochemistry 25 (1986) 6372.
23 D. Labuda, G. Striker and D. Porschke, J. Mol. Biol. 174 (1984) 587.
24 D. Porschke and D. Labuda, Biochemistry 21 (1982) 53.
25 R. Ehrlich, J.F. Lefevre and P. Remy, Eur. J. Biochem. 103 (1980) 145.
26 J.F. Lefevre, R. Ehrlich and P. Remy, Eur. J. Biochem. 103 (1980) 155.