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Identification of the magnesium, europium and lead binding sites in *E. coli* and lupine tRNA^{Phe} by specific metal ion-induced cleavages

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The Pb, Eu and Mg-induced cleavages in *E. coli* and lupine tRNA^{Phe} have been characterized and compared with those found in yeast tRNA^{Phe}. The pattern of lupine tRNA^{Phe} hydrolysis closely resembles that of yeast tRNA^{Phe}, while several major differences occur in the specificity and efficiency of the *E. coli* tRNA^{Phe} hydrolysis. The latter tRNA is cleaved with much lower yield in the D-loop, and interestingly, cleavage is also detected in the variable region, that is highly resistant to hydrolysis in eukaryotic tRNAs. The possible location of tight Pb, Eu and Mg binding sites in *E. coli* tRNA^{Phe} is discussed on the basis of the specific hydrolysis data.

tRNA cleavage; Metal-binding site; tRNA^{Phe}

1. INTRODUCTION

Metal ions are present in water solution in their hydrated form and some of the hydrates ionize at, or near, a physiological pH, being a source of highly nucleophilic hydroxyl groups [1,2]. The metal hydrates bind to suitable pockets formed by tRNA tertiary structure [3,4] and several metal ions, especially Pb, Mg and Eu are capable of cleaving the tRNA chain with a high degree of specificity [5–7]. The mechanism of the specific yeast tRNA^{Phe} hydrolysis by Pb was proposed some years ago [8–11]. It involves initial proton abstraction from a sugar 2′-OH group at the cleavage site by the ionized Pb hydrate, acting from its tight binding site. An analogous 2′-OH group activation mechanism was proposed recently for Eu- and Mg-induced hydrolysis of yeast tRNA^{Phe} [12,13].

A straightforward consequence of the fact that the specific tRNA hydrolysis occurs from the sites of tight metal ion binding is the possibility of probing the environment of these sites by the induced cleavages. Here, we describe the cleavage patterns and analyze the Pb, Eu and Mg binding sites in *E. coli* and lupine tRNA^{Phe}. The results are compared directly with those obtained for yeast tRNA^{Phe} [12–16].

2. MATERIALS AND METHODS

2.1. *tRNAs*

Yeast and lupine tRNA^{Phe} were purified from crude preparations by standard column chromatography procedures. The final purification was by HPLC on TSK-gel 2SW (Toyo Soda). *E. coli* tRNA^{Phe} was purchased from Subriden RNA.

2.2. *Enzymes*

T_4 polynucleotide kinase, snake venom phosphodiesterase and RNase T_1 were from Boehringer. Calf intestine alkaline phosphatase was from Sigma; tRNA nucleotidyltransferase was a gift from Dr R. Giege.

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Abbreviations: Y-base, α-(carboxyamino)-4,9-dihydro-4,6-dimethyl-9-oxo-1H-imidazolo[1,2-*a*]purine-7-butyric acid dimethyl ester; o_2yW, α-(carboxyamino)-4,9-dihydro-β-hydroperoxy-4,6-dimethyl-9-oxo-1H-imidazolo[1,2-*a*]purine-7-butyric acid dimethyl ester; ms^2i^6A, 2-methylthio-N^6-(Δ^2-isopentenyl)adenosine

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2.3. *Chemicals*

Europium chloride was from Koch-Light, lead acetate from Serva and magnesium chloride from Sigma. Acrylamide, *N,N'*-methylenebisacrylamide, Tris base, boric acid and urea were from Serva.

2.4. *End labeling of tRNAs*

Labeling at the 3'-end was performed on the tRNA deprived of its 3'-terminal sequence by snake venom phosphodiesterase treatment. Reconstruction of the natural CCA end was achieved with CTP, [α-^{32}P]ATP (400 Ci/mmol, Amersham) and tRNA-nucleotidyltransferase, as described in [13]. Labeling at the 5'-end was carried out with [γ-^{32}P]ATP (3000 Ci/mmol, Amersham) and T_4 polynucleotide kinase on tRNAs dephosphorylated with calf intestine alkaline phosphatase. The dephosphorylated tRNAs were gel-purified on 15% PA gel before reaction with kinase. Labeled tRNAs were purified by polyacrylamide gel electrophoresis (15% gel, 8 M urea), then eluted from the gel, and precipitated with ethanol. In typical experiments ~1 × 10^6 cpm/μg tRNA was obtained for both 5'- and 3'-end labeling.

2.5. *tRNA hydrolysis with metal ions*

The end-labeled tRNA was supplemented with cold tRNA of the same nucleotide sequence to obtain final tRNA concentrations of 8 μM in all reactions. Other constant reaction parameters were: 40 mM NaCl and 10 mM buffer Tris-HCl. The reaction temperature, pH of the buffer, concentrations of metal ions and reaction time are specified in the figure legends. Prior to reactions, tRNAs were subjected to the standard denaturation-renaturation procedure [14].

3. RESULTS

3.1. *Lead(II)-induced hydrolysis*

The cleavages occurring in $tRNA^{Phe}$ from yeast, lupine and *E. coli* are shown in fig.1a–c, respectively. The hydrolysis with Pb was analysed at 25°C, at three different concentrations of Pb ions: 0.1, 0.5 and 1.0 mM, and its effect is shown in lanes A–C for each tRNA. The pattern of yeast

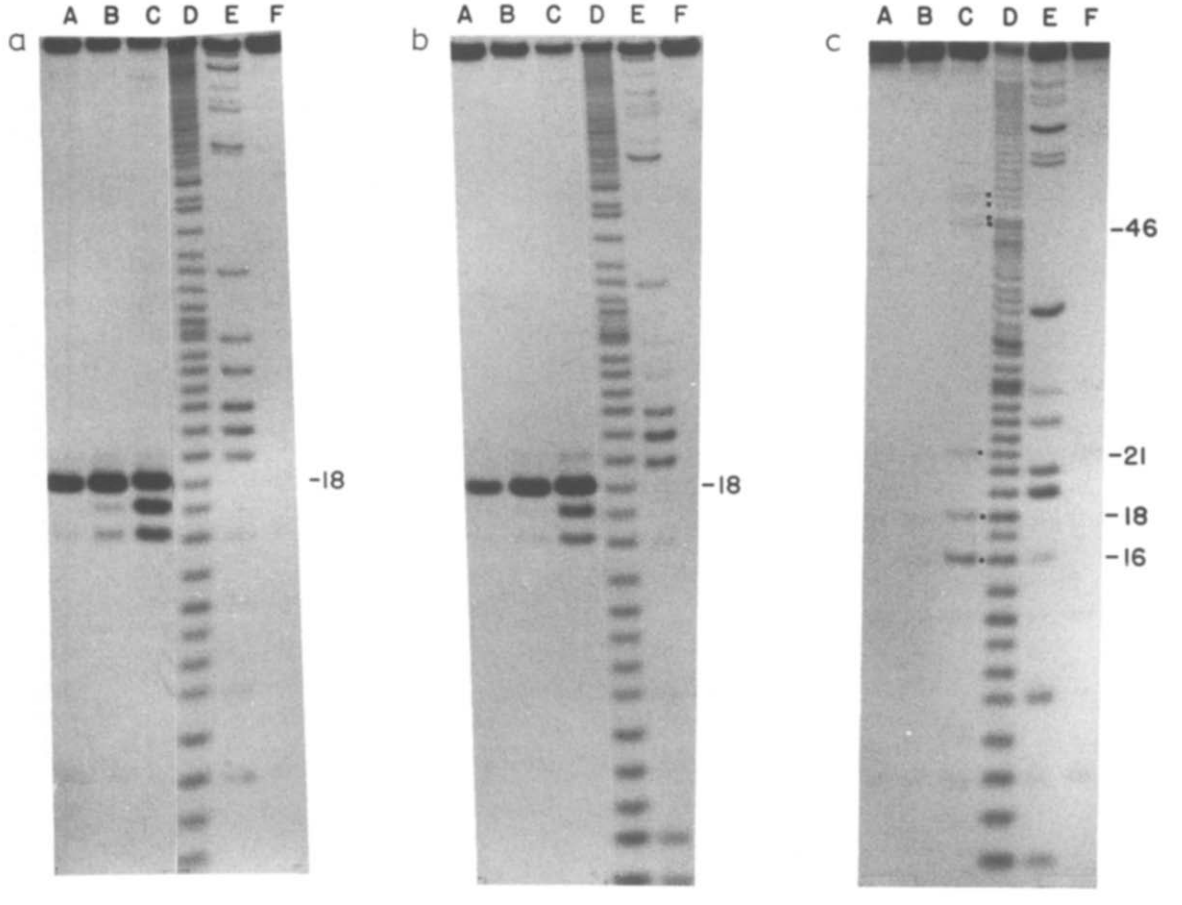


Fig.1. Specificity of lead-induced cleavages. Lanes: (A–C) 0.1, 0.5 and 1 mM Pb, respectively; (D) formamide ladder, (E) T_1 ladder, (F) control. Reactions were performed at pH 7.2 and 25°C for 15 min.

$tRNA^{Phe}$ hydrolysis obtained in this experiment is in perfect agreement with that observed earlier in the range of low concentrations of Pb ions [14]. The lupine $tRNA^{Phe}$ having the same nucleotide sequence as yeast $tRNA^{Phe}$ in both the T-loop and D-loop, i.e. at the Pb-ion-binding site and at the cleavage site [8–11], is cleaved with the same strength and specificity. On the other hand, *E. coli* $tRNA^{Phe}$ is significantly more resistant to the reaction with Pb. Three weak cleavages occur at phosphates 16, 18 and 21 in the D-loop and other weak cuts take place at phosphates 46–49 in the variable region.

3.2. *Europium(III)-induced hydrolysis*

The Eu-catalyzed reactions of the three tRNAs have been studied at 37°C at four different concentrations of Eu ions ranging from 10 to 100 μM. In fig.2b and c the cleavage patterns obtained for lupine and *E. coli* tRNAs are shown, while in fig.2a yeast $tRNA^{Phe}$ hydrolysis is presented for comparison. The lupine $tRNA^{Phe}$ (except for one minor difference consisting of the appearance of a weak cut at phosphate 47) reacts with Eu identically to yeast $tRNA^{Phe}$. The strongest cuts occur at phosphates 16 and 18 in the D-loop, and at phosphates 34 and 36 in the anticodon loop. Very weak cleavages at phosphates 17 and 19–21 are also detected. As in the case of the Pb reaction, the specificity of *E. coli* $tRNA^{Phe}$ hydrolysis by Eu ions is different from that found in the two eukaryotic tRNAs. Moreover, at higher concentrations of Eu the reaction significantly loses its specificity, and random hydrolysis takes place in all single-stranded regions, and to some extent also in the double-stranded stems. Therefore, we restrict this analysis to the specific cuts only, that occur in this tRNA at low concentrations of Eu

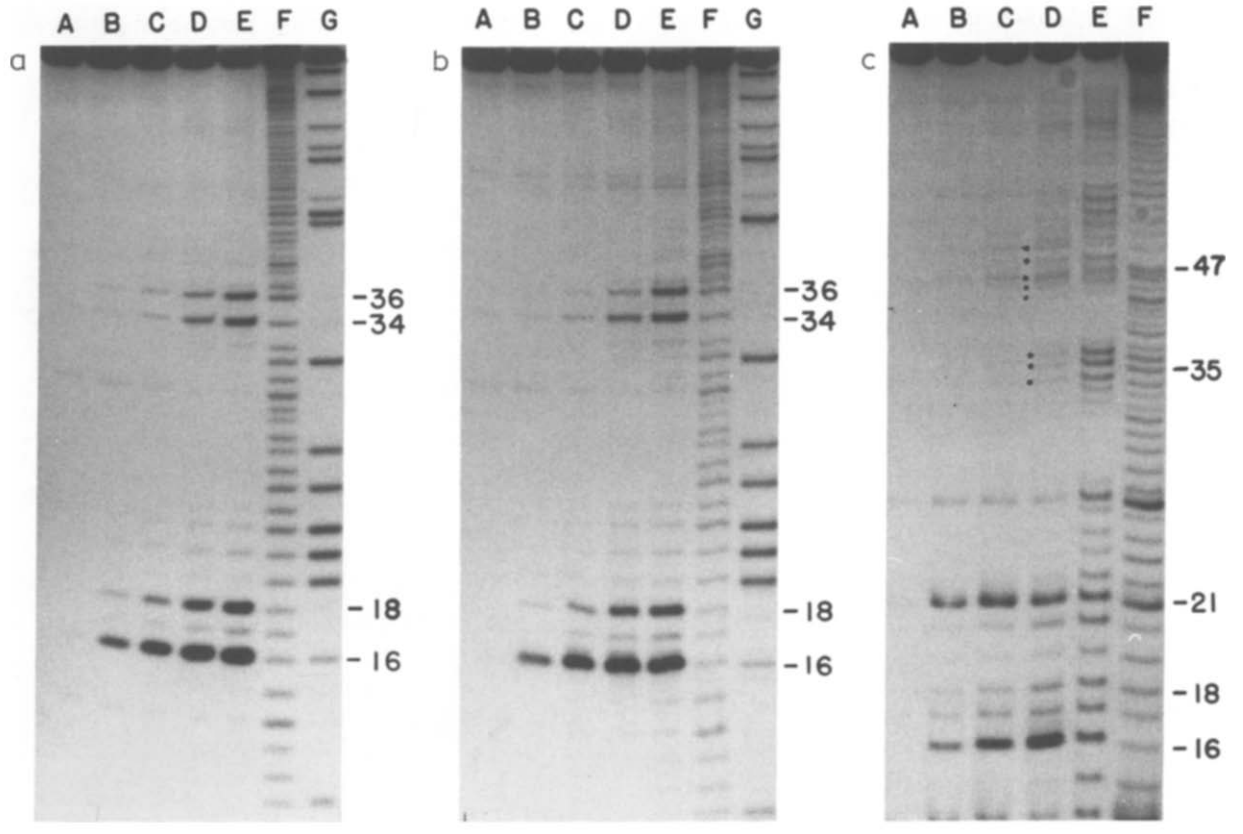


Fig.2. Specificity of europium-induced cleavages. Lanes: (A) control reaction; (B–E) reactions with 10, 30, 50 and 100 μM Eu, respectively; (F) formamide ladder; (G) T_1 ladder. Reactions were conducted at pH 7.2 and 37°C for 30 min.

(fig.2c, lanes B,C). The strongest cleavages are induced in the D-loop at phosphates 16 and 21. Some other cuts of low intensity occur at phosphates 14, 17–20, 26 and at phosphates 45–49 in the variable region. Cleavages in the anticodon loop of *E. coli* $tRNA^{Phe}$ are completely absent in lane B, and are barely discernible in lane C.

3.3. *Magnesium(II)-induced cleavages*

The Mg-induced cuts in the studied tRNAs show the highest degree of similarity. They are presented in fig.3a–c for yeast, lupine and *E. coli* tRNAs. In this case the reactions were performed with 3′-end-labeled tRNAs, at 37°C, with Mg ions used at concentrations ranging from 1 to 30 mM. Besides one qualitative difference, this time in the anticodon loop reactivity, the cleavage pattern is virtually identical in the two eukaryotic tRNAs. In *E. coli* $tRNA^{Phe}$ the two strongest cleavages also occur in the D-loop, at the same phosphates 16 and 20. Two other cleavages in this loop are seen at phosphates 17 and 18. What distinguishes, however, the *E. coli* $tRNA^{Phe}$ cleavages from those observed in eukaryotic tRNAs is their lower intensity. As concerns the anticodon loop hydrolysis, three weak cuts occur at phosphates 34, 36 and 37 and this resembles the cleavage pattern found in lupine $tRNA^{Phe}$. Thus, only hydrolysis in the variable region at phosphates 46 and 44, 45, 47, 48 clearly discriminates the *E. coli* $tRNA^{Phe}$ from the other two objects of this comparative study.

4. DISCUSSION

The nucleotide sequences of the studied tRNAs and a summary of the hydrolysis data are presented in table 1. Both eukaryotic tRNAs have identical nucleotide sequences in the single-stranded regions, except o_2yW-37 and D-47 in lupine tRNA that replace yW and U present in yeast $tRNA^{Phe}$ at the corresponding positions. It is therefore not surprising that these tRNAs are cleaved with similar specificity, and some minor differences in their cleavage patterns might be ac-

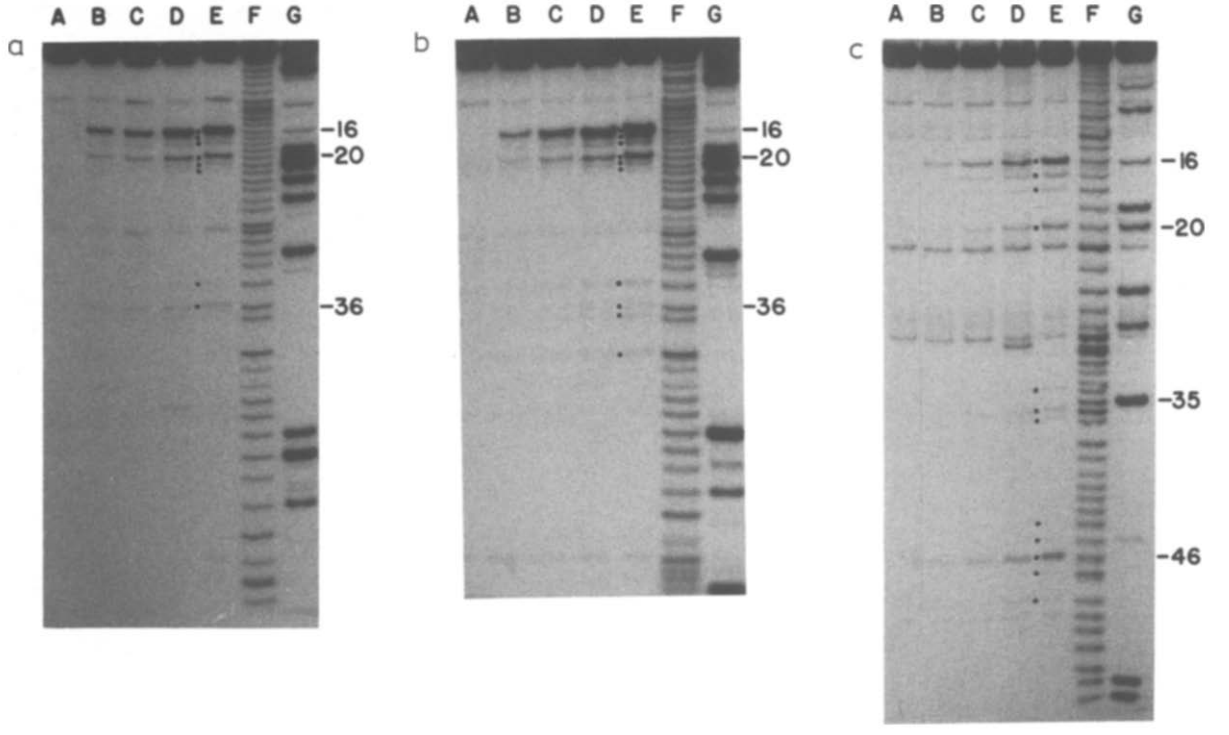
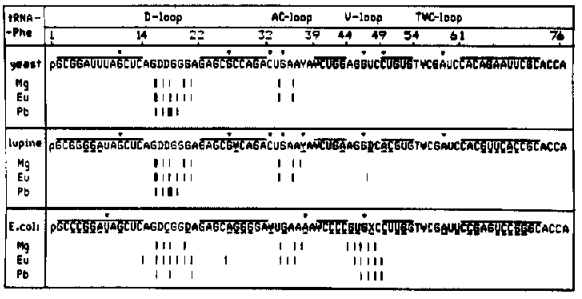


Fig.3. Specificity of magnesium-induced cleavages. Lanes: (A) EDTA-treated control reaction; (B–E) reactions in the presence of 1, 3, 10 and 30 mM Mg, respectively; (F) formamide ladder; (G) T_1 ladder. Reactions were performed at pH 8.5 in the presence of 0.1 mM EDTA, at 37°C, for 12 h.

Table 1

Cleavages induced by Mg, Eu and Pb ions in three tRNA^{Phe} molecules



Sequences involved in secondary structure formation are overlined. Numbering refers to phosphate residues and triangles denote modified nucleosides. Nucleosides in lupine and *E. coli* tRNAs that are different from those present in yeast tRNA^{Phe} at corresponding positions are underlined. Bar thickness is correlated with relative cleavage intensity

counted for by the two base substitutions mentioned above. More interesting, however, are the major differences found in cleavage patterns of yeast and *E. coli* tRNAs.

4.2. *D-loop cleavages*

The Pb cleavages in the D-loop are induced in yeast tRNA^{Phe} by a Pb ion tightly bound to the two T-loop bases U59 and C60 [8–10]. In *E. coli* tRNA^{Phe}, the latter base is replaced by U60, and most likely this single base substitution is sufficient to change the position and binding constant of the tightly bound Pb ion in a way that results in a much lower cleavage specificity and efficiency. Also the pattern of Eu-induced cuts in the D-loop of *E. coli* tRNA^{Phe} only weakly resembles that found in yeast tRNA^{Phe}. At a low excess of Eu ions, two sets of cleavages can be distinguished, one at the 5′-side of the D-loop with the strongest cut at phosphate 16, and the other at the 3′-side with the cut at phosphate 21. The two strongest D-loop cleavages in yeast tRNA^{Phe} at phosphates 16 and 18 were interpreted as being performed by Eu ions acting from two distinct binding sites [12] which could also be the case in *E. coli* tRNA^{Phe}. The two strongest Mg-induced cleavages in *E. coli* tRNA^{Phe} occur in the D-loop at the same positions as in yeast tRNA^{Phe}. Nevertheless, their efficiency is considerably lower. In yeast tRNA^{Phe} the cuts at phosphates 16 and 20 were thought to be effected from Mg3- and Mg2-binding sites, respectively [13]. In *E. coli* tRNA^{Phe}, D20 substitutes guanosine and U60 replaces cytidine at the Mg3 site. The phosphate oxygen atom of G20 also participates in the formation of the Mg2 site in yeast tRNA^{Phe} [17]. The above base changes are probably responsible for such an arrangement of the Mg-binding sites in *E. coli* tRNA^{Phe} that the cleavages occur with considerably lower yield.

4.3. *Anticodon-loop cleavages*

None of the studied tRNAs is cleaved by Pb in the anticodon loop under the conditions applied. Only two eukaryotic tRNAs are hydrolyzed by Eu at a low concentration of Eu ions, and all three tRNAs are cleaved by Mg in that region. Thus, the base changes in the upper part of the anticodon loop, i.e. Cm32 replacement by ψ and yW substitution for ms^{2}i^{6}A in *E. coli* tRNA^{Phe}, affect considerably only the Eu-binding site within that loop.

4.4. *Variable-loop cleavages*

The Pb ion at the Pb2-binding site in yeast tRNA^{Phe} has the shortest contacts to two bases of the variable region, A44 and G45, being directly coordinated to G45 O6 [8–10]. The absence of any Pb-induced cleavages in that region in yeast tRNA^{Phe} [14–16] was confirmed in the present study. There are also no europium and magnesium-induced cleavages in the variable loop. On the other hand, in *E. coli* tRNA^{Phe} where G45 is replaced by uridine and A44 by guanosine, a series of weak Pb, Eu and Mg-induced cleavages occur in that region. It is, therefore, tempting to assume that due to the above base substitutions a metal-ion-binding site is created in *E. coli* tRNA^{Phe} that is highly similar for Pb, Eu and Mg ions but different from the Pb2-(Sm4)-binding site in yeast tRNA^{Phe} [8–10,18,19].

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REFERENCES

[1] Burgess, J. (1978) in: Metal Ions in Solution, Horwood, Chichester.

[2] Fersht, A.R. (1977) Enzyme Structure and Mechanism, pp.49–51, W.H. Freeman, San Francisco.
[3] Teeter, M.M., Quigley, G.J. and Rich, A. (1980) in: Nucleic Acid-Metal Ion Interactions (Spiro, T. ed.) pp.146–177, Wiley, New York.
[4] Schimmel, P.R. and Redfield, A. (1980) Annu. Rev. Biophys. Bioeng. 9, 181–221.
[5] Werner, C., Krebs, B., Keith, G. and Dirheimer, G. (1976) Biochim. Biophys. Acta 432, 161–175.
[6] Wintermayer, W. and Zachau, G. (1973) Biochim. Biophys. Acta 299, 82–90.
[7] Rordorf, B.F. and Kearns, D.R. (1976) Biopolymers 15, 1491–1504.
[8] Brown, R.S., Hingerty, B.E., Dewan, J.C. and Klug, A. (1983) Nature 303, 543–546.
[9] Brown, R.S., Dewan, J.C. and Klug, A. (1985) Biochemistry 24, 4785–4801.
[10] Rubin, J.R. and Sundaralingam, M. (1983) J. Mol. Struct. Dyn. 1, 639–646.
[11] Sundaralingam, M., Rubin, J.R. and Cannon, J.F. (1984) Int. J. Quant. Chem. 11, 355–366.
[12] Ciesiołka, J., Marciniec, T. and Krzyżosiak, W.J. (1988) Eur. J. Biochem., submitted.
[13] Marciniec, T., Ciesiołka, J., Wrzesinski, J., Wiewiórowski, M. and Krzyżosiak, W.J. (1988) Eur. J. Biochem., submitted.
[14] Krzyżosiak, W.J., Marciniec, T., Wiewiórowski, M., Romby, P., Ebel, J.P. and Giege, R. (1988) Biochemistry 27, 5771–5777.
[15] Ciesiołka, J., Marciniec, T., Dziedzic, P., Krzyżosiak, W.J. and Wiewiórowski, M. (1987) in: Biophosphates and their Analogues: Synthesis, Structure, Metabolism and Activity (Bruzik, K.S. and Stec, W.J. eds) pp.409–414, Elsevier, Amsterdam, New York.
[16] Sampson, J.R., Sullivan, F.X., Behlen, L.S., DiRenzo, A.B. and Uhlenbeck, O.C. (1987) in: Proceedings of the Cold Spring Harbor Symposia on Quantitative Biology 52, pp.267–275.
[17] Hingerty, B., Brown, R.S. and Jack, A. (1978) J. Mol. Biol. 124, 523–534.
[18] Jack, A., Ladner, E., Rhodes, D., Brown, R.S. and Klug, A. (1977) J. Mol. Biol. 111, 315–328.
[19] Stout, C.D., Mizuno, H., Rao, S.T., Swaminathan, P., Rubin, J., Brennan, T. and Sundaralingam, M. (1978) Acta Crystallogr. B34, 1529–1544.