LACK OF SPECIFICITY IN THE AMINOACYL-tRNA SYNTHETASE-CATALYSED DEACYLATION OF AMINOACYL-tRNA

J. BONNET, R. GIEGÉ and J.P. EBEL

Laboratoire de Chimie Biologique, Université Louis Pasteur, Rue Descartes, 67000 Strasbourg, France

Received 27 July 1972

1. Introduction

It has been reported that aminoacyl-tRNA's can be deacylated by their cognate aminoacyl-tRNA synthetase in the absence of AMP and pyrophosphate [1–6]. A detailed study of this type of reaction has been performed by Schreier and Schimmel [4].

In the present study, we show that valyl-tRNA synthetase (VRS) and phenylalanine tRNA synthetase (PRS) from yeast deacylate either yeast Val- and Phe-$tRNA^{Val}$ or yeast Val- and Phe-$tRNA^{Phe}$, as well as numerous other aminoacyl-tRNA's. Our results suggest a lack of specificity in the aminoacyl-tRNA synthetase catalysed deacylation of aminoacyl-tRNA.

2. Materials and methods

PRS and VRS were prepared in our laboratory by F. Fasiolo and D. Kern as previously described [8, 9]. Yeast $tRNA^{Ala, Arg_{II}, Arg_{III}, Asp, Phe, Trp, Tyr, Val}$ were highly purified and yeast $tRNA^{Leu, Ser, Pro}$ were partially purified in our laboratory by G. Keith, J. Weissenbach and B. Kuntzel using a counter- current distribution followed by classical column chromatography techniques.

Correct aminoacylations were performed using the classical conditions [10]. Incorrect aminoacylations were performed as previously described [11]. Valyl-adenosine was prepared by hydrolysis of Val-$tRNA^{Val}$ by pancreatic RNAase according to Madison et al. [12]. The product was used without further purification.

Deacylation reactions were generally performed in the following medium: glutathione 2.5 mM; Mg^{2+} 25 mM; KCl 3.3 mM; Tris-HCl 5.5 mM, pH 7.4; bovine serum albumin 100 μg/ml. After incubation, aliquots were removed from the medium and put on Whatman 3MM paper discs which were submitted to several acid and alcoholic washes in order to eliminate the free amino acid. All the incubations were performed at 37°.

Deacylation of valyl-adenosine was conducted in the same way as for the aminoacyl-tRNA's with the difference that the reaction was stopped by lowering the pH of the medium to 4 at 0°, then [^{14}C]valine and [^{14}C]valyl-adenosine were separated by paper electrophoresis according to Sanger et al. [13].

3. Results

3.1. *Deacylation of Val- and Phe-$tRNA^{Val}$ and Val- and Phe-$tRNA^{Phe}$ by VRS and PRS*

The kinetics of the non enzymic and of the VRS and PRS catalysed deacylation of these aa-tRNA's are shown in fig. 1. The initial velocities which can be drawn from these data are summarised in table 1.

3.2. *Deacylation of valyl-adenosine by VRS and PRS*

Fig. 2 shows that neither VRS nor PRS deacylate at a significant extent valyl-adenosine, while the values of deacylation observed without enzyme show that Val-adenosine is slightly more labile than Val-tRNA in the conditions used.

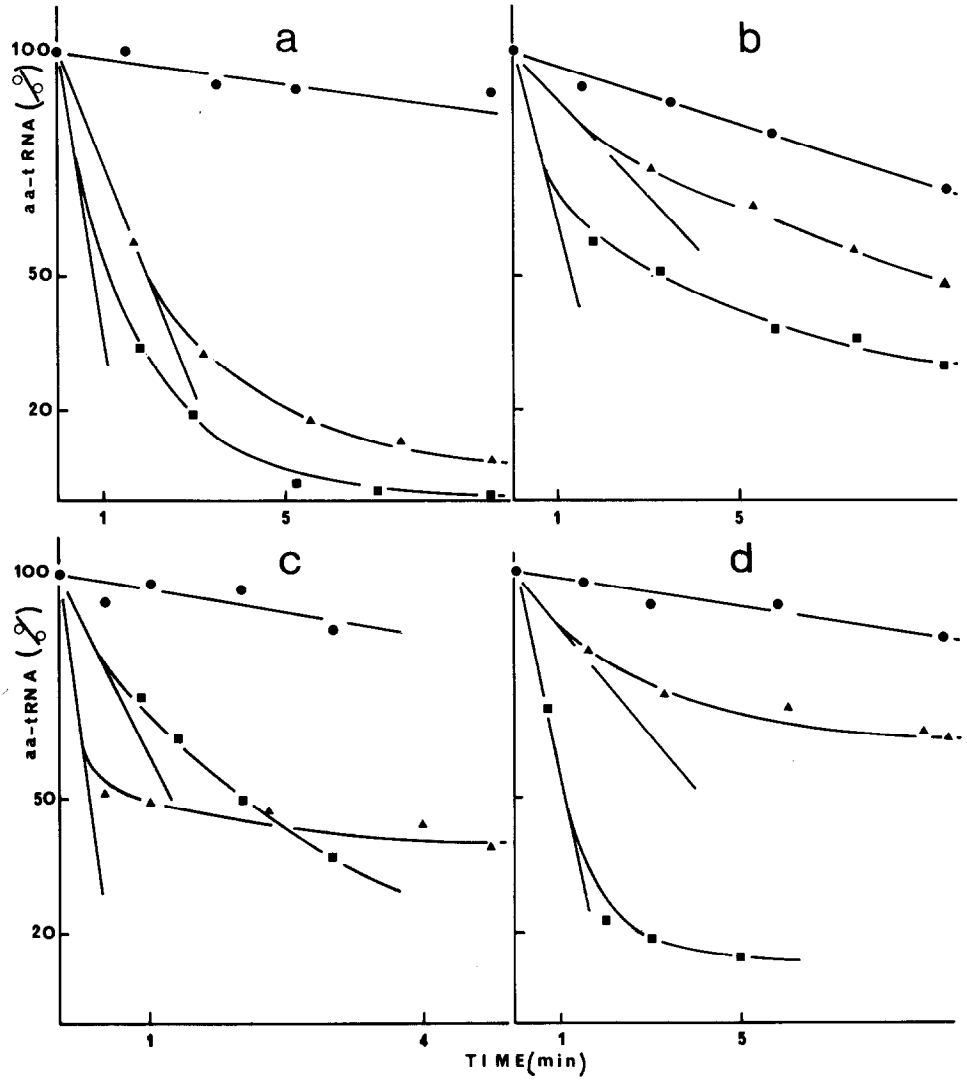

Fig. 1. Deacylation of Val-tRNA^{Val} (1a), Phe-tRNA^{Val} (1b), Phe-tRNA^{Phe} (1c) and Val-tRNA^{Phe} (1d). Aminoacyl-tRNA alone: (●—●—●); aminoacyl-tRNA + VRS:(▲—▲—▲); aminoacyl-tRNA + PRS:(■—■—■). As incorrect aminoacylations were not complate, aa-tRNA and tRNA concentrations were always adjusted respectively to 0.2 μM and 1.1 μM. PRS concentration was (1a): 0.6 μM, (1b): 1.5 μM; (1c) 0.3 μM and (1d): 3 nM. VRS concentration was (1a): 0.33 μM; (1b), (1c), (1d): 1.7 μM.

3.3. *Influence of the presence of amino acid in the VRS and PRS catalysed deacylation of Val-tRNA^{Val}*

The initial rates of the deacylation kinetics described in fig. 3 show that, in the case of either VRS or PRS, the amino acid has no inhibitory effect.

3.4. *VRS and PRS catalysed deacylation of various aa-tRNA's*

Figs. 4 and 5 show that the deacylation varies greatly from one aa-tRNA to another.

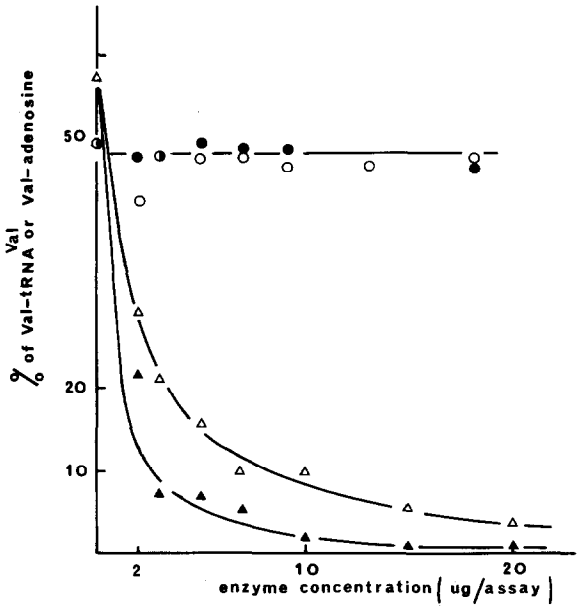

Fig. 2. VRS and PRS catalysed deacylation of Val-tRNA^{Val} and Val-adenosine. *Circles* : for each assay 2.3 μg of Val-tRNA^{Val} were first hydrolysed by 0.5 μg of pancreatic RNAase during 30 min in the standard medium without Mg^{2+}. Then Mg^{2+} was added at a concentration of 2.5 mM and the hydrolysate was incubated 30 min with different amounts of VRS (○—○—○) and PRS (●—●—●). The final volume was 20 μl. The deacylation was measured as described in Methods. *Triangles* : for each assay 2.3 μg of Val-tRNA^{Val} were treated as above, but the first incubation was performed without RNAase. (△—△—△): incubation with VRS; (▲—▲—▲) incubation with PRS.

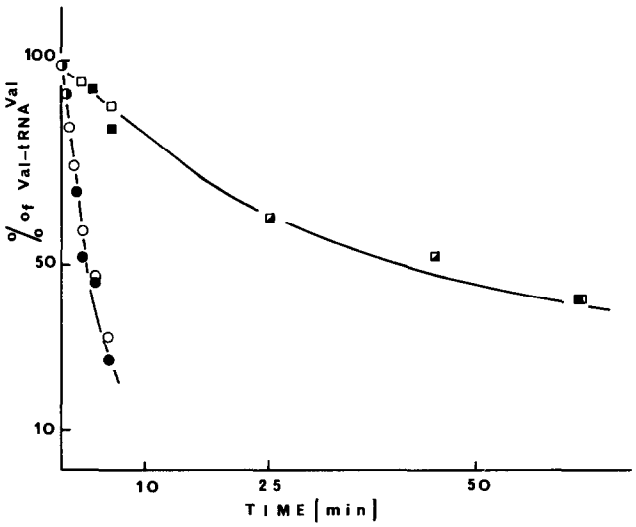

Fig. 3. Influence of the addition of valine and phenylalanine on the deacylation of Val-tRNA^{Val} by VRS and PRS. ○ Val-tRNA^{Val}: 0.72 μM; VRS: 0.21 μM. (●—●—●) Val-tRNA^{Val}: 0.72 μM; VRS: 0.21 μM; valine: 1 mM. (□—□—□) Val-tRNA^{Val}: 1.0 μM; PRS: 30 nM. (■—■—■) Val-tRNA^{Val} 1 μM; PRS: 31 nM; phenylalanine: 1 mM. The curves are corrected for the spontaneous deacylation.

4. Discussion and conclusion

The main observation to be pointed out is a broad lack of specificity in the aminoacyl-tRNA synthetase catalysed deacylation of the aminoacyl-tRNA's. Indeed, the results of table 1 show that VRS and PRS both deacylate equally well the Val-tRNA^{Val} and the Phe-tRNA^{Phe}. In the case of PRS, there is even a much greater deacylation of Val-tRNA^{Phe} than of Phe-tRNA^{Phe}. The results of figs. 4 and 5 make possible a generalisation of the preceding results with other aa-tRNA's. Although their tRNA moiety is neither tRNA^{Phe} nor tRNA^{Val}, they are deacylated by the VRS and the PRS to various extents, with a few exceptions.

An interesting observation has been reported by Eldred and Schimmel [7] who have shown that Ile-tRNA synthetase (IRS) from *E. coli* can deacylate *E. coli* Ile-tRNA^{Ile} and Val-tRNA^{Ile}, but not Phe-tRNA^{Ile}. Relevant to this observation is the fact that IRS is able to activate not only isoleucine, and also valine but not phenylalanine [2]. This could argue in favour of the necessity of a specific recognition of the amino acid by the enzyme in the deacylation reaction. However, our observations reported in table 1 and figs. 4 and 5 show that the aminoacyl-tRNA's with amino acids which are not activated by the aminoacyl-tRNA synthetase are strongly deacylated. Actually, it is impossible to derive any rule concerning the rates of deacylation according to the nature of the tRNA or of the aa-tRNA's.

This lack of specificity in the deacylation reaction of aminoacyl-tRNA's could lie either at the amino acid level or at the tRNA level. In the first case, it could be imagined that the aa-tRNA synthetase can recognise more or less any amino acid. In this case, the enzyme would bind the aminoacyl-tRNA through the amino acid moiety and it would split the ester bond without needing to recognise the tRNA. But many observations suggest that this hypothesis is

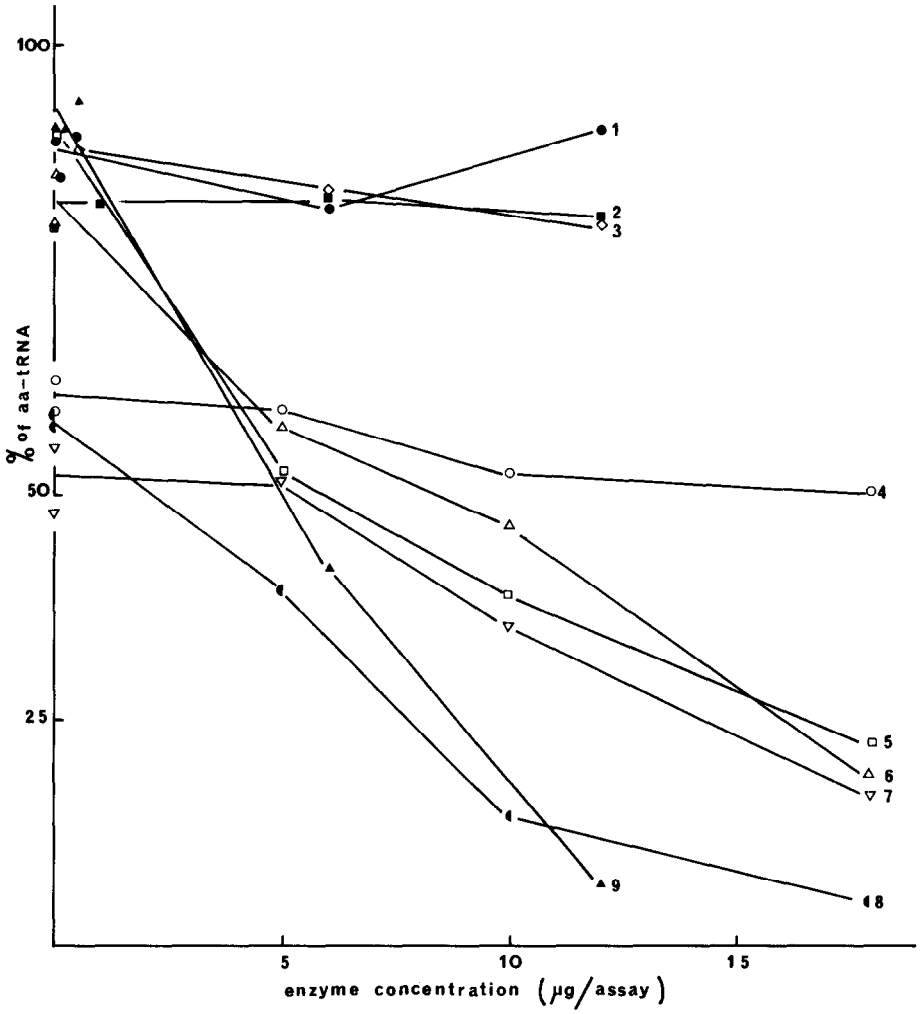

Fig. 4. Deacylation of various aminoacyl-tRNA's by VRS. The aminoacyl-tRNA's were incubated during 30 min with increasing amounts of VRS. The medium (100 µl) contained about 2 µg of nucleic material. 1: Asp-tRNA^{Asp}; 2: Gly-tRNA^{Gly}; 3: Tyr-tRNA^{Tyr}; 4: Arg-tRNA$^{Arg}_{II}$; 5: Trp-tRNA^{Trp}; 6: Arg-tRNA$^{Arg}_{III}$; 7: Pro-tRNA^{Pro}; 8: Ala-tRNA^{Ala}; 9: Ser-tRNA^{Ser}.

unlikely and that the tRNA moiety is involved in the recognition step.

It has been reported that the tRNA and the aminoacyl-tRNA bind to the enzyme at the same site [6, 14, 15]. In our hands, the Val-tRNA^{Val} behaved as a competitive inhibitor of tRNA^{Val} in the aminoacylation reaction catalysed by the yeast Val-tRNA synthetase [21]. On the other hand, it has been shown that tRNA^{Ile} from *E. coli* is a competitive inhibitor of Ile-tRNA^{Ile} in the Ile-tRNA synthetase-catalysed deacylation [4].

Another argument is the result shown in fig. 2 that valyl-adenosine is not enzymatically deacylated.

Lastly, a 100 to 500-fold K_m concentration of the cognate amino acid has no effect on the deacylation rate of Val-tRNA^{Val} by the VRS and PRS as shown in fig. 3. However, in the case of the deacylation of Ile-tRNA^{Ile} by IRS, it has been reported that isoleucinyl-AMP or isoleucine are inhibitors, but not competitive inhibitors, in the Ile-tRNA^{Ile} deacylation [4].

For all these reasons, it is unlikely that the amino acid is the single part of the aminoacyl-tRNA which is

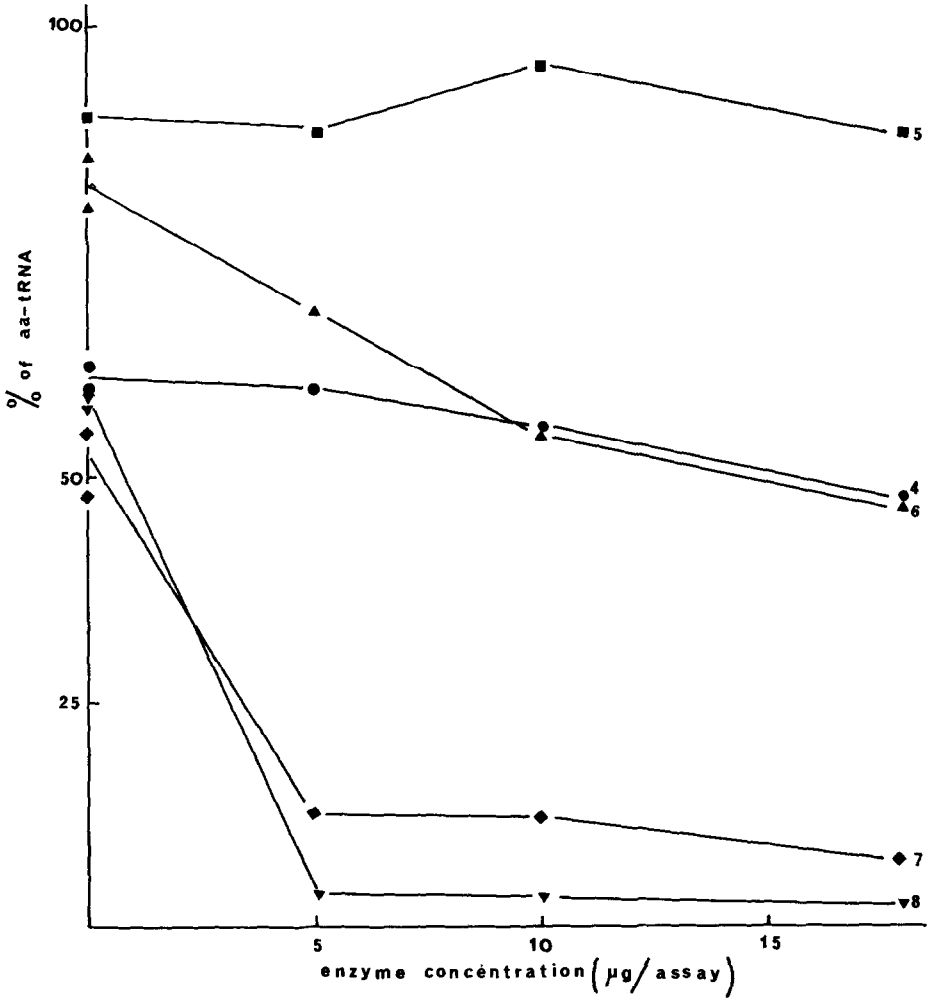

Fig. 5. Deacylation of various aminoacyl-tRNA's by PRS. The aminoacyl-tRNA's were incubated during 30 min with increasing amounts of PRS. The medium (100 µl) contained about 2 µg of nucleic material. 4: Arg-tRNA$^{Arg}_{II}$; 5: Trp-tRNA^{Trp}; 6: Arg-tRNA$^{Arg}_{III}$; 7: Pro-tRNA^{Pro}; 8: Ala-tRNA^{Ala}.

recognised by the enzyme and we must admit that in the deacylation reaction of aminoacyl-tRNA's a functional interaction takes place between the enzyme and the tRNA moiety. As non cognate aminoacyl-tRNA's are split by a given aminoacyl-tRNA synthetase (table 1, figs. 4 and 5), it can be concluded that the enzyme is able to recognise, in the deacylation reaction, non cognate tRNA's. It is interesting to mention, in relation to these results, observations made by Rigler et al., who described interactions between yeast seryl-tRNA synthetase and yeast tRNA^{Phe} and tRNA^{Val} [16].

This lack of specificity in the deacylation reaction is rather surprising compared to the high specificity of the aminoacylation reaction. As our results suggest that, in the deacylation reaction, the recognition of the aminoacyl-tRNA synthetase takes place on the tRNA moiety of this substrate, one should observe the same lack of specificity in the aminoacylation reaction and numerous incorrect aminoacylations should take place. This is not the case, as such incorrect

Table 1
Comparison of initial rates of deacylation of Val- and Phe-tRNA^{Phe} and Val- and Phe-tRNA^{Val} by VRS and PRS.

aa-tRNA	Rate of deacylation by VRS	Rate of deacylation by PRS
Val-tRNA^{Val}	73	100
Phe-tRNA^{Val}	21	7
Phe-tRNA^{Phe}	36	120
Val-tRNA^{Phe}	6	15 000

The rates (expressed in arbitrary units) were corrected for the rate of spontaneous deacylation and normalised for enzyme concentration.

aminoacylations are only observed in special aminoacylation conditions [11, 17–20]. Therefore, it must be questioned whether the high specificity in the aminoacylation reaction is exclusively linked to the recognition between the tRNA and the aminoacyl-tRNA synthetase, and if this recognition is as specific as is generally believed. One could imagine that the great difficulty experienced in performing an aminoacylation with an incorrect tRNA in normal aminoacylation conditions is not only due to the lower affinity of the wrong tRNA for the enzyme, but also to a non ideal position of the CCA end of the tRNA which lowers considerably or annuls the rate of the transacylation reaction. When special aminoacylation conditions are used [11, 17–20], conformational changes may be induced either at the tRNA or at the enzyme level which favour the transacylation reaction, thus leading to incorrect aminoacylations.

As for the deacylation reaction, our results suggest that the amino acid moiety of the aminoacyl-tRNA is not bound to the enzyme in the same way as in the aminoacylation reaction. Thus the steric requirements in the reaction between the tRNA and amino acid do not exist in the deacylation reaction. The differences in the specificity of the aminoacylation and the deacylation reactions could lie in this fact.

Acknowledgements

We thank Miss M. Delfau for her skillful technical assistance. This work was supported by the CNRS (Laboratoire associé 119), the Délégation Générale à la Recherche Scientifique et Technique (Action Concertée Interactions Moléculaires en Biologie) and by the Commissariat à l'Energie Atomique.

References

[1] N. Befort, F. Fasiolo, C. Bollack and J.P. Ebel, Biochim. Biophys. Acta 217 (1970) 319.
[2] P. Berg, F.H. Bergmann, E.J. Ofengand and M. Dieckmann, J. Biol. Chem. 236 (1961) 1726.
[3] U. Lagerkvist, L. Rymo and J. Waldenström, J. Biol. Chem. 241 (1966) 5391.
[4] A. Schreier and P. Schimmel, Biochemistry 11 (1972) 1583.
[5] J. Waldenström, European J. Biochem. 5 (1968) 239.
[6] M. Yaniv and F. Gros, J. Mol. Biol. 44 (1969) 17.
[7] E. Eldred and P.R. Schimmel, J. Biol. Chem. 247 (1972) 2961.
[8] F. Fasiolo, N. Befort, Y. Boulanger and J.P. Ebel, Biochim. Biophys. Acta 217 (1970) 305.
[9] D. Kern, Thesis (1972) Université L. Pasteur, Strasbourg.
[10] J.H. Weil, Bull. Soc. Chim. Biol. 51 (1969) 1479.
[11] D. Kern, R. Giegé and J.P. Ebel, European J. Biochem. (1972), submitted for publication.
[12] J.T. Madison, G.A. Everett and H. Kung, J. Biol. Chem. 246 (1967) 1318.
[13] F. Sanger, G. Brownlee and B. Barrell, J. Mol. Biol. 13 (1965) 373.
[14] M. Yarus and P. Berg, J. Mol. Biol. 42 (1969) 171.
[15] P. Rouget and F. Chapeville, European J. Biochem. 23 (1971) 443.
[16] R. Rigler, E. Cronvall, R. Hirsh, U. Pachmann and H. Zachau, FEBS Letters 11 (1970) 320.
[17] R. Taglang, J.P. Waller, N. Befort and F. Fasiolo, European J. Biochem. 12 (1970) 550.
[18] P. R. Ritter, F. Kull and K. Jacobson, Biochim. Biophys. Acta 179 (1969) 524.
[19] R. Giegé, D. Kern, J.P. Ebel and R. Taglang, FEBS Letters 15 (1971) 281.
[20] R. Giegé, D. Kern and J.P. Ebel, Biochimie (1972), submitted for publication.
[21] J. Bonnet and J.P. Ebel, unpublished results.