FRAGMENTS OF YEAST tRNA^{Phe} AND tRNA^{Ser} PREPARED BY PARTIAL DIGESTION WITH SPLEEN PHOSPHODIESTERASE

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Received 2 April 1971
Original figures received 29 April 1971

1. Introduction

Razzel and Khorana [1] characterized the phosphodiesterase from spleen (PDE) as an exonuclease which degrades nucleic acids from the 5′-end under the formation of 3′-mononucleotides. The enzyme which had been further purified by A. and G. Bernardi [2] was used for the digestion of yeast tRNA^{Ser} by Bernardi and Cantoni [3]. The degradation of tRNA with and without the 5′-phosphate was compared and the loss of biological activities during the course of digestion was followed [3].

A detailed investigation by disc electrophoresis revealed that the PDE degrades some sequences of the tRNA quite fast while it is retarded at certain other regions of the tRNA structure. This allowed us to isolate a number of otherwise unavailable fragments of yeast tRNA^{Phe} and tRNA^{Ser} which extended from the retardation points to the 3′-terminal CCA sequence (fig. 1). The results of amino acid acceptance assays with various fragment combinations are reported.

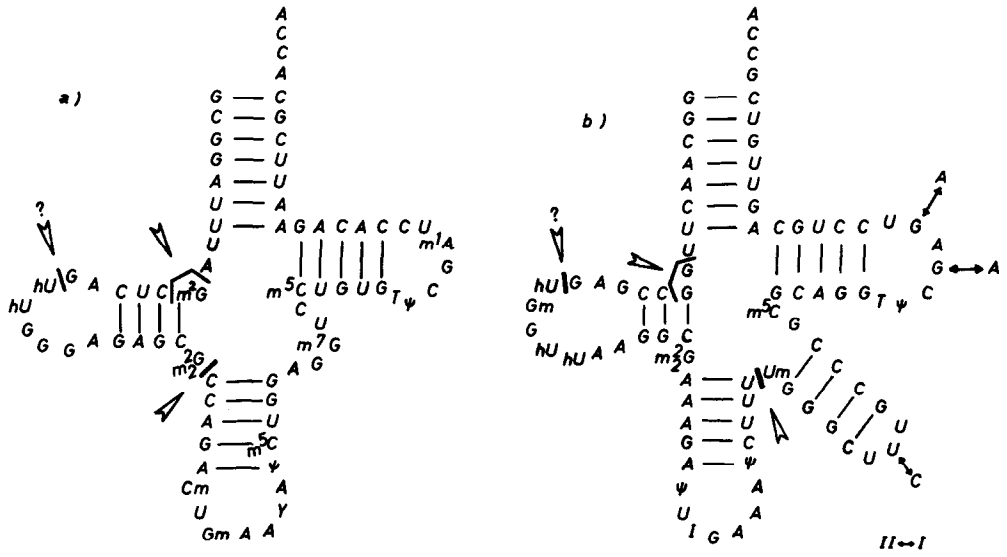

Fig. 1. Cloverleaf models of tRNA^{Phe} (a) and tRNA$^{Ser}_{I+II}$ (b) from yeast. The points or regions of retarded degradation by PDE are indicated.

2. Materials and methods

$tRNA^{Ser}_{I+II}$ and $tRNA^{Phe}$ were prepared from brewer's yeast tRNA [4, 5]. Only tRNAs from which the 5'-terminal phosphate had been removed [6] were used. The fragments Phe 1–36, Phe 1–15, and Ser 1–34 were kindly donated by R. Thiebe, K. Harbers, and F. Fittler, respectively. Endonuclease free PDE was prepared from hog spleen following the procedure [2] up to the Sephadex G 75 step (specific activity 400 units/A_{280} unit) and stored at +4° as suspension in 80% $(NH_4)_2SO_4$. 200-fold purified phenylalanyl and seryl tRNA synthetases and 125-fold purified CCA-nucleotidyl transferase from yeast were kindly provided by R. Hirsch and H. Overath, respectively.

tRNA degradation with PDE was at 37° (see legends to figs. 2 and 3). In analytical experiments aliquots containing about 0.1 A_{260} units were pipetted into an equal volume of cold 50% sucrose, 7 M urea, and submitted to disc electrophoresis in gels of 12% acrylamide and 1% bisacrylamide with the pH 8.9 buffer system of [7] in 7 M urea. The electrophoresis was performed as in [5]. On preparative scale, PDE digests of tRNAs were separated by electrophoresis in the same buffer on 1 cm thick gel slabs containing 11% acrylamide and 0.5% bisacrylamide. The tRNA fragments were eluted and further purified by filtration over small DEAE cellulose urea columns.

3. Results

Digests of $tRNA^{Phe}$ with spleen PDE showed a number of peaks in densitograms of disc electrophoreses (e.g. fig. 2a). From kinetic studies (fig. 2b) it is clear that the tRNA itself was degraded rather fast while the other peaks appear in different amounts at the various times. There were no absolute 'stop points'; with more PDE or longer incubation times tRNA could be degraded completely to mononucleotides. Obviously the course of degradation was retarded at certain positions of the $tRNA^{Phe}$ structure. A similar situation was observed with $tRNA^{Ser}$ (fig. 3), $tRNA^{Tyr}$, and $tRNA^{Val}$ from yeast. The degradation patterns depended strongly on the specific salt concentrations in the incubation mixture, on the pH and on the temperature. The effect of ammonium salts is shown in fig. 3. Mg^{2+} (1 mole/mole nucleotide) inhibited the degradation largely or, under certain conditions, completely.

Table 1
Phe and Ser incorporations into fragment combinations.

Fragment combination		Amino acid incorporation
1. Phe 10/11–76	+ Phe 1–15	930 (65%)
2. Phe 10/11–76	+ Phe 1–36	550 (38%)
3. Phe 16(?)–76	+ Phe 1–15	540 (34%)
4. Phe 16(?)–76	+ Phe 1–36	400 (25%)
5. Phe 27–76	+ Phe 1–15	< 50 (< 2.5%)
6. Phe 27–76	+ Phe 1–36	970 (51%)
7. Ser 9/11–85	+ Ser 3–9	810 (65%)
8. Ser 9/11–85	+ Ser 1–34	1050 (83%)
9. Ser 16(?)–85	+ Ser 1–34	830 (62%)
10. Ser 44–85	+ Ser 1–34	1020 (46%)

0.02–0.04 A_{260} units CCA-fragment were preincubated with pG-fragment (2 to 3-fold molar excess, 6-fold in no. 5, 10-fold in no. 7) in 0.08 ml 90 mM KCl, 3 mM $MgCl_2$, 6 mM cacodylate, pH 7.0, at 37° for 15 min. After addition of 5 nmoles ^{14}C-Phe or ^{14}C-Ser, 1 μmole ATP, 1.5 μmole $MgCl_2$, 2.5 μmoles tris-HCl, pH 7.5, 100 μunits Phe-, 70 μunits Ser-tRNA synthetases [11] and, for the $tRNA^{Phe}$ fragments, 20 μunits CCA-nucleotidyl transferase [12] in 20 μl, the mixtures were incubated at 20° (10° in no. 7). Work-up and counting were as in [13]. Incubations were up to 140 min. All numbers were plateau values (except no. 5) and are expressed in pmoles Phe (1–6) or Ser (7–10) per A_{260} unit of the respective CCA-fragment. Values in parenthesis are percent incorporation relative to 1250 pmoles Phe/A_{260} unit $tRNA^{Phe}$ or 1100 pmoles Ser/A_{260} unit $tRNA^{Ser}$. There was no incorporation into the CCA-fragments alone except into Ser 9/11–85 (see fig. 5 and text).

For preparative purposes the same conditions were used as in the analytical experiments ($tRNA^{Phe}$ fragments 2 min incubation; Ser 9/11–85 and Ser 16 (?)–85 3 min and Ser 44–85 15 min incubation). From a degradation of 100 A_{260} units $tRNA^{Phe}$ 15 A_{260} units Phe 10/11–76, 6 A_{260} units Phe 16(?)–76 and 15 A_{260} units Phe 27–76 were isolated on the average. 10 A_{260} units Ser 9/11–85 and 17 A_{260} units of Ser 16(?)–85 were obtained from 70 A_{260} units $tRNA^{Ser}$ and 40 A_{260} units Ser 44–85 from 200 A_{260} units $tRNA^{Ser}$.

Phe 27–76 and Ser 44–85 were homogeneous on analytical disc electrophoreses. In the oligonucleotide analysis of Phe 27–76 (fig. 4a) CpCpApGp was found in the usual amount, while no m^2_2G could be detected.

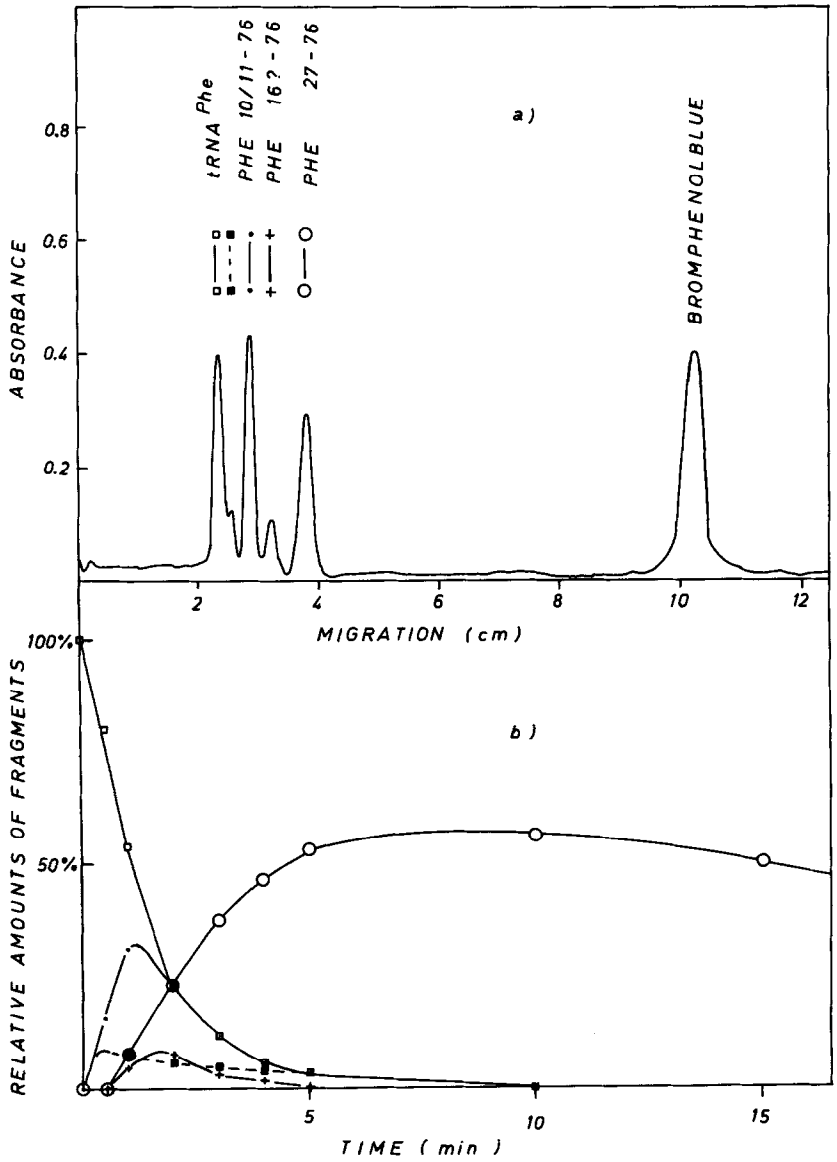

Fig. 2. Degradation of $tRNA^{Phe}$ with PDE. 1.8 A_{260} units tRNA and 4.6 units [2] PDE in 0.23 ml 75 mM K-phosphate, pH 6.0, 25 mM $(NH_4)_2SO_4$. (a) Densitograms of a disc electrophoretic separation of one aliquot after 2 min incubation. (b) Kinetics of the degradation. The peak areas of a series of densitograms are plotted. The symbols correspond to those indicated in part (a) of this figure.

In Ser 44–85 (fig. 4b) 1.0 mole UmpGp was present. The other fragments were contaminated, according to analytical disc electrophoresis, by up to 5% of material from neighboring bands. In the oligonucleotide analysis of Phe 10/11–76 m^2G was detected but we are not sure yet whether it is present in the fragment in a molar amount. In Phe 16(?)–76 and Ser 16(?)–85 the same amounts of hU were found [8] as in the respective tRNAs. The amount of Ser 16(?)–85 was greatly enhanced when the hU residues were reduced with $NaBH_4$ [9] prior to the PDE digestion. The oligonucleotide analysis of the longest $tRNA^{Ser}$ fragment did not allow a more exact designation than Ser 9/11–85. No analytical data were obtained for

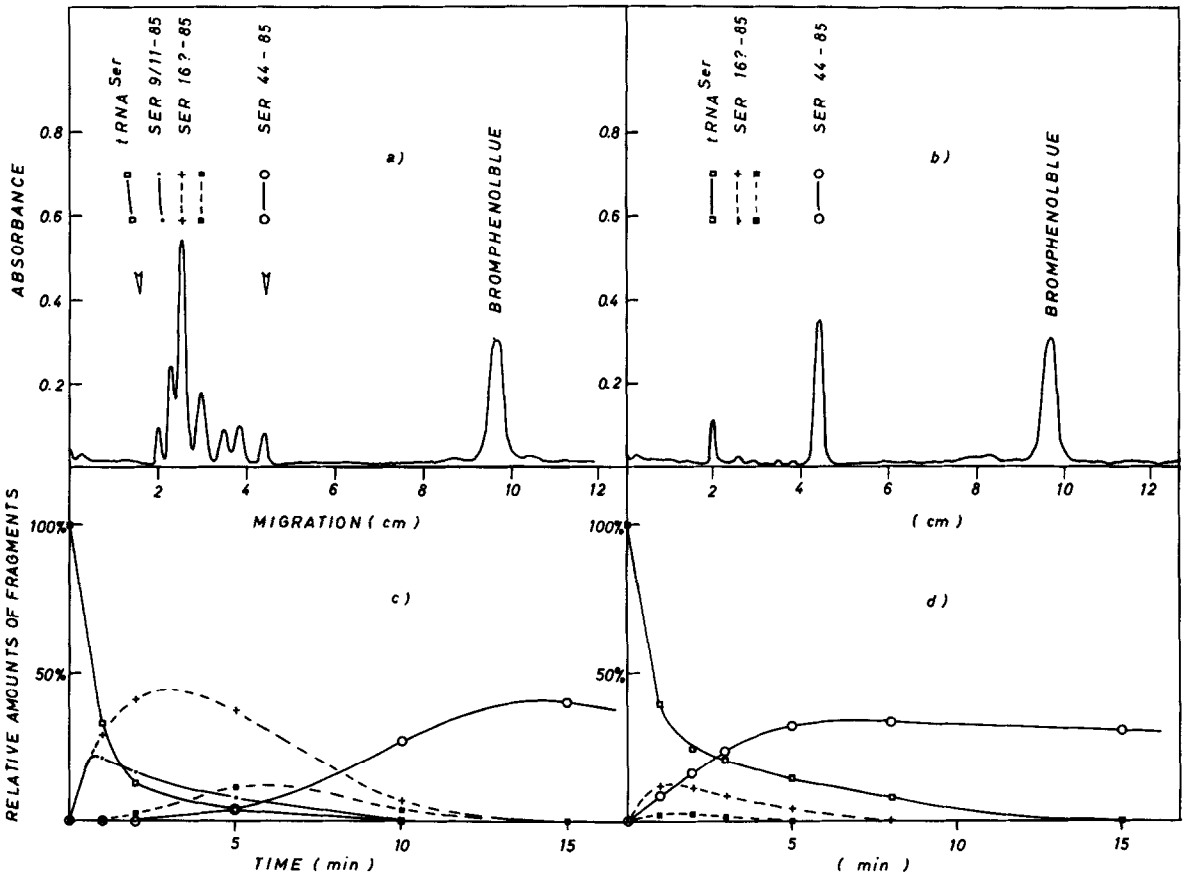

Fig. 3. Degradation of $tRNA^{Ser}$ with PDE. 4.4 A_{260} units tRNA and 20 units [2] PDE in 0.21 ml of 0.1 M sodium acetate, pH 6.2, with (a, c) and without (b, d) 35 mM ammonium acetate–15 mM $(NH_4)_2SO_4$. (a, b) Densitograms of disc electrophoretic separations after 5 min. (c, d) Kinetics of the degradations plotted as in fig. 2b.

the fragment in the shoulder of the $tRNA^{Phe}$ peak (fig. 2a) and for the fragments in the three peaks between Ser 16(?)–85 and Ser 44–85 (fig. 3a).

The results of acceptor activity assays are reported in table 1. Some values may still be increased by variations in the assay conditions. The Ser incorporation into fraction Ser 9/11–85 (fig. 5) was partly due to contaminating $tRNA^{Ser}$ (less than 7% according to disc electrophoresis and oligonucleotide analysis). From the analytical data we would have estimated an incorporation of less than 100 pmoles Ser/A_{260} unit of the fragment fraction. The much higher incorporation, particularly at low temperatures, may be attributed to aminoacylation of the fragment itself. An unequivocal statement, however, will be possible only with a completely pure fragment. The decrease of incorporation with time at the higher temperatures (fig. 5) was probably due to a slow inactivation of the synthetase. In the experiments with fragment combinations a high excess of Ser 3–9 was necessary since association of this heptanucleotide with itself competed with the association to Ser 9/11–85.

4. Discussion

The results reported in this paper contribute to the understanding of spleen PDE action. Apparently certain odd nucleotides as well as some base paired regions of the cloverleaf models retard and, with that, synchronize the degradation. A similar situation was observed in degradations of tRNAs with snake venom PDE [14].

Fragment combinations which contained more nucleotides than the tRNAs themselves accepted the amino acids quite well. Parts of the hU arm of

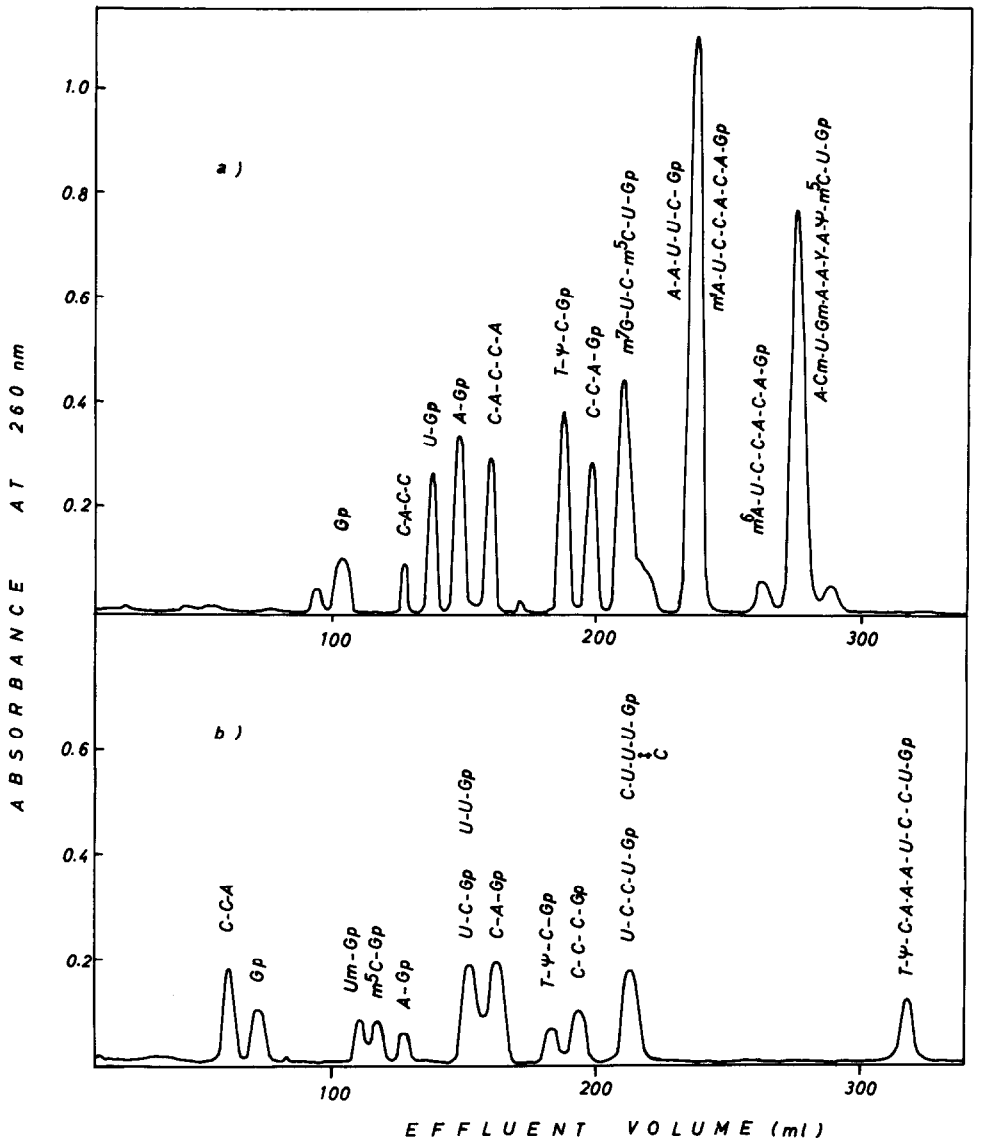

Fig. 4. Chromatographies of complete T1-RNase digests of 22 A_{260} units Phe 27–76 (a) and 9 A_{260} units Ser 44–85 (b). Conditions as in fig. 6 of ref. 9.

$tRNA^{Phe}$ seem to be important for activity. In $tRNA^{Ser}$ the two 5′-terminal nucleotides and parts of the anticodon stem could be excised without complete loss of activity. These findings are interesting also in relation to recent work on fragments of yeast $tRNA^{Val}$ [15] and *E. coli* $tRNA^{fMet}$ [16]. The results will be discussed in more detail at a later date together with other experiments on fragments of yeast $tRNA^{Phe}$ and $tRNA^{Ser}$ (R. Thiebe, K. Harbers, F. Fittler, unpublished).

Acknowledgements

We thank Miss S. Notz for help with the incorporation assays. The work was supported by Deutsche Forschungsgemeinschaft, SFB 51, and by Fonds der Chemischen Industrie.

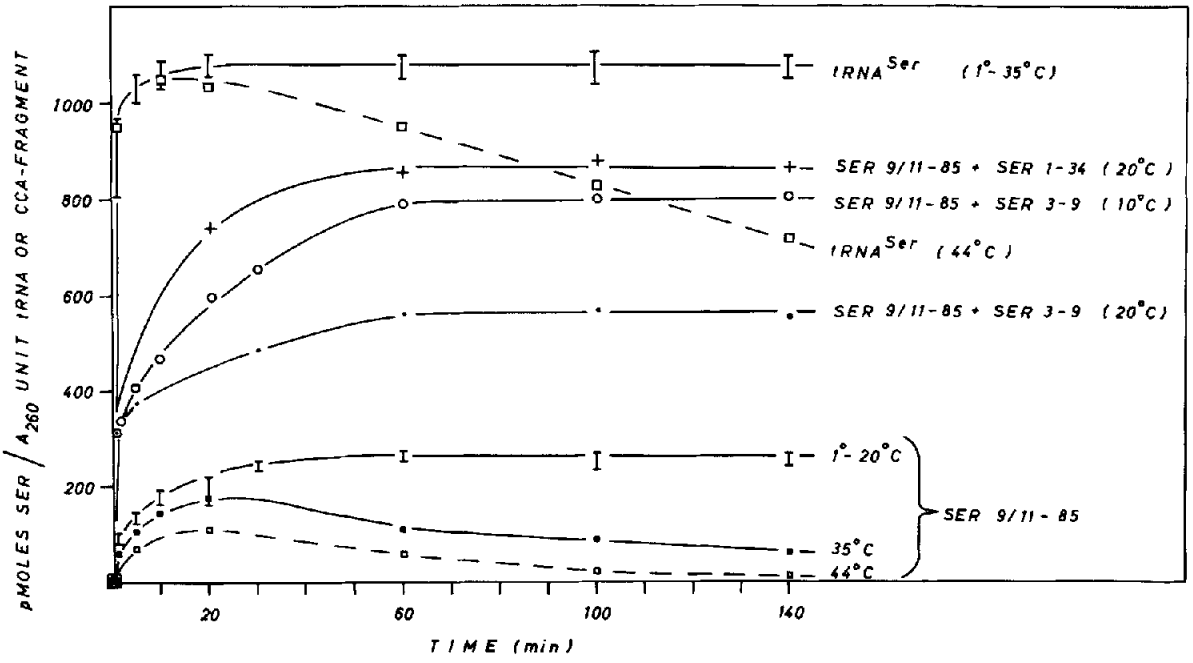

Fig. 5. Acceptor activity of tRNA^{Ser}, fragments, and fragment combinations. Conditions were as in table 1.

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