ACCEPTOR ACTIVITY OF VALINE tRNA MODIFIED WITH CME-CARBODIIMIDE. HETEROGENEITY OF MODIFIED tRNA*

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Received 22 February 1971

It was found recently in this laboratory that the valine-acceptor activity of unfractionated yeast tRNA was largely retained after extensive modification with CME-carbodiimide in the presence of Mg^{2+} ions [1]. At increased Mg^{2+} or NaCl concentrations this modified tRNA incorporated even more valine than did native tRNA. The result was explained in terms of the model [1] proposed to explain the unusual kinetics of tRNA reaction with CME-carbodiimide [2]. The model assumes that the three-dimensional structure of modified tRNA is fixed by intramolecular interaction of the positively charged CME-nucleoside residues with the negatively charged polyribose phosphate backbone.

The present paper is concerned with studies of the acceptor activity of pure $tRNA^{Val}$ modified with CME-carbodiimide.

Valine tRNA was isolated by chromatography on DEAE-Sephadex [2] followed by reverse-phase chromatography [3]. The preparation obtained incorporated 1400 pmoles of valine per $A_{260\ nm}$ unit (measured in 0.02 M $MgSO_4$ – 0.1 M *N*-methylmorpholinium chloride pH 7.5) on aminoacylation with crude valine-tRNA-ligase from rat liver†. The relative content of $tRNA_1^{Val}$ determined by terminal oligonucleotides analysis [4] was 65%. ^{14}C-CME-carbodiimide (approx. 1 Ci/mole) was obtained as described earlier [5] from ^{14}C-methyl *p*-toluenesulphonate.

Fig. 1 shows the kinetics of $tRNA^{Val}$ modification. The experimental data are in accord with the assumption that the apparent average second-order rate constant of the reaction of tRNA with CME-carbodiimide decreases exponentially with increasing extent of modification [1]. The low value of k_0 in the presence of Mg^{2+} ions indicates that no more than 1–2 exposed nucleosides are present in native tRNA conformation, assuming that exposed nucleosides have the same reactivity towards CME-carbodiimide as free nucleosides in solution; cf [7, 8].

Fig. 2 shows the change of acceptor activity of $tRNA^{Val}$ in the course of modification. Each value of acceptor activity plotted in fig. 2A was obtained in a kinetic experiment (fig. 2B, C) in order to be sure that the maximum extent of aminoacylation was achieved. It is seen that $tRNA^{Val}$ like unfractionated tRNA [1] retains high valine-acceptor activity after extensive modification in the presence of Mg^{2+} ions. On the contrary, modification in the absence of Mg^{2+} ions results in a rapid loss of valine-acceptor activity. In terms of our model [1] this can be due to fixation of non-native conformations of tRNA that predominate under the conditions. It should be mentioned, however, that even at extremely high *average* extent of modification (n = 17.4) modified tRNA still retains measurable acceptor activity (about 5%) (fig. 2A, B).

The partial drop of valine-acceptor activity on modification suggested that modified tRNA could be significantly heterogenous. To study the heterogenity, we obtained in the presence of Mg^{2+} ions

* CME-carbodiimide is *N*-cyclohexyl,*N'*-β-(4-methylmorpholinium)-ethylcarbodiimide *p*-toluenesulphonate; CME-nucleosides: nucleosides modified with CME-carbodiimide. The present results have been partially reported at the Presymposium on tRNA held by IUPAC in Riga, 1970.

† Valine-tRNA obtained was not specially treated to recover the terminal –C–C–A sequence.

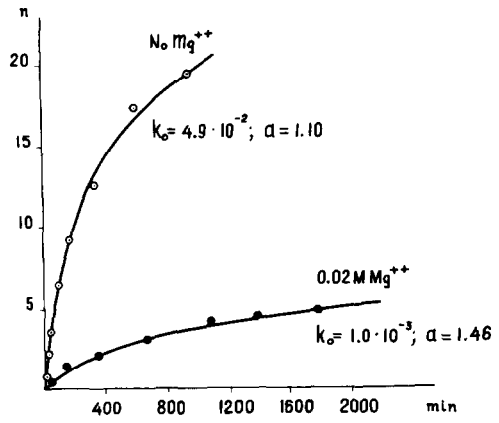

Fig. 1. Kinetics of $tRNA^{Val}$ reaction with CME-carbodiimide. ○, ● experimental data; solid lines, best fit functions obtained by choice of parameters k_0 (apparent average second-order rate constant at zero time, M^{-1} min^{-1}) and a (inhibition coefficient) in equations assuming exponential drop of apparent average second-order rate constant in the course of tRNA modification with CME-carbodiimide [1] (for mathematics, see also accompanying paper [6]); n is the extent of modification, moles of CME-nucleosides per mole of tRNA. The reactions were run at 25° and pH 8.0 in 0.1 M *N*-methylmorpholinium chloride buffer. The extents of modification were determined by paper disc method (precipitation with ethanol–2 M NaCl, 2:1) as described earlier [1]. The molar extinction coefficient of $tRNA^{Val}$ in 0.1 M *N*-methylmorpholinium buffer–0.02 M $MgSO_4$ was assumed to be 5×10^5 M^{-1} cm^{-1} on the basis of the calculated extinction coefficient of $tRNA_1^{Val}$ alkaline hydrolysate in acidic medium 7.9×10^5 M^{-1} cm^{-1} [2] and of specially determined hyperchromicity coefficient.

○ $tRNA^{Val}$ 17 A units per ml; 0.05 M ^{14}C-CME-carbodiimide;

● $tRNA^{Val}$ 110 A units per ml; 0.02 M $MgCl_2$; 0.19 M ^{14}C-CME-carbodiimide.

an extensively modified (n = 7.8) preparation of $tRNA^{Val}$ (fig. 3A). This tRNA retained about 75% of the starting valine-acceptor activity (fig. 2A, triangles); the relative content of $tRNA_1^{Val}$ estimated by analysis of terminal oligonucleotides was also the same as that in the starting preparation (65%). The preparation was chromatographed on DEAE-cellulose at pH 5. As seen in fig. 3B, the preparation in fact appeared heterogenous. In the left-hand part of the profile the extent of modification is very high (n = 24) and there is no acceptor activity. In the right-hand part there is a large, relatively homogenous fraction (n = 6) with acceptor activity practically equal to that

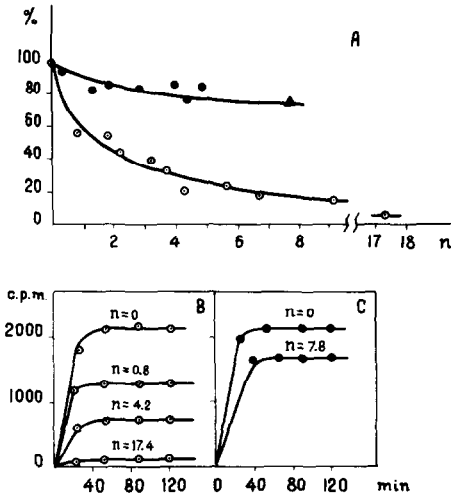

Fig. 2. Inactivation of $tRNA^{Val}$ caused by modification with CME-carbodiimide. (A) dependence of the acceptor activity on the extent of modification n, % of the starting preparation. ○ modification in the absence of Mg^{2+}; ● modification in the presence of Mg^{2+} (kinetics of the modification of $tRNA^{Val}$ in this experiment is shown in fig. 1). ▲ modified $tRNA^{Val}$ from another experiment (fig. 3). To study the acceptor activity, aliquots were removed from the modification reaction mixture and passed after 10-fold dilution with water through small columns with CM-Sephadex C-50 Na^+ 'medium'. Modified tRNA was eluted with water together with *p*-toluenesulphonate. This solution of modified tRNA was used directly for enzymatic aminoacylation. Each value of the acceptor activity plotted is the maximum extent of aminoacylation determined in a kinetical experiment. A few examples of the kinetics of aminoacylation are presented in fig. 2B (modification in the absence of Mg^{2+}) and fig. 2C (modification in the presence of Mg^{2+}). The aminoacylation conditions were: pH 7.5, 25°; tris-HCl 25 mM; ^{14}C-L-valine (approx.180 Ci/mole) 10^{-2} mM; ATP 3 mM; KCl 10 mM; tRNA ca. 0.3 A units per ml; enzyme (solution of rat liver pH 5 fraction passed through Sephadex A-25 Cl^- and Sephadex G-25 [1]) 2 A_{280nm} units per ml. Radioactivity of valyl-tRNA was assayed by paper disc method (precipitation with cold 5% TCA); the radioactivity of discs prepared immediately after enzyme addition was assumed as control value and subtracted from the radioactivities of the following discs along each set of kinetic values.

of starting tRNA (95%; 1330 pmoles of valine per A unit). Analysis of terminal oligonucleotides of the active fraction [4] revealed that the relative content of $tRNA_1^{Val}$ was 65%.

Heterogenity of modified $tRNA^{Val}$ can be explained in two ways. First, tRNA in the presence of

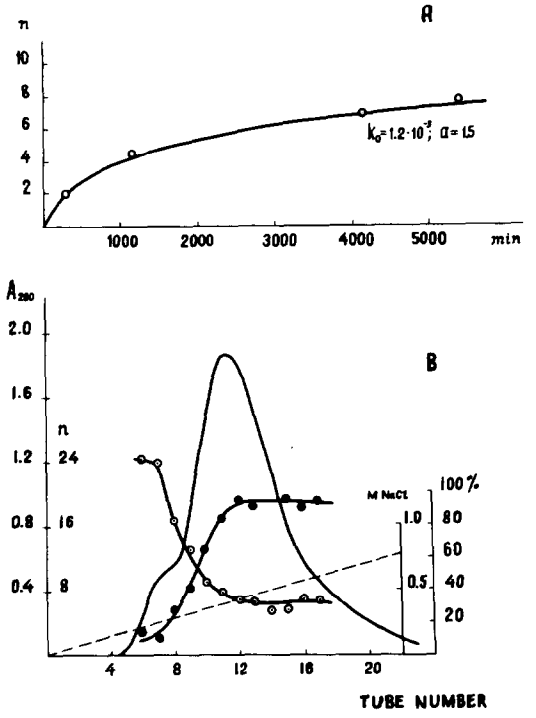

Fig. 3. Studies of the heterogenity of $tRNA^{Val}$ modified with ^{14}C-CME-carbodiimide in the presence of Mg^{2+}ions.

(A) Kinetics of $tRNA^{Val}$ modification. Conditions the same as in fig. 1 (lower curve, + 0.02 M $MgCl_2$) but concentration of tRNA 150 A units per ml. Parameters k_0 and a found are close to those obtained in experiment presented in fig. 1.

(B) Chromatography of the modified $tRNA^{Val}$ obtained on DEAE-cellulose. The time of modification was 5400 min (fig. 3A), the final extent of modification n = 7.8, the acceptor activity 75% of the starting preparation (triangle in fig. 2A). Column 0.5 × 20 cm, 10 ml of 0.02 M CH_3COONa, pH 5.0, 10 ml of 1 M NaCl in the same buffer, 2.8 ml per hr, fraction volume 0.73 ml; amount of tRNA about 10 A units. Aliquots of fractions were counted in dioxan scintillator and studied for the acceptor activity. - - - - sodium chloride gradient; ⊙——⊙ extent of tRNA modification (n); ●——● acceptor activity (% of that of the starting tRNA preparation).

Mg^{2+} ions could exist as a mixture of two stable conformations, one with a greater, and the other with a smaller number of exposed nucleosides. There are in fact some indications of two stable conformations of tRNA [9] that differ in the availability of their 5′-terminus to phosphorylation with polynucleotide kinase. However, chemical modifications, as far as we know, have up till now not manifested significant heterogenity arising from individual tRNA conformations.

The alternative explanation seems to us simpler since it does not assume the presence of two stable conformations but is rather based on the assumption that tRNA in aqueous solution exists as an equilibrium mixture of conformers. In terms of our model [1] individual tRNA may become extensively heterogenous after the modification since the final extent of a given tRNA molecule modification may depend strongly on the site of the first attack. The first act of modification may sometimes lead to fixation of the three-dimensional structure close to the native one and containing a small number of exposed nucleosides, whereas sometimes it may result in fixation of a non-native conformation with a considerably greater number of exposed nucleosides.

It should be mentioned that heterogenity of individual tRNAs modified with CME-carbodiimide was strongly suggested by the results of structural [10] and functional [10, 11] studies of other workers, but the fact has as yet escaped proper attention. The obvious conclusion is the necessity of extensive fractionation of modified tRNA prior to structural analysis; it is especially important that the structural data must be interpreted bearing in mind that fractions homogenous with respect to extent of modification may well appear somewhat heterogenous with respect to sites of modification, if our model [1] is correct.

However, in our opinion, the most interesting result of the present studies is the isolation of a completely active fraction of $tRNA^{Val}$ that contains 6 residues of CME-nucleosides. This complete activity in an extremely specific enzymatic reaction with amino acid-tRNA-ligase is the more amazing since introduction of the bulky positively charged substituent resulting from CME-carbodiimide modification completely prevents substrates of a number of less selective enzymes from entering into enzymatic reactions [12, 13]. In our opinion, this indicates that internal hydrophobic regions of tRNA rather than its outside surface are important for the recognition. This conclusion is supported by the known activity of numerous dissected molecules [14]; it is in line with the established importance of tRNA stem regions for recognition [15–17].

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