GLYCYL-tRNA SYNTHETASE FROM RAT LIVER: THE ROLE OF tRNA IN FORMATION OF GLYCYLHYDROXAMATES

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1. Introduction

Two forms of glycyl-tRNA-synthetase (E.C.6.1.1) have been found in rat liver: the first is unable to catalyse glycylhydroxamate formation in the absence of $tRNA_{gly}$ whereas the activity of the second enzyme is not affected by the tRNA [1]. It is shown here that glycyl-tRNA is an intermediate product of glycylhydroxamate synthesis which proceeds in the presence of the tRNA-dependent glycyl-tRNA-synthetase.

2. Materials and methods

Enzyme and tRNA preparations were isolated as described earlier [1]. Free hydroxylamine was obtained according to Beinert et al. [2] followed by additional distillation under reduced pressure. It was stored in sealed ampoules under N_2 at $-60°C$. Concentration of NH_2OH was determined with hydroxyquinoline [3]. The accumulation of glycylhydroxamates was determined after its sorption on the CM-cellulose discs according to the method developed in this laboratory [4]. Other experimental details are found in the preceding paper [1] and in the legends to figures.

3. Results and discussion

It is known that [see ref. 5,6] α-aminohydroxamic acids are formed as a result of a non-enzymatic reaction between NH_2OH and the carboxylic group of activated amino acid.

Since tRNA-dependent glycyl-tRNA synthetase catalyzes hydroxamate formation only in the presence of tRNA, glycylhydroxamate could be formed either directly through hydroxylaminolysis of glycyladenylate (eq. 1) or after transfer of the glycyl residue to the tRNA molecule (eqs. 2 and 3).

$$\begin{array}{ccc} & E + ATP + Gly + tRNA & \\ & \downarrow & \\ NH_2OH\ (1) & tRNA_{gly} + NH_2OH & (2) \\ & \downarrow & \\ E + AMP + PP + Gly\text{-}NHOH + tRNA & & (3) \end{array}$$

During further investigation of this enzyme a certain lag-period was noticed when kinetics of the glycylhydroxamate formation were measured at low NH_2OH concentrations (fig. 1). Under these conditions a stationary concentration of glycyl-tRNA was detected, indicating that part of the $tRNA_{gly}$ is in the acylated state (fig. 1). These observations together with the inability of the tRNA with oxidized terminal ribose to stimulate glycylhydroxamate synthesis [1] suggest that glycylhydroxamate is formed by non-enzymatic hydroxylaminolysis of enzymically synthesized glycyl-tRNA.

If hydroxamate synthesis proceeded via tRNA the rate of glycylhydroxamate accumulation in the presence of tRNA should be equal to the rate of hydroxylaminolysis of that part of the $tRNA_{gly}$ which was acylated by the enzyme at a given NH_2OH concentration. To compare these two rates it is necessary to determine (a) the rate constant of a non-enzymatic hydroxylaminolysis of glycyl-tRNA, (b) the stationary concentration of glycyl-tRNA at a given NH_2OH

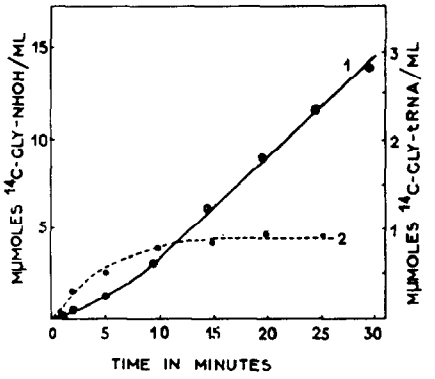

Fig. 1. Kinetics of accumulation of glycylhydroxamates (1) and glycyl-tRNA (2) in the presence of NH_2OH at 25°C. Concentration of the components of incubation mixture: tRNA 1.08 mg/ml; NH_2OH 0.254 M; enzyme preparation 3.1 mg/ml; ATP 15 mM; ^{14}C-glycine (7.5 mC/mmole) 2.2 mM; $MgCl_2$ 25 mM; tris HCl pH 7.6, 2.5 mM. Aliquots (0.1 ml) taken at indicated intervals for determination of glycylhydroxamate formation were heated at 100°C for 15 sec, the denatured protein was removed by centrifugation, 0.05 ml of the supernatant solution was applied on the CM-cellulose disc, dried, washed with distilled water, dried and counted in the toluene scintillator in the counter SL-40 (Intertechnique). To determine the ^{14}C-glycyl-tRNA formation, 1 ml of 10% TCA was added to each aliquot then the suspension was filtered through a nitrocellulose filter (AUFS, Czechoslovakia), washed with 3% TCA, ethanol and counted as described. Since the efficiencies of counting on the CM and AUFS disks was different, reduced efficiency of counting was determined in a separate experiment. To calculate this value, radioactivity of the samples was solubilized and counted in the dioxane scintillator.

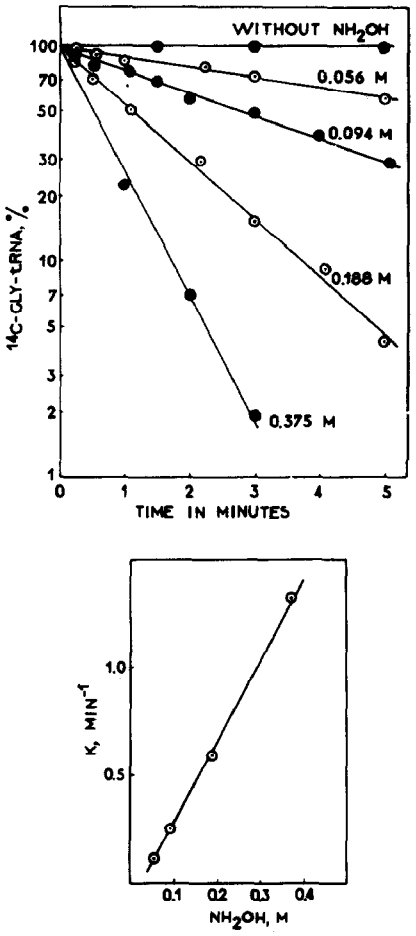

Fig. 2. Determination of the rate constant of glycyl-tRNA hydroxylaminolysis. A. Kinetics of hydroxylaminolysis at various NH_2OH concentrations. B. The rate constant (K) of hydroxylaminolysis as a function of NH_2OH concentration. The values of K at various NH_2OH concentrations were calculated from the data presented in fig. 2A according to eq. (5).

concentration, (c) the rate of hydroxamate formation in the presence of tRNA.

(a) Non-enzymatic hydroxylaminolysis of the glycyl-tRNA is a second order reaction

$$\frac{d\,[^{14}C\text{-}gly\text{-}tRNA]}{dt} =$$

$$\alpha\,[^{14}C\text{-}gly\text{-}tRNA]\,[NH_2OH].$$

Concentration of the ^{14}C-gly-tRNA in the incubation mixture is equal to 1.5×10^{-6} M and that of NH_2OH to $(5.6\text{–}37.5) \times 10^{-2}$ M. In other words $[NH_2OH] \gg [^{14}C\text{-gly-tRNA}]$ and, therefore, a decrease in NH_2OH concentration can be neglected.

Hence,

$$\frac{d\,[^{14}C\text{-}gly\text{-}tRNA]}{dt} = K\,[^{14}C\text{-}gly\text{-}tRNA],$$

where $K = \alpha\,[NH_2OH]$ – const. (eq. 4).

Thus the equation for pseudomonomolecular reaction can be applied

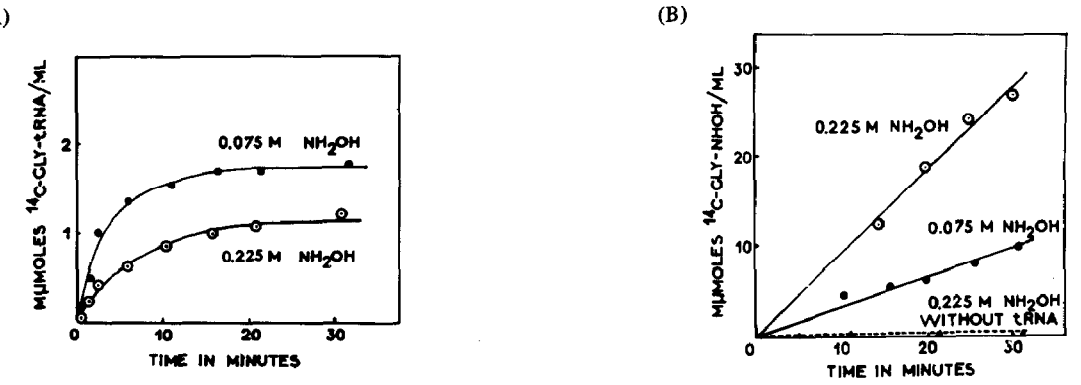

Fig. 3. Kinetics of simultaneous formation of glycyl-tRNA (A) and glycylhydroxamate (B) in the presence of 1.08 mg/ml tRNA at various NH_2OH concentrations at 25°C. All tubes contained also other components of the incubation mixture as indicated in the legend to fig. 1. Samples were treated as described in the legend to fig. 1.

$$K = \frac{1}{t} \ln \frac{A}{A_0} \quad \text{(eq. 5).}$$

Based on these considerations rate constants of hydroxylaminolysis at various NH_2OH concentrations were determined (fig. 2). From the curve obtained it is clear that equation 4 was valid.

(b and c) In fig. 3A kinetics of the glycyl-tRNA formation is shown at two NH_2OH concentrations. The same is demonstrated for the glycylhydroxamate synthesis in the presence and absence of tRNA (fig. 3B).

The stationary concentration of the glycyl-tRNA corresponds to the plateau level in fig. 3A. The rate of glycylhydroxamate formation is determined from the slope of the curves presented in fig. 3B.

Summarizing all the available data, rates of the glycyl-tRNA hydroxylaminolysis and of the glycyl-hydroxamate formation can be compared (table 1). Obviously the rates of these two reactions are almost identical irrespective of the NH_2OH concentration. In other words, the stationary concentration of the glycyl-tRNA, in the presence of NH_2OH, is sufficient to support the observed rate of hydroxamate formation at various NH_2OH concentrations. Thus glycyl-hydroxamate is formed according to equations 2 and 3 whereas hydroxamate formation according to equation 1 does not proceed at a measurable rate.

Threonyl-tRNA synthetase from *E. coli* also does not catalyze hydroxamate formation without tRNA [7]. Qualitatively the picture resembles those for glycyl-tRNA synthetase; however, Hirsh and Lipmann did not perform the analysis quantitatively. Based on their measurements, we calculated the rate constant

Table 1

Comparison of the rates of glycyl-tRNA hydroxylaminolysis and glycyl-hydroxamate accumulation at various conditions.

No.	NH_2OH,	Rate constant of gly-tRNA hydroxyl-aminolysis,	Gly-tRNA	I $\frac{d\,[\text{gly-tRNA}]}{dt}$	II $\frac{d\,[\text{gly-NHOH}]}{dt}$	I:II
	(M)	(min^{-1})	(m μM/ml)	(m μM/ml/min)		
1	0.075	0.19	1.76	0.33	0.32	1.03
2	0.180	0.59	2.22	1.31	1.35	0.97
3	0.225	0.75	1.08	0.81	0.90	1.11
4	0.254	0.85	0.88	0.75	0.71	1.06

for threonyl-tRNA hydroxylaminolysis at 1.1 M NH_2OH to be 0.61/min. The rate of hydroxylaminolysis was 26.4 M/0.1 ml/min and that of threonyl-hydroxamate accumulation was about 30 μM/0.1 ml/ min. Therefore, these two rats are similar and both enzymes are identical in the sense that hydroxamate formation catalyzed by these enzymes proceeds via and after formation of the corresponding aminoacyl-tRNA.

4. Summary

One glycyl-tRNA synthetase from rat liver does not form glycylhydroxamates in the absence of tRNA. Glycyl-hydroxamate accumulated during the incubation of enzyme preparation with glycine, ATP, tRNA and NH_2OH is formed by non-enzymatic hydroxylaminolysis of glycyl-tRNA.

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