MODIFICATION OF AN ESSENTIAL ARGININE IN *ESCHERICHIA COLI* DNA-DEPENDENT RNA POLYMERASE

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1. Introduction

The chemical modification of specific amino acids within enzymes can offer important information regarding the structure–function relationship of these enzymes. The use α-dicarbonyl reagents such as phenylglyoxal [1] and 2,3-butanedione [2] as specific probes for arginyl residues ([3] and references cited therein) is now well established, and such residues appear to be involved in the binding of negatively charged substrates. Our interest in the identification of active site amino acids of *E. coli* DNA-dependent RNA polymerase [4], an enzyme that binds both a negatively charged DNA template and nucleoside triphosphates has therefore led us to undertake a preliminary investigation of the inactivation of this enzyme by phenylglyoxal.

2. Materials and methods

E. coli DNA-dependent RNA polymerase holo enzyme was isolated using matrix bound heparin [5]. Enzymic activity was measured in a reaction mixture (0.1 ml) containing 40 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, 5 mM DTE, 200 mM KCl, 1 mM [^{14}C]ATP (2000 cpm/nmol), 1 mM UTP, and 0.1 A_{260} units poly(d(A–T)). Activity was measured as the amount of [^{14}C]AMP incorporated into acid-insoluble product [6] after a 15 min incubation at 37°C.

Phenylglyoxal monohydrate was obtained from Fluka AG. [7-^{14}C]Phenylglyoxal (2000 cpm/nmole) was synthesized from [7-^{14}C]acetophenone (New England Nuclear) essentially following a published procedure [7].

For the determination of the incorporation of [^{14}C]phenylglyoxal into RNA polymerase 450 μg of the enzyme was incubated at 37°C in a final volume of 1 ml with 0.36 mM [^{14}C]phenylglyoxal, 100 mM bicine buffer (pH 8.0), and 10 mM $MgCl_2$. After various times 100 μl aliquots were removed and diluted with 1 ml of 5% trichloroacetic acid. The mixture was then filtered over a nitro cellulose membrane filter (pore size 0.45 μ, 25 mm diameter, Schleicher and Schüll). The filter was washed with 20 ml of 5% trichloroacetic acid, dried under an infrared lamp, and the radioactivity determined by standard liquid scintillation techniques.

3. Results

RNA polymerase is rapidly inactivated upon incubation with a 10 mM solution of phenylglyoxal. As shown in fig.1, the inactivation rate obeys pseudo frist-order kinetics and is dependent upon the phenylglyoxal concentration. The second-order rate constant for the inactivation of the enzyme is 44 M^{-1} min^{-1}. From a plot of log $(1/t_{0.5})$ versus log [phenylglyoxal] [8,9] a straight line was obtained (fig.2) whose slope was equal to 1.07 suggesting that the inactivation is the result of reaction of one arginyl residue on RNA polymerase.

The stoichiometry of incorporation of phenylglyoxal was determined using ^{14}C-labelled material. As can be seen from fig.3, an increasing amount of [^{14}C]-phenylglyoxal is bound to the enzyme with a corresponding loss of activity. The plot of activity versus phenylglyoxal incorporated per mole enzyme is curved indicating that arginyl or other residues not essential

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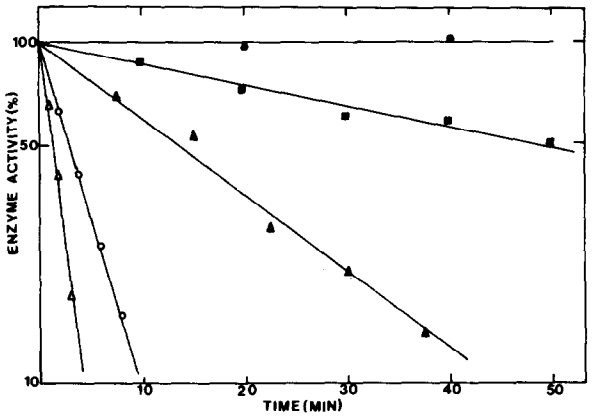

Fig.1. Semilogarithmic plot of the kinetics of the inactivation of RNA polymerase by phenylglyoxal. The incubation mixture (0.1 ml) contained 100 mM bicine buffer (pH 8.0), 10 mM $MgCl_2$, 2.25 μg enzyme and the following concentrations of phenylglyoxal: 0 mM (○), 0.36 mM (□), 1.25 mM (△), 5.4 mM (○), and 10 mM (△). Incubations were performed at 37°C and 10 μl aliquots were removed after various times and assayed as described in Materials and methods.

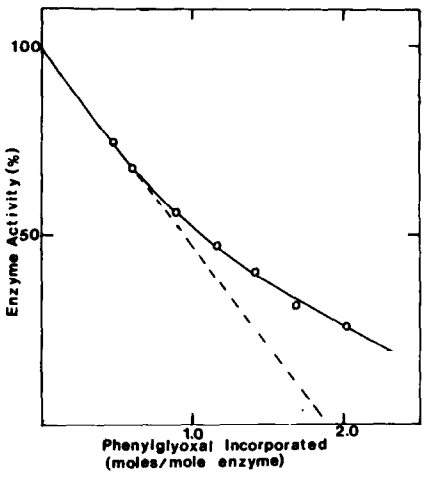

Fig.3. Correlation of inactivation of RNA polymerase with incorporation of [^{14}C]phenylglyoxal. The amount of [^{14}C]-phenylglyoxal incorporated and the enymatic activity were determined as described in Materials and methods.

for activity are also reacting. However, extrapolation of the initial portion of the curve to zero activity showed that the incorporation of two moles of phenylglyoxal per mole of enzyme correlates to complete loss of enzymic activity.

4. Discussion

Phenylglyoxal is a reasonably specific reagent for arginine residues [1], although reaction also occurs with α-amino groups, with sulphydryl groups [10] and on longer incubation times with the ε-amino group of lysine. On reaction with arginine, two moles of phenylglyoxal condense with one mole of arginine. The incorporation data of [^{14}C]phenylglyoxal into RNA polymerase show that the incorporation of two moles of phenylglyoxal per mole of enzyme would be required for complete inactivation, suggesting that the modification of one essential arginine residue is responsible for the loss of enzymatic activity. This is further supported by the inactivation data.

In order to elucidate the role of this arginyl residue we are at present investigating the effect of substrate and template on the inactivation, and are attempting to determine to which subunit of the enzyme the arginine is attached.

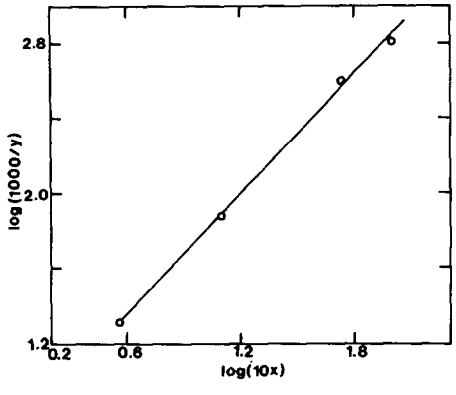

Fig.2. Plot of log (1000/y) versus log (10x) from the data in fig.1, where y is the half time of inactivation ($t_{0.5}$) and x the phenylglyoxal concentration. The slope of the plot is 1.07.

Acknowledgements

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