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HIGH MOLECULAR WEIGHT COMPLEX FORMATION OF RAT LIVER LYSYL-tRNA SYNTHETASE REDUCES ENZYME LABILITY TO THERMAL INACTIVATION

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Eukaryotic aminoacyl-tRNA synthetases tend to occur in high molecular weight multienzyme complexes in contrast to the prokaryotic counterparts. The functional significance of complex formation is not understood but stabilization of enzyme activities in the complex(es) has been suggested as one of many possible functions. We provide evidence that the 24S complex lysyl-tRNA synthetase activity is more resistant to thermal inactivation than the 6S free lysyl-tRNA synthetase activity at 30°, 37°, and 43°C.

Introduction

The aminoacyl-tRNA synthetases catalyze the formation of aminoacyl-tRNA from amino acid, tRNA, and ATP providing activated amino acids for protein biosynthesis (1). The eukaryotic aminoacyl-tRNA synthetases tend to occur as high molecular weight (HMW) complexes with molecular weights up to over 10^6 in contrast to prokaryotic enzymes with molecular weights less than 250,000 (1-18, for a review see Ref. 4). The HMW complexes appear ubiquitous in eukaryotes occurring in many cell types from yeast (11) and Drosophila (8) to human placenta (8). Evidence from conventional and affinity chromatography indicate that these HMW complexes are multienzyme complexes of aminoacyl-tRNA synthetases (2, 5, 10). The functional significance of these HMW complexes is presently not understood. One possible advantage of complex formation is stabilization of enzyme activities from thermal inactivation. Recently it has been suggested that complex formation may provide intermolecular stabilization *in vivo* (16).

We have reported the isolation and characterization of a rat liver 24S complex (3) and its disassembly by hydrophobic chromatography to yield

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free 6S enzymes (3, 5). We report in this communication in vitro evidence suggesting that complex formation of aminoacyl-tRNA synthetases reduces lability of enzyme activity to thermal inactivation by comparing the inactivation kinetics of the 6S and 24S lysyl-tRNA synthetase activity.

Materials and Methods

Materials: Sources for materials are described previously (2, 3, 5).

Enzyme Preparation and Assay: The rat liver 24S lysyl-tRNA synthetase was prepared in the presence of phenylmethane sulfonyl fluoride (PMSF) as described by Dang and Yang (3). The 6S lysyl-tRNA synthetase was prepared by chromatography of the 24S complex on a diaminooctyl-Sepharose 4B column and centrifugation in a sucrose gradient as described (3, 5). Enzyme assays were according to (3) in presence of 3 mg/ml of bovine serum albumin which protects the enzyme activity completely at 37°C for at least 10 minutes. One unit of enzyme activity is that amount catalyzing the formation of 1 nmole of aminoacyl-tRNA in 1 minute at 37°C.

Thermal Inactivation of Synthetase Activities: Pooled 24S lysyl-tRNA synthetase was diluted to give comparable concentration of enzyme activity (2-4 units/ml) as the pooled 6S enzyme. The 24S and 6S enzymes (1 ml each) were dialyzed in 1 liter of buffer (50 mM Tris-HCl (pH 7.5 at 25°C), 2 mM dithiothritol, 25 mM KCl, 5 mM $MgCl_2$, 0.2 mM PMSF) containing 10% glycerol at 4°C for 6 hours to assure identical buffer conditions. Aliquots (0.2 ml) of 6S and 24S enzymes were incubated in 10 x 75 mm glass test tubes in water baths at 30°C, 37°C, and 43°C. Aliquots of 25 µl were removed at appropriate times and immediately assayed at 37°C for 5 minutes in presence of 3 mg/ml BSA.

Results and Discussion

The 24S lysyl-tRNA synthetase activity is less labile than the 6S lysyl-tRNA synthetase activity at 30°, 37°, and 43°C (Figs. 1A, B, C). Increasing lability of the 6S Lys-tRNA synthetase activity is seen with increasing temperatures, but the 24S lys-tRNA synthetase activity has similar rates of inactivation at 30° and 37°C. At 43°C there is a markedly increased rate of inactivation of the 24S Lys-tRNA synthetase activity. The inactivation rate of the 6S Lys-tRNA synthetase activity appears to be first-order at the three temperatures. The 24S Lys-tRNA synthetase inactivates to what appears to be a residual activity at the three different temperatures.

The results show that the 24S Lys-tRNA synthetase activity is more resistant to thermal inactivation than the 6S enzyme activity at 30°, 37°, and 43°C. The curvilinear semilog plots of the 24S Lys-tRNA synthetase inactivation suggest a residual activity in contrast to the 6S enzyme. The nature of this residual activity is not understood, but it may result

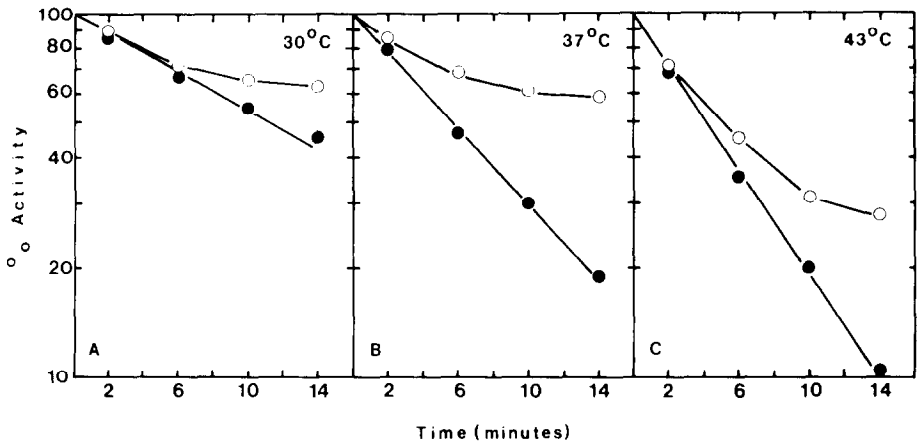

Fig. 1 Thermal inactivation of 24S (-O-O-) and 6S (-●-●-) lysyl-tRNA synthetase activity at 30°C (panel A), 37°C (panel B), and 43°C (panel C). The ordinate represents the logarithm of the percent of initial activity remaining after a certain incubation time (abscissa).

from the equilibrium between complexed and free Lys-tRNA synthetase in the 24S enzyme preparation. The residual activity may represent the complexed enzyme activity which is more resistant to thermal inactivation than the free enzyme. This phenomenon has been reported for the thermal inactivation of the dimeric wheat germ leucyl-tRNA synthetase (17). The possibility of exchange between complexed and free enzymes may now be more definitely investigated with the availability of highly purified aminoacyl-tRNA synthetase complex preparation (5, 10).

The presence of other synthetases specific for Arg, Gln, Glu, Ile, Leu, Met and Pro in the 24S complex (3) may provide stability for the Lys-tRNA synthetase by intermolecular interactions. This concept of intermolecular stabilization has been substantiated by *in vivo* studies. A temperature-sensitive Leu-tRNA synthetase Chinese hamster ovary (CHO) cell mutant has been shown to be protected by the homologous amino acid leucine and synergistically protected by Ile, Val, and Leu (16). This suggests the occurrence of intermolecular stabilization in the synthetase complex *in vivo*. The *in vitro* inactivation experiments with 6S and 24S Lys-tRNA synthetase in this com-

munication show *in vitro* stabilization of synthetase activity by complex formation. The similar rates of inactivation of the 24S Lys-tRNA synthetase activity at 30° and 37°C and a faster rate of inactivation at 43°C have been previously observed with the rabbit reticulocyte 16S complex (13). In summary, among the many possible functions of HMW aminoacyl-tRNA synthetases, we demonstrate in this communication that complex formation decreases the lability of the enzyme activities to thermal inactivation.

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References

1. Schimmel, P.R. and Söll, D. (1979) Ann. Rev. Biochem. 48, 601-648.
2. Dang, C.V. and Yang, D.C.H. (1978) Biochem. Biophys. Res. Commun. 80, 709-714.
3. Dang, C.V. and Yang, D.C.H. (1979) J. Biol. Chem. 254, 5350-5356.
4. Dang, C.V. and Yang, D.C.H. (1982) Int. J. Biochem., in press.
5. Johnson, D.L., Dang, C.V. and Yang, D.C.H. (1980) J. Biol. Chem. 255, 4362-4366.
6. Johnson, D.L. and Yang, D.C.H. (1981) Proc. Nat. Acad. Sci., U.S.A. 78, 4059-4062.
7. Bandyopadhyay, A.K. and Deutscher, M.P. (1971) J. Mol. Biol. 60, 113-122.
8. Denney, R.M. (1977) Arch. Biochem. Biophys. 183, 157-167.
9. Glinski, R.L., Gainey, P.C., Mawhinney, T.P., and Hilderman, R.H. (1979) Biochem. Biophys. Res. Commun. 88, 1052-1061.
10. Kellerman, O., Brevet, A., Tonetti, H., and Waller, J.-P. (1979) Eur. J. Biochem. 99, 541-550.
11. Dimitrijevic, L. (1977) FEBS Lett. 79, 37-41.
12. Shafer, S.J., Olexa, S. and Hillman, R. (1976) Insect. Biochem. 6, 405-411.
13. Som, K. and Hardesty, B. (1975) Arch. Biochem. Biophys. 166, 507-517.
14. Ussery, M.A., Tanaka, W.K. and Hardesty, B. (1977) Eur. J. Biochem. 72, 491-500.
15. Vennegoor, C. and Bloemendal, H. (1972) Eur. J. Biochem. 26, 462-473.
16. Molnar, S.J. and Rauth, A.M. (1979) J. Cell. Physiol. 98, 315-326.
17. Carias, J.R., Mouricout, M., Quintard, B., Thomas, J.C. and Julien, R. (1978) Eur. J. Biochem. 87, 583-590.
18. Ritter, P.O., Enger, M.D. and Hampel, A.E. (1979) Biochim. Biophys. Acta 562, 377-385.