Biochemical Genetics, Vol. 22, Nos. 3/4, 1984

A Chinese Hamster Ovary Leucyl-tRNA Synthetase Mutant with a Uniquely Altered High Molecular Weight Leucyl-tRNA Synthetase Complex

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Received 3 Aug. 1983—Final 30 Nov. 1983

The Chinese hamster ovary (CHO) cell culture temperature-sensitive mutant ts*025Cl with a defect in leucyl-tRNA synthetase (LeuRS) does not have an inherently more thermolabile LeuRS, but instead the mutation causes the complete loss of the LeuRS high molecular weight complexes which are present in normal wild-type cells. The mutant cell LeuRS has a single 8 S enzyme form which corresponds hydrodynamically to the 8 S free form of wild-type enzyme. Both 8 S forms have the same thermostability and the same* K_m *for leucine, indicating that there is no inherent defect in the catalytic activity of the enzyme. The temperature-sensitive phenotype can be explained by the lack of thermostable high molecular weight forms of LeuRS.*

KEY WORDS: leucyl-tRNA; Chinese hamster ovary enzyme complexes.

INTRODUCTION

The Chinese hamster ovary (CHO) cell line has proven useful for the isolation of temperature-sensitive conditionally lethal mutants (Thompson *et al.*, 1973, 1975, 1977). The mutant *ts*025Cl had a temperature-sensitive phenotype with low levels of LeuRS activity and was used to assign the location of the human leucyl-tRNA synthetase (LeuRS) genetic locus to chromosome 5 of the human genome (Giles *et al.*, 1980). Others had previously shown that the

This work was supported by NIH Grant GM 19506 to A.E.H. and GM 20737 to D.E.O.

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0006-2928/84/0400-0349$03.50/0

mutation was recessive (McBurney and Whitmore, 1975), expressed a leucine phenotypic protective effect, and did not complement the well-characterized LeuRS mutant *ts*H1 (Thompson *et al.*, 1975). The mutant *ts*H1 has a thermolabile LeuRS with low levels of enzyme activity (Thompson *et al.*, 1973). The enzyme is kinetically altered (Haars *et al.*, 1976; Farber and Deutscher, 1976), with a leucine K_m about 4× that of wild type (WT) (Klekamp *et al.*, 1981). When the defective LeuRS is analyzed on sucrose gradients, only the low molecular weight 8 S form of the enzyme is seen, while wild-type cells have three hydrodynamic forms of LeuRS—a low molecular weight form at 8 S and two high molecular weight forms at 20 S and 30 S which account for about 70% of the total LeuRS activity (Hampel *et al.*, 1978; Ritter *et al.*, 1976, 1979).

In this report we show that the temperature-sensitive LeuRS mutant *ts*025Cl does not have an inherently thermolabile LeuRS. Only the 8 S form of LeuRS exists, which has the same thermostability as wild-type 8 S and the same leucine K_m as wild-type 8 S. The reason for the *ts* phenotype can be explained by the lack of thermostable high molecular weight complexes. These results are important in understanding which component of LeuRS was mapped on the human genome and in our eventual understanding of the nature and function of the high molecular weight aminoacyl-tRNA synthetase complexes commonly found in mammalian cells (Bandyopadhyay and Deutscher, 1971; Schimmel and Soll, 1979).

MATERIALS AND METHODS

Size Distribution of Leucyl-tRNA Synthetase on Sucrose Gradients

Chinese hamster ovary (CHO) cells were grown as previously described (Klekamp *et al.*, 1981). Cells (10^8) were lysed with Nonidet-P40, nuclei and cell debris were removed, and supernatant (0.7 ml) was layered on 12-ml 10–30% (w/v) linear sucrose gradients in 10 mM KCl, 1 mM $MgCl_2$, 0.1 mM dithiothreitol, and 10 mM Tris, *p*H 7.5, at 25°C. The gradients were centrifuged in a Beckman SW 28.1 rotor at 28,000 rpm for 16 hr, 40 min at 4°C, fractionated, and assayed for LeuRS activity using [^{3}H]leucine as a substrate and counting [^{3}H]Leu-tRNA^{Leu} as a product of the reaction (Klekamp *et al.*, 1981). Sedimentation coefficients ($s_{20,w}$) were calculated on a computer program as previously described (Dingman, 1972). The total protein in 10^8 cells was 35 mg as determined by the method of Lowry *et al.* (1951).

Thermostability of the 8 S Form of LeuRS from Three Cell Types

Protein concentrations in tubes containing the 8 S LeuRS peaks from the sucrose gradients were determined and the protein was adjusted to 10 mg/ml

by the addition of bovine serum albumin (Sigma). Purified rat liver tRNA was added to a final concentration of 3.0 mg/ml and the tubes were heated at 40.5°C. Aliquots (10 μl) were removed after 0, 2, 4, 6, 8, 10, 12, and 14 min and assayed at 34°C for LeuRS activity in a 50-μl incubation mixture as described (Klekamp *et al.*, 1981).

Leucine Michaelis Constants of Wild-Type 8 S LeuRS and the Mutant ts*025Cl 8S*

Peak tubes from sucrose gradients were assayed for leucyl-tRNA synthetase activity over a range of leucine concentrations, 2, 4, 8, 12, 16, and 20 μM, as described previously (Haars *et al.*, 1976, Klekamp *et al.*, 1981). Assay times of 0.5, 1.0, and 1.5 min gave increasing linear product formation with respect to time under the conditions employed. Double reciprocal plots (1/v vs 1/S) were analyzed by linear regression and the calculated K_m values confirmed by standard statistical kinetic analysis (Wilkinson, 1961).

RESULTS AND DISCUSSION

The presence of only the low molecular weight form of the LeuRS in the CHO cell LeuRS mutant *ts*025Cl can be seen in Fig. 1. Wild-type cells show the three regular forms of the enzyme at 8 S, 20 S, and 30 S. This mutant profile shows no evidence of any high molecular weight LeuRS complexes and contains about 12% of wild-type LeuRS activity. The mutant 8 S LeuRS regularly moves one tube slower than wild-type LeuRS on sucrose gradients, a behavior which is very similar to that of the mutant *ts*H1 which we analyzed previously and indicates that the 8 S forms are not identical but that *ts*025Cl 8S is physically different than wild type.

The thermolability of the respective enzyme forms is shown in Fig. 2. The gradient tubes from the 8 S LeuRS peak of the sucrose gradient in Fig. 1 were heated at enzyme-inactivating temperature as described in Materials and Methods and were assayed for loss of LeuRS activity over time. Wild-type 8 S LeuRS and *ts*025Cl 8 S LeuRS showed the same thermolability. The mutant *ts*H1 8 S LeuRS was run as a control and shows its typical temperature sensitivity, with a thermal inactivation rate about 3× that of wild-type 8 S (Klekamp *et al.*, 1981). The thermostability of wild-type 30 S and 20 S LeuRS relative to wild-type 8 S has been previously published by us, and we showed that wild-type 20 S LeuRS has the same thermostability as 30 S LeuRS, with both being 2× more stable than wild-type 8 S. We also showed that the greatest stability occurs when all three forms are incubated together. In the case of the mutant described here, however, only the most inherently thermolabile form (8 S) of LeuRS exists. The similarity between *ts*025Cl 8 S LeuRS and the wild-type 8 S form was further confirmed by leucine K_m

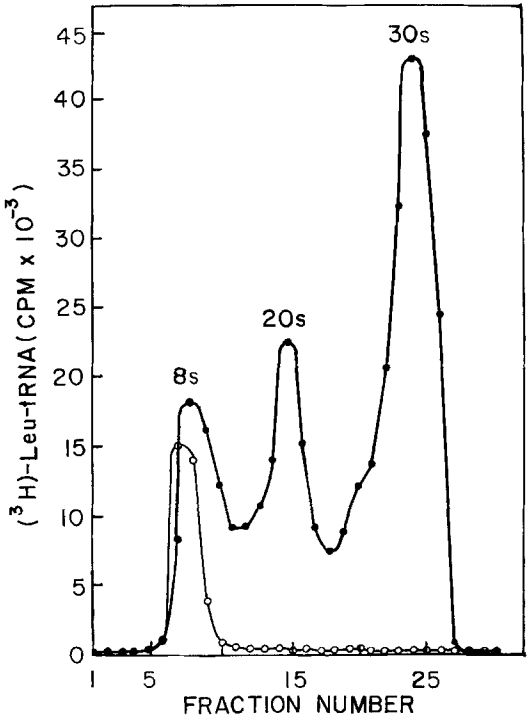

Fig. 1. Size distribution of leucyl-tRNA synthetase on sucrose gradients. LeuRS enzyme activity from wild type (—●—) and mutant *ts*025Cl (—○—) CHO cells. The same number of cells (10^8) and total protein (35 mg) were added to each gradient. Further details are as in Materials and Methods.

determinations (Fig. 3). Both enzymes showed the same leucine K_m of 16 μM, which is similar to that we previously published for wild-type 8 S (Klekamp *et al.*, 1981). The temperature-sensitive LeuRS mutant *ts*025Cl clearly contains an altered LeuRS, but the alteration affects the ability of the mutant LeuRS to form complexes and does not affect the catalytic activity of the free form (8 S) of the enzyme. The mutant clearly has only the 8 S form, with no traces of the higher molecular weight complexes found in wild-type cells. The respective 8 S forms from these cell types show the same thermolability and have the same leucine K_m. This is in contrast to the previously characterized CHO LeuRS *ts*H1, which has a very thermolabile 8 S form of the enzyme with a fourfold larger K_m (Klekamp *et al.*, 1981).

The question, then, What is the basis of the temperature-sensitive phenotype of *ts*025Cl? is posed. We previously showed that the high molecular weight 20 S and 30 S LeuRS complexes of wild-type cells are 2× more

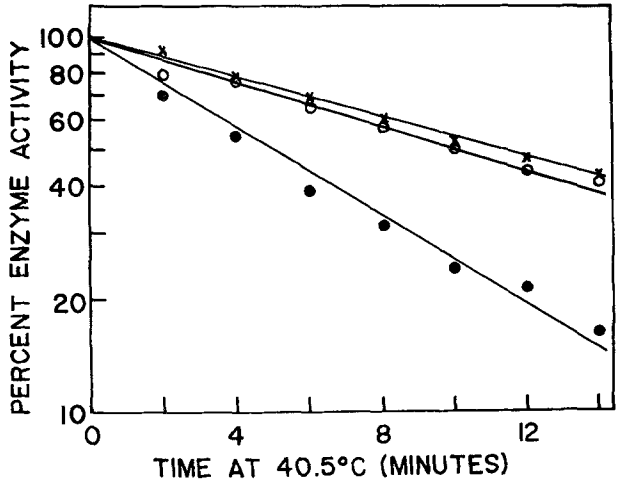

Fig. 2. Thermostability of the 8 S form of LeuRS from three cell types: wild type (—X—); LeuRS mutant *ts*025Cl (—○—); and LeuRS mutant tsH1 (—●—).

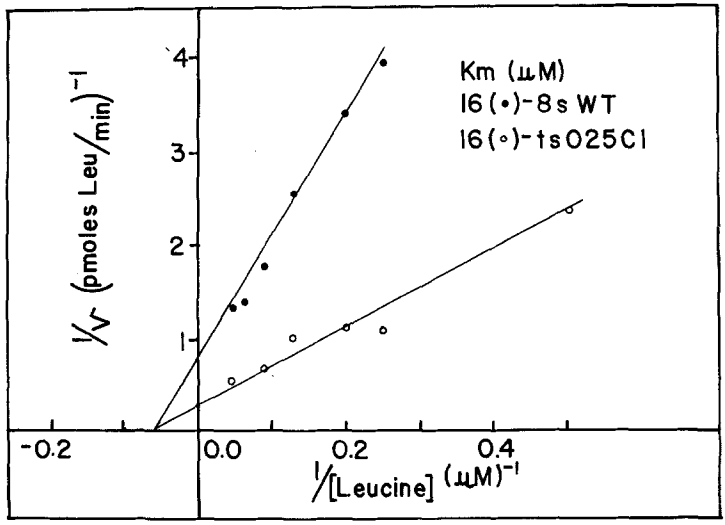

Fig. 3. Leucine Michaelis constants of wild-type 8 S LeuRS and mutant *ts*025Cl 8 S LeuRS.

thermostable than the 8 S form of the enzyme (Klekamp *et al.*, 1981). The fact that *ts*025Cl contains none of these thermostable forms and low levels of 8 S can readily account for the temperature-sensitive phenotype. This is further supported by the phenotypic protective effect on the parent of *ts*025Cl, *ts*025, of high levels of leucine (Thompson *et al.*, 1975), which we have shown is seen in mutants when no corresponding high molecular weight synthetase complexes are present (Klekamp *et al.*, 1981; Pahuski *et al.*, 1983). Since the parent of *ts*025Cl, *ts*025, does not genetically complement the mutant *ts*H1, it is most likely that the LeuRS enzyme in *ts*025Cl is altered, and indeed the gene locus for LeuRS was genetically mapped to human chromosome 5 (Giles *et al.*, 1980). This is further supported by mapping studies with human × *ts*H1 hybrids which also defined the LeuRS locus at human chromosome 5 (Dana and Wasmuth, 1982).

What, then, is the molecular basis for the *ts*025Cl mutation? The evidence argues that most likely *ts*025Cl LeuRS contains as alteration in the LeuRS structural protein itself which does not affect the catalytic activity or structure but does prevent the formation of complexes. It now becomes important to analyze the LeuRS of the human × hamster (*ts*025Cl) temperature-resistant hybrid to determine if human gene products other than LeuRS are stabilizing a CHO LeuRS complex in the hybrid. This possibility is further supported by the observation that human gene products can enter hamster multicomponent complexes in interspecific hybrids (Dana and Wasmuth, 1982). A number of possible combinations of human hamster LeuRS and complex proteins could exist to confer the temperature-resistant phenotype of the hybrid which are amenable to study with the *ts*025Cl mutant.

ACKNOWLEDGMENTS

We thank Lynn Isoye, Dorthy Ling, and Wiley Taylor for technical assistance.

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