PEPTIDYL-tRNA AND PEPTIDYL TRANSFERASE ACTIVITY OF SKELETAL MUSCLE RIBOSOMES. EFFECT OF DIABETES

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The enzyme that catalyzes peptidyl transfer is a component of the large subunit of ribosomes from prokaryotes [1] and eukaryotes [2,3]. The activity of the enzyme can be measured apart from the other steps in protein synthesis in the "fragment reaction" [1,4]. With a view to understanding the basis for the deficiency in the ability of ribosomes from the muscle of alloxan-diabetic rats to synthesize protein [5], we have compared the extent to which ribosomes and ribosome subunits will carry out the fragment reaction. In the course of the study we discovered that the peptidyl transferase activity of ribosomes, as measured in the fragment reaction, was contingent on the amount of peptidyl-tRNA bound to the particles.

Peptidyl transferase activity, measured by the formation of Ac-^{3}H-Leu-puromycin from CACCA-^{3}H-Leu-Ac in the fragment reaction, was directly proportional to the concentration of rat skeletal muscle ribosomes (fig. 1). At concentrations between 0.1 and 0.5 μM, diabetic 80 S ribosomes were twice as efficient as normal in catalyzing the fragment reaction. The same difference in the activity of the ribosomes was observed no matter the duration of incubation (between 5 and 60 min; results not shown). However, equal concentrations of the 60 S subunits (0.42 μM in fig. 2; 0.1, 0.2, and 0.4 μM in other experiments) from normal and diabetic ribosomes had exactly the same capacity to effect peptidyl transfer (fig. 2). Moreover, we calculated that 60 S subunits were on a molar basis more active than 80 S ribosomes in the fragment reaction. We found, as had others before [2], that the 40 S subunit was inactive in the reaction.

That peptidyl transfer was greater with equimolar amounts of 60 S subunits indicated that some component of the 80 S ribosome interfered with the fragment reaction. Peptidyl-tRNA bound to the ribosome

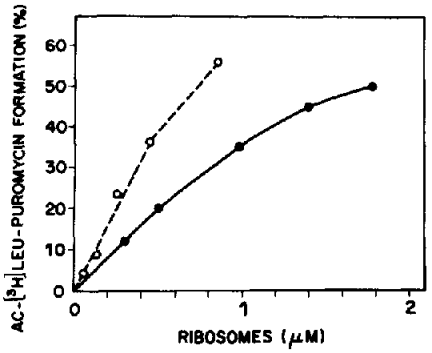

Fig. 1. Effect of concentration of skeletal muscle ribosomes from normal and diabetic rats on the catalysis of the fragment reaction. Skeletal muscle ribosomes were prepared from normal and alloxan-diabetic rats [5]. Peptidyl transfer was measured in the fragment reaction: The reaction mixture contained (prior to the addition of methanol) ribosomes (in the concentrations indicated), 0.05 M tris-HCl (pH 7.5), 0.4 M KCl, 0.04 M Mg acetate, 1 mM puromycin, and 19 nM CACCA-^{3}H-Leu-Ac (specific activity 4.1 Ci/mmole) prepared as described by Monro, Cerna and Marcker [6] except that acetylation of ^{3}H-leucyl-tRNA was carried out according to Lapidot, DeGroot and Fry-Shafrir [7]. The fragment reaction was initiated by the addition of one-half volume of methanol and terminated after 20 min of incubation at 0° by adding an equal volume of 0.3 M Na acetate (pH 5.5) saturated with $MgSO_4$ [8]. Ac-^{3}H-Leu-puromycin was extracted with ethyl acetate [8] and its radioactivity determined. The results are expressed as the percentage of radioactivity in CACCA-^{3}H-Leu-Ac transferred to Ac-^{3}H-Leu-puromycin. The amount of radioactivity soluble in ethyl acetate in the absence of puromycin (less than 4% of the total) was substracted from the values.

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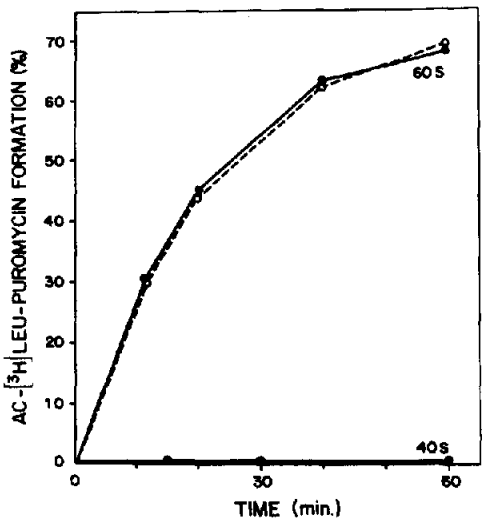

Fig. 2. Time course of Ac-^{3}H-Leu-puromycin formation in the fragment reaction by 60 S subunits from normal and diabetic rats. Muscle ribosome subunits were prepared [9] and the formation of Ac-^{3}H-Leu-puromycin was determined as described in fig. 1. The concentration of 60 S and 40 S subunits before adding methanol was 0.42 and 2.7 μM respectively.

might decrease the formation of Ac-^{3}H-Leu-puromycin by preventing access of CACCA-^{3}H-Leu-Ac to the catalytic center; since the 60 S subunit has less attached peptidyl-tRNA it would appear more efficient in the fragment reaction. Moreover, 80 S ribosomes from the muscle of diabetic rats bear half as many nascent peptide chains as normal ribosomes [10] – that might then account for the greater activity of the former. To test the possibility ribosomes were preincubated in 880 mM KCl with or without puromycin (1 mM) – conditions that release peptidyl-tRNA from the ribosome (Stirewalt, Castles and Wool, in preparation). After preincubation the activity of the ribosomes in the fragment reaction was determined (fig. 3). Preincubation for 1 hr at 37° in low (80 mM) potassium buffer did not affect the activity of the particles; however, preincubation in high potassium (880 mM) increased Ac-^{3}H-Leu-puromycin formation 2.5 fold and addition of puromycin (1 mM) to the high potassium buffer increased activity 4 fold. The peptidyl transferase activity of normal ribosomes was increased to a greater degree than that of diabetic ribosomes by preincubation in 880 mM KCl and 1 mM puromycin (table 1) – but the critical observation is

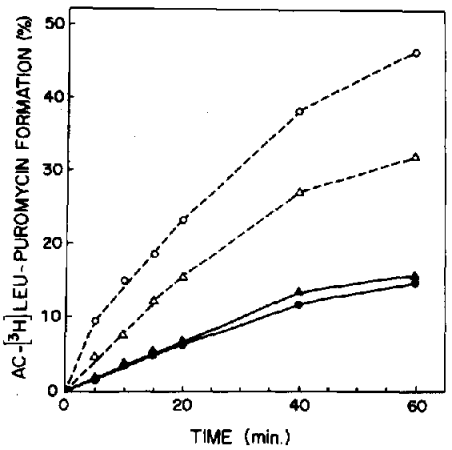

Fig. 3. Effect of preincubation of normal 80 S ribosomes on the time course of Ac-^{3}H-Leu-puromycin formation in the fragment reaction. Ribosomes were preincubated at 37° for 1 hr in:
▲–▲ 50 mM-tris HCl (pH 7.5), 12.5 mM $MgCl_2$, 80 mM KCl;
△–△ 50 mM-tris HCl (pH 7.5), 12.5 mM $MgCl_2$, 880 mM KCl;
○–○ 50 mM-tris HCl (pH 7.5), 12.5 mM $MgCl_2$, 880 mM KCl, 1 mM puromycin;
●–● control, no preincubation.
The concentration of ribosomes was 0.25 μM. Other conditions are described in the legend of fig. 1.

Table 1
Effect of preincubation of 80 S normal and diabetic muscle ribosomes on Ac-^{3}H-Leu-puromycin formation in the fragment reaction.

Conditions of preincubation	Ac-^{3}H-Leu-puromycin formation (%)	
	80 S ribosomes	
	Normal	Diabetic
Exp. 1		
None	8	21
37°, 880 mM KCl, puromycin	26	26
Exp. 2		
None	6	18
37°, 80 mM KCl	6	19
37°, 880 mM KCl	15	24
37°, 880 mM KCl, puromycin	27	28

Preincubation was for 1 hr in 50 mM tris, 12.5 mM $MgCl_2$, and the concentration of KCl indicated; 1 mM puromycin was added where indicated. Ac-^{3}H-Leu-puromycin formation in the fragment reaction was measured after 20 min of incubation at 0°. The concentration of ribosomes was 0.25 μM. Other conditions are described in the legend of fig. 1.

that after preincubation in high potassium and puromycin the activity of normal and diabetic ribosomes is the same (table 1). Moreover, we calculate that preincubated normal and diabetic ribosomes had the same activity as equimolar amounts of 60 S subunits. Silverstein [11] had also found that preincubation in high potassium buffer enhanced the ability of *E. coli* ribosomes to carry out the fragment reaction.

In 880 mM KCl, approximately half the peptidyl-tRNA bound to muscle ribosomes is released; if puromycin is added the release of nascent chains is increased to 90% (Stirewalt, Castles and Wool, in preparation). That similar treatment increased the activity of the ribosomes in the fragment reaction to a like degree, accords with the conclusion that peptidyl-tRNA prevents interaction of either the fragment or puromycin (or both) with peptidyl transferase. Different amounts of attached peptidyl-tRNA may also explain the difference in activity of canine liver [12] or human tonsil [3] and rat liver 80 S ribosomes. At any rate the results underscore the importance of peptidyl-tRNA in estimating the true peptidyl transferase activity of ribosomes.

Ribosomes from the muscle of diabetic rats are less efficient than normal in endogenous protein synthesis; administration of insulin to diabetic animals restores activity of the particles to normal [5,13]. Modulation of protein synthesis by insulin is not likely to be mediated by an effect on peptidyl transferase since normal and diabetic ribosomes, and normal and diabetic 60 S subunits, had equal activity in the fragment reaction, once proper allowance was made for the disproportionate amounts of bound peptidyl-tRNA. One reservation need be kept in mind: the alcohol used in the fragment reaction may generate maximum peptidyl transferase activity, whereas in physiological circumstances a portion of that activity may be restrained. If that is the case, there could be a difference in the peptidyl transferase activity of normal and diabetic ribosomes which would not be revealed in the fragment reaction.

Acknowledgements

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