THE REASSOCIATION OF RIBOSOMAL SUBUNITS FROM THE MUSCLE OF NORMAL AND DIABETIC ANIMALS

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SUMMARY

Ribosomal subunits from the muscle of diabetic animals were less effective than normal in forming 80S couples, either spontaneously (e.g. in the absence of factors) or in the reaction catalyzed by EIF-1 (G-25 fraction); those couples that did form were more sensitive to pressure induced dissociation during centrifugation. The diabetic defect was carried by the 60S subunit; the 40S particle appeared unaltered by diabetes.

The synthesis of protein is diminished in the muscle of diabetic animals (1) and the defect is reflected in the decreased activity of the ribosomes (2). Diabetes reduces the number of polysomes in muscle (3) and the proportion of the particles that are active in protein synthesis (4). However, diabetic ribosomes seem not to suffer a deficiency in their capacity to carry out the elongation reactions (5-8). Those observations have led to the view that the change in protein synthesis in muscle from diabetic animals results from a decreased capacity to initiate the synthesis of peptides (9). The assumption is supported by the demonstration of increased numbers of ribosomal subunits in the muscle of diabetic animals (10), and by the finding that in circumstances where the synthesis of protein requires the initiation of new peptide chains (e.g. the translation of encephalomyocarditis virus RNA and the synthesis of virus-specific peptides

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in the presence of initiation factors) diabetic ribosomes are only half as efficient as normal (9). Thus we have been led to undertake a systematic analysis of the effect of diabetes on the ability of ribosomes to participate in the partial reactions required for the initiation of protein synthesis.

The cell cytosol contains a factor (or factors) that catalyzes the reassociation of 40S and 60S ribosomal subunits to form 80S ribosomes (11,12). The reaction ordinarily requires a template, aminoacyl-tRNA and is stimulated by the initiation factor EIF-1, which suggests that reassociation of ribosomal subunits is a part of the initiation of protein synthesis. We report now on the capacity of ribosomal subunits from diabetic muscle to reassociate to form 80S couples.

MATERIALS AND METHODS

The following have been described before: the method of inducing diabetes with alloxan in male Sprague-Dawley rats (2); the isolation of ribosomes from skeletal muscle (13,14); the formation of ribosomal subunits (14,15); the preparation of a factor from rat liver cytosol that catalyzes binding of aminoacyl-tRNA to 40S particles and subunit reassociation--referred to as G-25 fraction (16); the preparation of rat liver tRNA (17) which was aminoacylated with twenty different amino acids (18).

The assay of the reassociation of ribosomal subunits has been described (19). Just prior to the experiments the suspension of subunits was clarified by centrifugation at 10,000 g for 5 min at 4°. The subunits (40S, 4.28 µg of RNA; 60S, 10.71 µg of RNA) were incubated for 15 min at 30° in 100 µl of Medium A (10 mM Tris-HCl, pH 7.6; 120 mM KCl; 3.5 mM $MgCl_2$) containing 30 µg of aminoacyl-tRNA, 10 µg of poly (U) and 0.29 mg of G-25 fraction protein. After incubation the ribosomal particles were fixed with glutaraldehyde (19). When G-25 was omitted from the reassociation mixture, the same amount of bovine serum albumin was added to prevent loss of ribosomal particles during glutaraldehyde fixation (20). An 80 µl sample was layered onto a 5.2 ml linear 10 to 30% sucrose gradient in Medium A. Centrifugation was in a SW 50.1 rotor

at 45,000 rpm for 100 min at 4°. In some experiments no glutaraldehyde was added and the samples (95 µl) were analyzed after the reaction was stopped by cooling. The distribution of ribosomal particles in the gradient was determined with an ISCO density gradient fractionator and ultraviolet analyzer (12,19). The extent of reassociation was estimated by cutting out and weighing the 40S, 60S and 80S peaks of the optical density tracing.

RESULTS AND DISCUSSION

When ribosomal subunits are incubated in buffer alone unstable 40S-60S couples are formed, the couples are dissociated by hydrostatic pressure during centrifugation unless they are fixed with glutaraldehyde (Fig. 1a and 1a' and reference 19). We compared the ability of ribosomal subunits from the skeletal muscle of normal and diabetic rats to associate. Diabetes reduced the capacity of subunits to form 80S monomers (Fig. 1, compare 1a and 1d). Moreover, the couples that formed in the reassociation reaction were less stable if the subunits were from diabetic ribosomes and, therefore, more liable to dissociate during centrifugation if they were not fixed with glutaraldehyde (compare now Fig. 1a' and 1d'). The defect is carried by the 60S subunit. When reassociation was assayed with a heterologous mixture of normal and diabetic ribosomal subunits, the number of 80S monomers formed (Fig. 1c) and their stability (Fig. 1c') was decreased if the 60S particle was from diabetic animals, whereas reassociation did not differ appreciably from normal if the small subunit was from diabetic animals (Fig. 1b and 1b'). Thus diabetes alters the 60S subparticle in a way that makes it less capable of forming a couple with the 40S subunit, and hybrid 80S ribosomes containing a diabetic 60S subunit are less stable.

An initiation factor (EIF-1) present in the cell cytosol catalyzes the formation of stable 80S ribosomes in the presence of poly (U) and aminoacyl-tRNA (Fig. 2a and 2a', and references 11, 12, 19). We tested now the capacity of diabetic ribosomal subunits to respond to the initiation factor (G-25 fraction). Diabetic ribosomal subunits were less effective than normal in forming 80S

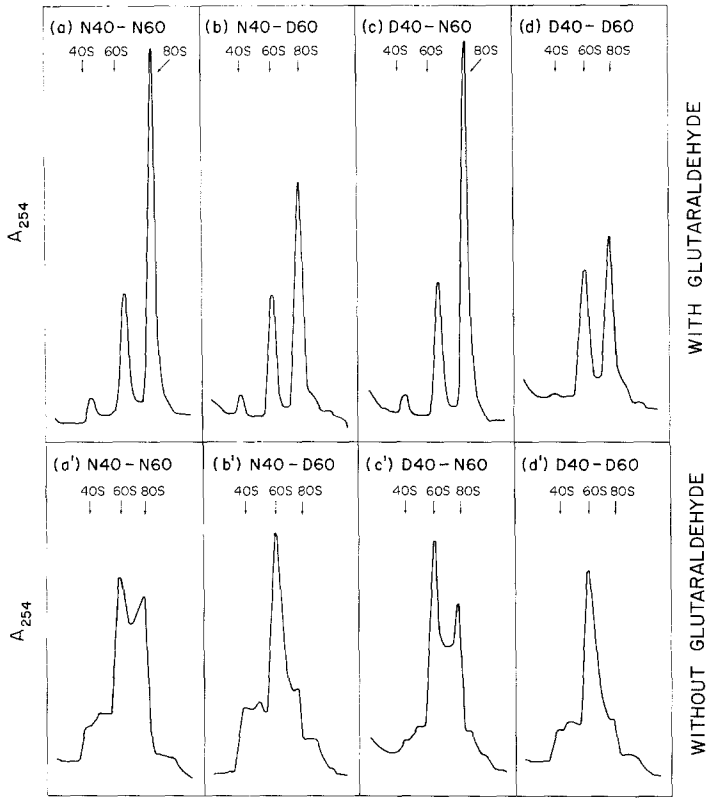

Fig. 1. Non-enzymic reassociation of ribosomal subunits from muscle of normal (N) and diabetic (D) rats. Ribosomal subunits (40S, 4.28 µg of RNA; 60S, 10.71 µg of RNA) were incubated for 15 min at 30° in 100 µl of Medium A containing 0.29 mg of bovine serum albumin. In (a-d), after incubation, the samples were cooled, fixed with 25 µl of 5% glutaraldehyde solution, and 80 µl was layered on a 10 to 30% sucrose gradient in Medium A; centrifugation was at 45,000 rpm for 100 min at 4° in a SW 50.1 rotor. In (a'-d') the conditions for the analysis were the same except the particles were not fixed with glutaraldehyde before centrifugation. The amount of 40S subunits does not appear to increase when reassociation is inhibited by diabetes for, as was shown before (12), the relative size of the 40S peak depends on whether or not G-25 fraction is present. In the medium which was used, the 40S subunits dimerize and sediment at 55S; the dimerization is prevented by some component present in G-25 fraction. Moreover, when unstable 80S monomers dissociate during centrifugation (in the absence of glutaraldehyde fixation) the 40S and 60S subunits form a broad peak between 80S and 60S.

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couples in the reaction catalyzed by EIF-1 (Fig. 2a and 2d and 2a' and 2d'); not only was the number of 80S monomers decreased by diabetes (Fig. 2d), but once again they were less stable (Fig. 2d'); indeed, the defect was more apparent when the particles were not fixed with glutaraldehyde (Fig. 2d'). The decreased responsive of diabetic subparticles to the initiation factor EIF-1

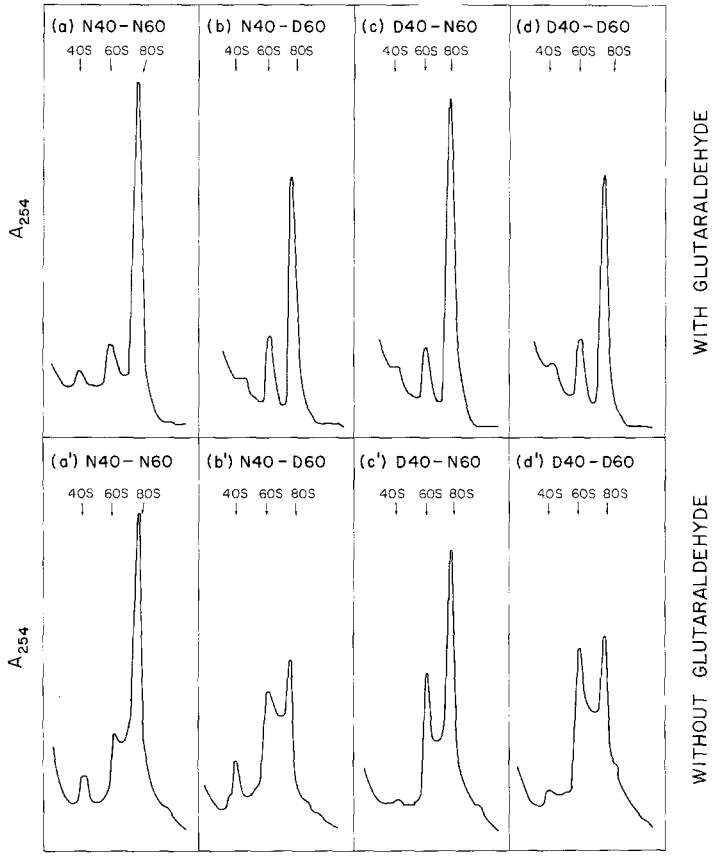

Fig. 2. Initiation factor catalyzed reassociation of ribosomal subunits from muscle of normal (N) and diabetic (D) rats. Ribosomal subunits (40S, 4.28 µg of RNA; 60S, 10.71 µg of RNA) were incubated for 15 min at 30° in 100 µl of Medium A containing 30 µg of aminoacyl-tRNA, 10 µg of poly (U) and 0.29 mg of G-25 fraction protein. Reassociation was assayed as in Fig. 1.

is carried by the 60S subunit (Fig. 2b and 2b'); the 40S subparticle appears normal in this reaction too (Fig. 2c and 2c').

We next tested whether polyphenylalanine synthesis was decreased in the conditions used to assay reassociation (Table 1). In those circumstances, diabetic ribosomes reconstituted from subunits were less efficient than normal; when hybrid ribosomes were constructed the defect in phenylalanine polymerization resided entirely in the 60S subunit. Similar observations had been made before (9, 21) but not in the same conditions.

The 60S subunit of diabetic ribosomes is less efficient than normal in

TABLE I

Polyphenylalanine synthesis by hybrid 80S particles formed from subunits of muscle ribosomes from normal and diabetic rats

Source of ribosomal subunits		[^{3}H]polyphenylalanine synthesis
40S	60S	(cpm)
N	N	6,637
N	D	2,698
D	N	8,109
D	D	1,845

Ribosomal subunits (40S, 4.28 µg of RNA; 60S, 10.71 µg of RNA) were incubated for 45 min at 30° in 100 µl of Medium A containing: 30 µg of [^{3}H] Phe-tRNA (21,000 cpm); 10 µg of poly (U); 0.25 µmole GTP; 0.05 µmole ATP; 0.25 µmole creatine phosphate; 10 µg creatine kinase; 0.29 mg G-25 fraction protein. The synthesis of polyphenylalanine was determined as described before (2). N, normal; D, diabetic.

forming 80S couples with the small subunit. The defect appears to be intrinsic to the particles since it is manifest even in the absence of the factor that catalyzes reassociation. Moreover, the putative structural change in the diabetic 60S subunit confers an instability (e.g. sensitivity to pressure induced dissociation) on 80S ribosomes which contain that subparticle. Finally, the diabetic 60S subunit is less responsive than normal to the action of EIF-1 in catalyzing reassociation. While the results accord with the idea that diabetes causes a change in the structure of the 60S particle, we cannot be sure that the defect is not the result of some fortuitous contaminant unequally distributed between normal and diabetic 60S subunits. We have taken precautions (the ribosomes were treated with puromycin and high concentrations of monovalent cations; references 13-15), but until the structural change is identified some uncertainty must remain.

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