DISSOCIATION OF SKELETAL MUSCLE RIBOSOMES FROM NORMAL AND DIABETIC ANIMALS BY INITIATION FACTOR EIF-3

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SUMMARY

80S ribosomes from skeletal muscle of diabetic rats were more susceptible than normal to dissociation by the initiation factor EIF-3; the increased sensitivity to dissociation was a property of the 60S subunit. Increased dissociation may be a reflection of the instability of diabetic 80S couples resulting from a change in the structure of the large subunit.

Ribosomes isolated from the muscle of diabetic rats are less effective than normal in the synthesis of protein (1), and the defect appears to reside entirely in the function of the 60S subunit (2,3). It is not certain, however, that the altered activity of the large subunit is a reflection of a change in the structure of the particle. There is no difference in the ability of normal and diabetic ribosomes to catalyze the elongation reactions of protein synthesis (4-7), thus it is to be expected that the fault lies in one of the reactions required to initiate protein synthesis (8,9). We have begun a study of the effect of diabetes on those partial reactions (3). Ribosomal subunits from the muscle of diabetic animals are less able to form 80S couples, either spontaneously or in the reaction catalyzed by the initiation factor EIF-1: in this reaction too the defect is a property of the 60S subunit (3).

We report now the effect of the initiation factor EIF-3 on the dissociation of muscle ribosomes from normal and diabetic rats. Dissociation of ribosome into subunits is necessary for initiation of protein synthesis. In

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prokaryotic cells the reaction is catalyzed by IF-3 (10-12). A factor (EIF-3) isolated from ascites cell ribosomes and resolved free of EIF-1 and EIF-2 activity catalyzes dissociation of rat liver 80S ribosomes into subunits (13).

MATERIALS AND METHODS

The following have been described before: the method of inducing diabetes with alloxan in male Sprague-Dawley rats (1); the isolation of ribosomes from skeletal muscle (14,15); the formation of ribosomal subunits (15,16); preparation of initiation factor EIF-3 from mouse ascites cell ribosomes (13).

The assay of dissociation of ribosomes by EIF-3 was carried out as described before (13). The 80S monomers which were used as substrate in the dissociation reaction were prepared from normal or diabetic ribosomal subunits (4.28 µg of rRNA of 40S and 10.71 µg of 60S) by incubating 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 0.29 mg of bovine serum albumin. The albumin was added to prevent loss of ribosomal particles during glutaraldehyde fixation. EIF-3 was added to the mixture and incubation was continued for an additional 5 min. The dissociation reaction was terminated by cooling the sample on ice and fixing the particles with 1% glutaraldehyde (13,17). A sample (80 µl) 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°. The distribution of ribosomal particles in the gradient was determined with an ISCO density gradient fractionator and ultraviolet analyzer. The percentage of dissociation was estimated by cutting out and weighing the 40S, 60S and 80S peaks of the optical density tracing.

RESULTS AND DISCUSSION

Ribosomal subunits from the skeletal muscle of normal or diabetic rats associate spontaneously when incubated in Medium A (Fig. 1a-d and reference 13). The 80S monomers formed in the reassociation reaction are free of peptidyl-tRNA and mRNA (18) and thus suitable as substrate to assay dis-

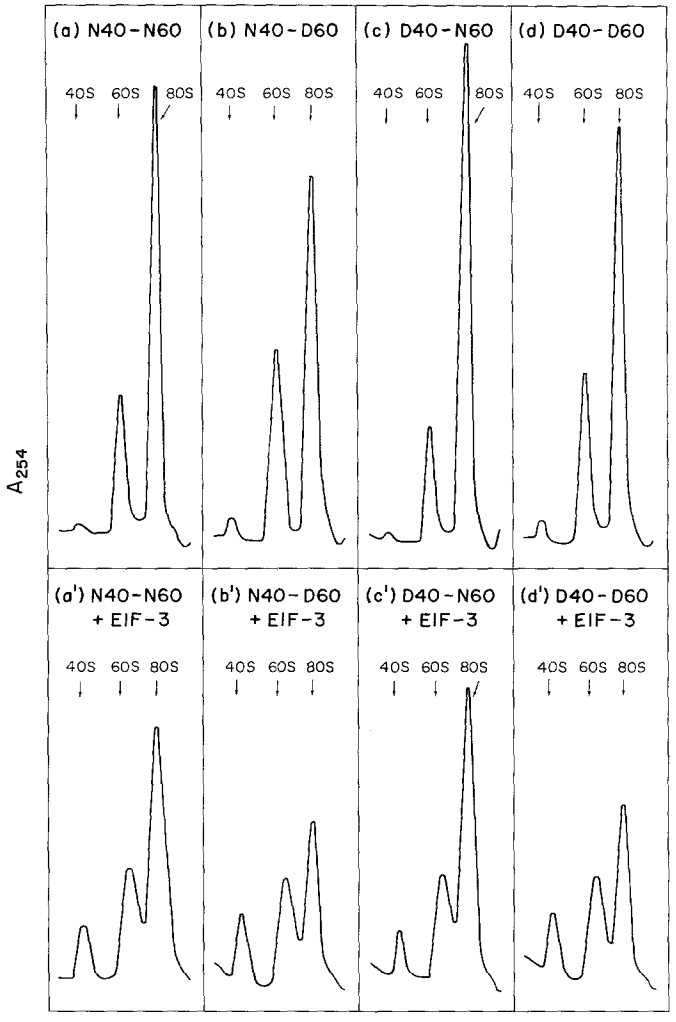

Fig. 1. Dissociation by EIF-3 of hybrid particles formed from subunits of muscle ribosomes from normal and diabetic rats. Ribosomal subunits (4.28 µg of 40S and 10.71 µg of 60S) were incubated for 15 min at 30° in 100 µl of Medium A containing 0.29 mg of bovine serum albumin (a-d). After incubation, 22.4 µg of EIF-3 was added to some of the sample (a'-d') and incubation was continued for another 5 min. The samples were chilled on ice and fixed with 25 µl of 5% glutaraldehyde solution. A sample (80 µl) was layered on a 10 to 30% sucrose gradient in Medium A. The gradients were centrifuged at 4° for 100 min at 45,000 rpm in a SW 50.1 rotor and the sedimentation of the particles was determined with an ISCO gradient fractionator and ultraviolet analyzer.

sociation by initiation factor EIF-3. It is important that there will be less substrate (80S ribosomes) for EIF-3 in the dissociation reaction whenever diabetic 60S subunits are present, since they are less effective than normal in the reassociation reaction (Fig. 1a-d).

Dissociation of 80S monomers from skeletal muscle, catalyzed by EIF-3 from ascites cells, had the same characteristics as with liver ribosomes (when account was taken of variations in the specific activity of different EIF-3 preparations). Dissociation was rapid and the extent of the reaction was proportional to the amount of EIF-3. In the experiments that follow we used less than maximal amounts of EIF-3, e.g. dissociation was limited by the availability of initiation factor.

Diabetic ribosomes were more sensitive than normal to the dissociating activity of EIF-3 (Fig. 1a' and 1d'); that sensitivity was a property of the 60S subunit, for hybrid ribosomes with a diabetic 40S subparticle responded to the initiation factor in a way that was appreciably normal (Fig. 1c'), whereas those with a diabetic 60S were more susceptible to dissociation (Fig. 1b'). There was increased dissociation, in response to EIF-3, of ribosome containing a diabetic 60S subparticle even when account was taken of the initial concentration of substrate (Table I); thus the actual dissociation catalyzed by EIF-3 was 1.5 to 2.5 times greater if the 80S ribosomes contained a diabetic large subunit (Table I).

Dissociation of ribosomes by EIF-3 is presumed to be a part of the initiation of the synthesis of peptide chains, indeed to be required for the process. Diabetic ribosomes are less efficient than normal in the translation of encephalomyocarditis virus RNA where the formation of virus-specific peptides requires the initiation of protein synthesis (9). Diabetic ribosomal subunits are less effective than normal in forming 80S couples in the reaction catalyzed by the initiation factor EIF-1 (3); the reassociation reaction is also a part of initiation. It is perhaps surprising then that diabetic ribosomes are more susceptible than normal to the dissociating activity of EIF-3. However, it is possible that increased dissociation of diabetic ribosomes by EIF-3 is actually a reflection of the instability of 80S couples containing a diabetic 60S subunit, rather than an actual increase in efficiency of one of the partial reactions of initiation. Increased dissociation of diabetic 80S

TABLE I

Dissociation by EIF-3 of hybrid 80S particles formed from subunits of muscle ribosomes from normal and diabetic rats

Expt.	Source of ribosomal subunits in 80S particles		80S particles (%)[a]		Dissociation (%)[b]
	40S	60S	Without EIF-3	With EIF-3	
1	N	N	70.6	60.0	15.0
	N	D	62.7	46.0	26.6
	D	N	76.6	63.8	16.7
	D	D	65.8	48.9	25.7
2	N	N	73.9	56.9	23.0
	N	D	62.9	35.7	43.3
3	N	N	68.8	56.0	17.2
	N	D	65.1	36.5	43.9

[a]The 80S particles are given as a percentage of all the material on the gradient that absorbs at 254 nm.

[b]The percentage dissociation is A-B/A X 100: where A is the percentage of 80S particles in the absence of EIF-3 and B is the percentage with EIF-3.

The data for Expt. 1 are from Fig. 1; in Expts. 2 and 3 the same amount (22.4 µg) of EIF-3 was used but it had a higher specific activity. N, normal; D, diabetic.

ribosomes by EIF-3 is viewed then as a manifestation of an alteration in the structure of the 60S subunit as a result of insulin lack. It is important that even if dissociation of ribosome monomers is increased in diabetes there will be no improvement in the synthesis of protein if subsequent reactions in the initiation process (such as reassociation) are decreased. Increased sensitivity of diabetic ribosomes to dissociation could account for the increased numbers of ribosomal subunits in the muscle of diabetic animals (8). The results then are consistent with the view that the decreased synthesis of muscle protein that diabetic animals suffer is the result of a defect in the initiation of peptide

formation following on a change in the structure of the 60S ribosomal subunit; the change in the large subparticle probably affects one of the partial reactions of initiation that follows dissociation of 80S monomers.

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