Binding and Translation of Turnip-Yellow-Mosaic-Virus Ribonucleic Acid by Skeletal-Muscle Ribosomes from Normal and Diabetic Rats

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Ribosomes from skeletal muscle of diabetic rats were less active than normal ribosomes in protein synthesis directed by turnip-yellow-mosaic-virus RNA. The proportion of ribosomes from muscle of diabetic rats capable of binding turnip-yellow-mosaic-virus RNA was greater than normal, but there was no difference in the equilibrium constants for the binding reaction. The turnip-yellow-mosaic-virus RNA was bound preferentially to the small (40S) ribosomal subunit, whereas the decrease due to diabetes in its translation was associated with the large (60S) subunit. Thus the diminished capacity of ribosomes from muscle of diabetic rats to translate turnip-yellow-mosaic-virus RNA was not the result of decreased binding of the template.

Ribosomes isolated from the muscle of alloxan-diabetic rats are less active than normal ribosomes in synthesizing protein (Wool & Cavicchi, 1967). The difference is the result of a decrease in the proportion of ribosomes from muscle of diabetic rats active in endogenous protein synthesis rather than of a decrease in the number of peptide bonds synthesized by each active ribosome (Wool & Kurihara, 1967). Diabetes also decreases the proportion of polyribosomes (Stirewalt, Wool & Cavicchi, 1967). Insulin will reverse all these changes even if RNA synthesis has been suppressed by actinomycin D (Wool & Cavicchi, 1966; Wool *et al.* 1968), indicating that DNA-dependent RNA synthesis is not required for the effect of insulin on muscle ribosomes. This suggests that decreased protein synthesis by ribosomes from muscle of diabetic rats may be due to a decreased ability to bind mRNA. We therefore have measured the efficiency of binding of TYMV‡ RNA by normal and diabetic-muscle ribosomes. We report here results showing that, although diabetic-muscle ribosomes are less active than normal in translation of TYMV RNA, there is no defect in their binding to this message.

We studied TYMV RNA binding to the ribosomes for the following reasons: (1) TYMV RNA can easily

be made radioactive and then isolated in a pure form (Haselkorn, Fried & Dahlberg, 1963); (2) each intact strand of TYMV RNA contains only four binding sites for *Escherichia coli* ribosomes (Dahlberg & Haselkorn, 1966) and for ribosomes from other sources, including heart muscle (Dahlberg, 1966); (3) binding of TYMV RNA to ribosomes in solution can be accurately measured by a convenient Millipore-filter technique (Dahlberg & Haselkorn, 1967).

The limited number of binding sites for ribosomes on TYMV RNA and the ability to measure binding that has occurred in solution permit measurement of the equilibrium constants of the binding reaction. This would not have been possible with a synthetic mRNA such as poly U for the number of binding sites for ribosomes per strand of the synthetic homopolymers is not limited and therefore the number of messenger-ribosome complexes formed cannot be measured (Dahlberg & Haselkorn, 1967). Moreover, poly U is adsorbed on Millipore filters and the methods used to circumvent this (Moore, 1966) preclude measurement of the binding of the RNA to the ribosomes in solution and determination of equilibrium constants.

MATERIALS AND METHODS

General. The following have been described previously: the sources of the materials, the animals used, the method of inducing diabetes with alloxan and the preparation of aminoacyl-tRNA (Wool & Cavicchi, 1967); the preparation of ribosomes, their subunits and the reassociation of subunits (Martin & Wool, 1968; Martin, Rolleston, Low & Wool, 1969); the growth of TYMV and the isolation and

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‡ Abbreviation: TYMV, turnip-yellow-mosaic-virus.

extraction of its RNA (Haselkorn, *et al.* 1963; Dahlberg & Haselkorn, 1967). L-[^{14}C]threonine (0.163 Ci/mmol) and L-[^3H]threonine (1.35 Ci/mmol) were purchased from New England Nuclear Corp., Boston, Mass., U.S.A.

Assay of protein synthesis. The ribosomes, at concentrations given in the Results section, were incubated for 30 min at 37°C in 1 ml containing 50 mM-tris-HCl buffer, pH 7.8, 80 mM-KCl, 5–20 mM-MgCl₂ (as indicated), 5 mM-ATP, 10 mM- β -mercaptoethanol, 0.4 mM-GTP, 1 mM-phosphoenolpyruvic acid, 10 μg of pyruvate kinase, 100 μg of aminoacyl-tRNA and 2 mg of skeletal muscle supernatant protein (Wool & Cavicchi, 1967). The concentration of TYMV RNA or poly U when present was 100 $\mu\text{g}/\text{ml}$. The reaction was initiated by addition of ribosomes and was stopped by adding 3 ml of cold 10% (w/v) trichloroacetic acid. After heating at 90°C for 15 min to hydrolyse aminoacyl-tRNA, protein was collected by filtration on glass fibre discs (24 mm diam.) and washed with 40 ml of 5% (w/v) trichloroacetic acid containing 10 mg of phenylalanine/ml. Protein on the filters was dissolved in 0.7 ml of formic acid in counting vials and the scintillation fluid [15 ml of 0.5% 2,5-bis-(5-*tert*-butylbenzoxazol-2-yl)thiophen in toluene-methylCello-solve (1:1, v/v)] was added. The concentration of ribosomes (measured as rRNA) was determined by a modification (Wool & Cavicchi, 1967) of the method of Fleck & Munro (1962). The efficiencies of counting in a Packard Tri-Carb liquid-scintillation spectrometer were 8.2% for ^3H and 63% for ^{14}C .

Binding of TYMV RNA to muscle ribosomes. The binding of TYMV RNA to muscle ribosomes was measured by using the technique of Dahlberg & Haselkorn (1967). Ribosomes were incubated with ^{32}P -labelled TYMV RNA for 4–5 min at 37°C in 0.22 ml (except for the experiments in Figs. 1 and 4, where the volume was 1 ml) of tris-KCl buffer [50 mM-tris-HCl buffer (pH 7.8)–80 mM-KCl] containing the MgCl₂ concentration indicated. The ribosomes and any bound TYMV RNA were separated from free TYMV RNA by rapid filtration through 25 mm Millipore filters (type HA, 0.45 μm pore size) after dilution of the reaction mixture with 5 ml of the same buffer. The filters were washed with a further 20 ml of the same buffer, transferred to scintillation bottles and dried at 110°C for 15 min; 15 ml of scintillation fluid [0.5% of 2,5-bis-(5-*tert*-butylbenzoxazol-2-yl)thiophen in toluene] was added and the samples were counted in a Packard Tri-Carb liquid-scintillation spectrometer.

The concentration of TYMV RNA and of ribosomes (measured as rRNA) were calculated from the extinction at 260 nm. For TYMV RNA $E_{0.1\%}^{1\text{cm}}$ 23 at 260 nm (Haselkorn *et al.* 1963) and a molecular weight of 2×10^6 daltons (Dahlberg & Haselkorn, 1967) were assumed; for the RNA of ribosomes $E_{0.1\%}^{1\text{cm}}$ was taken to be 22 at 260 nm [determined by using the procedure of Fleck & Munro (1962) as modified by Wool & Cavicchi (1967)] and the molecular weight to be 2.2×10^6 daltons (Petermann & Pavlovic, 1963).

(i) Temperature and time of incubation. Preliminary experiments showed that binding of TYMV RNA by rat muscle ribosomes was greater at 37°C than at 0°C or 21°C (Fig. 1), in contrast with binding by *E. coli* ribosomes, which increased as the temperature decreased (Dahlberg & Haselkorn, 1967). The difference may be related to the reversible aggregation of ribosomes from rat tissue

Fig. 1. Temperature-dependence and time-course of binding of TYMV RNA by normal skeletal-muscle ribosomes. Incubations contained ribosomes (9 μg of rRNA) and 0.32 μg of ^{32}P -labelled TYMV RNA (6030 c.p.m.) in 1 ml of tris-KCl buffer containing 12.5 mM-MgCl₂. ○, 0°C; △, 21°C; ●, 37°C.

that is observed at low temperatures (Reader & Stanners, 1967). Accordingly, the binding of TYMV RNA to rat muscle ribosomes was assayed at 37°C; the binding reaction at that temperature was complete in 4 min.

(ii) Hydrolysis of TYMV RNA. TYMV RNA was partially hydrolysed in 0.11 M-KOH at 23°C for 6 min so that each polynucleotide chain contained no more than one ribosome-binding site (Dahlberg & Haselkorn, 1967); hydrolysis was terminated by neutralization with HCl. The solution was then made 50 mM with respect to tris-HCl buffer, pH 7.8, and 80 mM with respect to KCl. The ^{32}P -labelled TYMV RNA was used to assay binding directly after hydrolysis.

(iii) Measurement of the extent of hydrolysis of TYMV RNA. TYMV RNA (0.3–0.5 μg) was incubated in 0.2 ml with increasing amounts of ribosomes in tris-KCl buffer containing 12.5 mM-MgCl₂; maximal binding of TYMV RNA was obtained with 120–180 μg of rRNA (the capacity of the filters for efficient adsorption of muscle ribosomes was 180–200 μg of rRNA).

The amount of TYMV RNA bound by an excess of ribosomes was the same for ribosomes from muscle of normal and diabetic rats (see Fig. 4). The fraction of partially hydrolysed TYMV RNA (generally 30–40%) that was bound in the presence of an excess of ribosomes was divided by four [the number of ribosome-binding sites per intact molecule of TYMV RNA (Dahlberg & Haselkorn, 1967); see the Results section] to obtain the weight-average size of the polynucleotide chains that could bind to ribosomes.

(iv) Measurement of the number of ribosomes capable of binding TYMV RNA. The maximal amount of partially hydrolysed TYMV RNA [whose average molecular weight had been determined in (iii)] that could bind to a limiting amount of ribosomes was measured by incubating ribosomes (2.5–3.5 μg of rRNA) with increasing amounts of hydrolysed TYMV RNA in tris-KCl buffer containing

12.5 mM-MgCl₂. The ribosomes were saturated by 30–40 μ g of partially hydrolysed TYMV RNA. The fraction of added ribosomes capable of binding TYMV RNA was calculated from the number of ribosomes added and the average chain length (molecular weight) of each strand of partially hydrolysed TYMV RNA containing a binding site (Dahlberg & Haselkorn, 1967); it was assumed that each ribosome binds only one strand of mRNA (Moore, 1966).

(v) Determination of equilibrium constants of the binding reaction. The reaction mixture used to determine the equilibrium constants of the binding reaction generally contained 6–10 μ g of ribosomal RNA and 2–4 μ g of partially hydrolysed TYMV RNA; in the assays 10–30% of the added ribosomes and 0.5–3% of the added TYMV RNA were bound. Calculation of the equilibrium constant for binding required the values measured above for the number of ribosomes capable of binding TYMV RNA and the average molecular weight of each strand of partially hydrolysed TYMV RNA containing a binding site.

(vi) Sample calculation of equilibrium constants for TYMV RNA binding. (a) Molecular weight of partially hydrolysed TYMV RNA. After hydrolysis for 6 min in 0.11 M-KOH at 23°C, 0.935 μ g (11131 c.p.m.) of partially hydrolysed TYMV RNA (specific radioactivity 11900 c.p.m./ μ g) were incubated with excess of ribosomes from muscle of normal and diabetic rats (90 and 180 μ g of rRNA); 5665 c.p.m. was bound to the filter (45 c.p.m. in the absence of ribosomes). Therefore after hydrolysis (5665–45)/11131 = 50.5% of the TYMV RNA is in polynucleotide strands containing ribosome-binding sites. Given a molecular weight of 2×10^6 daltons, and four ribosome-binding sites for intact TYMV RNA the polynucleotides in the partially hydrolysed TYMV RNA that carry ribosome-binding sites have a weight-average molecular weight of $(50.5/100) \times (2 \times 10^6/4) = 2.53 \times 10^5$ daltons.

(b) Fraction of ribosomes binding TYMV RNA. $0.0735 E_{260}$ unit = $(0.0735 \times 10^3)/22 \mu$ g of rRNA = $(0.0735 \times 10^3)/(2.2 \times 22) = 1.50$ pmol of ribosomes was incubated with 5.05×10^5 c.p.m. = 42.4 μ g of partially hydrolysed TYMV RNA; 4513 c.p.m. was bound to the filter (703 c.p.m. in the absence of ribosomes). Therefore 1.50 pmol of ribosomes bound $(4513 - 703)/11900 = 0.320 \mu$ g of TYMV RNA. From (a), 0.320μ g of partially hydrolysed TYMV RNA = $0.320/(2.53 \times 10^5) = 1.27$ pmol of partially hydrolysed TYMV RNA strands carry a ribosome site. Therefore $1.27/1.50 = 85\%$ of the ribosomes can bind TYMV RNA.

(c) Equilibrium constant of the binding reaction. $0.147 E_{260}$ unit = 3.00 pmol of ribosomes were incubated with 28708 c.p.m. of partially hydrolysed TYMV RNA in 0.22 ml; 2608 c.p.m. was bound (262 c.p.m. in the absence of ribosomes). Therefore $(2608 - 262)/(11900 \times 0.253) = 0.529$ pmol of ribosome–partially hydrolysed TYMV RNA complex was formed. Of the ribosomes added, 85% = 2.55 pmol was able to bind TYMV RNA. Of the TYMV RNA radioactivity added 50.5% = 14498 c.p.m. = 1.218 μ g = 4.81 pmol contain a ribosome binding site. Therefore the equilibrium constant = $0.529/(2.55 - 0.529) \times (4.81 - 0.529) (\text{pmol}/0.22 \text{ ml})^{-1} = 0.0611 (\text{pmol}/0.22 \text{ ml})^{-1} = 2.77 \times 10^8 \text{ M}^{-1}$.

RESULTS

Translation of TYMV RNA. Addition of TYMV RNA to muscle ribosomes increased the incorporation into protein of radioactivity from [¹⁴C]threonyl-tRNA. At each Mg²⁺ concentration tested the incorporation due to addition of TYMV RNA (Fig. 2b) [obtained by subtracting endogenous incorporation (Fig. 2a) from that in the

Fig. 2. Effect of TYMV RNA on protein synthesis by ribosomes from muscle of normal and diabetic rats. Incubations contained ribosomes (30 μ g of rRNA) and the [¹⁴C]threonine-labelled aminoacyl-tRNA contained 21400 c.p.m./100 μ g. (a) Incorporation in the presence and absence of 100 μ g of TYMV RNA: —, normal-muscle ribosomes incubated with ($\blacktriangle$) and without ($\bullet$) TYMV RNA; ---, diabetic-muscle ribosomes incubated with ($\triangle$) and without ($\circ$) TYMV RNA. (b) Incorporation due to the addition of TYMV RNA (the values are the differences between pairs of curves in a): $\blacksquare$, normal; $\square$, diabetic.

presence of TYMV RNA] was less with ribosomes from muscle of diabetic rats than with normal ribosomes.

Fraction of ribosomes binding TYMV RNA. The difference between ribosomes from muscle of normal and diabetic rats in translation of TYMV RNA could be due to a decrease in the ability of the latter to bind the mRNA, resulting either from a decrease in the fraction of ribosomes with the capacity to bind mRNA or a decrease in the affinity of all the ribosomes for mRNA.

The possibility that ribosomes from muscle of diabetic rats interact with fewer binding sites on TYMV RNA than normal was tested with partially hydrolysed polynucleotide in the presence of an excess of normal and diabetic ribosomes; the fraction of partially hydrolysed TYMV RNA that could be bound and the ribosome concentration giving maximal binding of TYMV RNA were the same for normal- and diabetic-muscle ribosomes (results not shown; the curves are very similar to those in Fig. 4). Thus, normal- and diabetic-muscle ribosomes bind to the same number of sites on TYMV RNA. The same experiment indicates that there are four sites on TYMV RNA to which skeletal-muscle ribosomes can bind. The hydrolysis of TYMV RNA was controlled, so the number of breaks per intact chain could be calculated (Dahlberg & Haselkorn, 1966). The points correlating the number of breaks with the fraction of partially hydrolysed TYMV RNA bound by an excess of ribosomes lay on the top curve in Fig. 6 of Dahlberg & Haselkorn (1966).

The fraction of ribosomes capable of binding partially hydrolysed TYMV RNA was measured in the presence of an excess of the polynucleotide. Assuming four binding sites per intact chain, 87% of normal-muscle ribosomes and 109% of diabetic-muscle ribosomes could bind TYMV RNA (Table 1); the difference was significant at the 3% level (Mann Whitney U Test; see Siegel, 1956). The diabetic defect in translation of TYMV RNA is therefore not related to the number of ribosomes

able to bind the message. The observation that more than 100% of diabetic-muscle ribosomes could bind TYMV RNA is inconsistent with the assumption that each ribosome binds one strand of messenger RNA. The calculation depends on the accuracy of published molecular weights of intact TYMV RNA and of rRNA; the combined error of these two values could be as great as 20%. Within these limits the fraction of ribosomes active in the binding reaction was in the range expected if all the ribosomes not active in endogenous protein synthesis (Wool & Kurihara, 1967) could bind TYMV RNA (Table 1).

Equilibrium constant of the binding of TYMV RNA to ribosomes. There was no significant difference between normal- and diabetic-muscle ribosomes in the equilibrium constants for their binding of hydrolysed TYMV RNA over the range of Mg^{2+} concentrations tested. The results in Fig. 3 are the average of four separate comparisons between normal- and diabetic-muscle ribosomes. As these were directly compared in each experiment, the means and standard deviations of the paired values were determined; in no case did the average difference between paired values deviate significantly from zero.

TYMV RNA contains four ribosome-binding sites that can be separated from each other by hydrolysis. These may have different affinities for ribosomes. Therefore the number of sites contributing to measurement of equilibrium constants will depend on the degree of saturation of each ribosome-binding site by ribosomes. This view is supported by comparison between the theoretical and experimental curves for binding of a fixed amount of hydrolysed TYMV RNA with various amounts of ribosomes (Fig. 4). If one assumes an equilibrium constant of $1.2 \times 10^8 M^{-1}$ at $12.5 mM-Mg^{2+}$ for all ribosome-binding sites on TYMV RNA there is very little agreement between the theoretical binding curve and the experimental observation. However, if it is assumed that this equilibrium constant refers only to one site and

Table 1. *Fractions of ribosomes from muscle of normal and diabetic rats active in binding TYMV RNA and in endogenous protein synthesis*

Fractions of ribosomes active in binding TYMV RNA were measured as described in the Materials and Methods section. The Mann Whitney U test (Siegel, 1956) was used to test for significance of difference. The values for fractions active in endogenous protein synthesis are from Wool & Kurihara (1967). Results are expressed as percentages of added ribosomes $\pm$ S.D. with the numbers of determinations in parentheses.

Source of muscle ribosomes	Percentage active in TYMV RNA binding	Percentage active in endogenous protein synthesis
Normal rats	87 ± 11 (4)	26.3 ± 5.4 (17)
Diabetic rats	109 ± 4 (4)	8.8 ± 1.7 (8)
	$P < 0.03$	$P < 0.001$

Fig. 3. Equilibrium constants for the binding of TYMV RNA to skeletal-muscle ribosomes from normal and diabetic rats. Muscle ribosomes ($6\text{--}10\mu\text{g}$ of rRNA) were incubated at 37°C for 4 min with partially hydrolysed ^{32}P -labelled TYMV RNA ($2\text{--}4\mu\text{g}$ and $10000\text{--}20000\text{ c.p.m.}$) in 0.22 ml of tris-KCl buffer containing the concentration of MgCl_2 indicated. The values for the binding of ^{32}P -labelled TYMV RNA by *E. coli* ribosomes are taken from Dahlberg & Haselkorn (1967). ●, Normal; ○, diabetic; ▲, *E. coli*.

Fig. 4. Effect of ribosome concentration on the binding of hydrolysed TYMV RNA; comparison between experimental observation and binding predicted from measured equilibrium constants. Ribosomes from muscle of normal (●) and diabetic (○) rats were incubated in 1 ml at 12.5 mM-Mg^{2+} with $0.70\mu\text{g}$ of TYMV RNA hydrolysed such that 47.4% (----) could bind to an excess of ribosomes (determined in a separate incubation in a smaller volume). The amounts of TYMV RNA and ribosomes on ordinate and abscissa refer to strands containing binding sites and ribosomes active in TYMV RNA binding respectively. Curves are calculated for an apparent equilibrium constant of $1.2 \times 10^8\text{ M}^{-1}$ (Fig. 3) assuming that all four binding sites have this constant (----) or that only one has this constant and the others do not bind (—).

that there is no binding by the other three, the theoretical curve agrees with the experimental points at low ribosome concentrations. As not more than 7% of the TYMV RNA containing ribosome-binding sites actually was bound to ribosomes in the measurements of equilibrium constants, it is probable that the equilibrium constants obtained refer mainly to the site with the greatest affinity for ribosomes. However, the close similarity over a wide concentration range between ribosomes from muscle of normal and diabetic rats (Fig. 4) suggests that they do not differ significantly in binding to any of the other three sites on TYMV RNA.

Binding of TYMV RNA to ribosomal subunits. mRNA has been shown to bind to the small subunit of bacterial ribosomes (Gilbert, 1963; Takanami & Okamoto, 1963), and nascent vaccinia-virus mRNA associates with native 40S particles in HeLa cells (Joklik & Becker, 1965). In accordance with these observations we find TYMV RNA binds preferentially to the small (40S) subunit of skeletal-muscle ribosomes (Table 2). There was, however, some binding of TYMV RNA to the large (60S) subunit, and this was not entirely due to contamination with 40S subunits.

Identification of the subunit responsible for defective translation of TYMV RNA. Subunits were prepared from ribosomes from muscle of normal and diabetic rats and reassociated to give homologous or hybrid ribosomes by the methods of Martin & Wool (1968). These methods involve treatment with potassium chloride, density-gradient centrifugation to separate the subunits and dialysis of the required subunit mixtures to remove the added potassium chloride. Treatment with potassium chloride followed by dialysis caused marked loss of protein synthesis by ribosomes in the presence of

Table 2. Binding of TYMV RNA to subunits of skeletal-muscle ribosomes from normal rats

Ribosomes were dissociated and reassociated as described by Martin & Wool (1968). Incubations contained 4.1 pmol of ribosomes or subunits and $5.8\mu\text{g}$ of TYMV RNA (not hydrolysed) in 0.22 ml of tris-KCl buffer containing 12.5 mM-MgCl_2 . Results are expressed as μg of TYMV RNA bound/ pmol of ribosomes. The binding by the 60S subunit is corrected for 20% contamination of the preparation with 40S subunits.

Ribosomes or subunits	TYMV RNA bound
Control	0.038
KCl-treated	0.046
Small subunit (40S)	0.053
Large subunit (60S)	0.018
Reassociated	0.048

Table 3. *Protein synthesis due to TYMV RNA by subunits and hybrid 80S particles formed from subunits of ribosomes from muscle of normal and diabetic rats*

Ribosomes were dissociated and reassociated as described by Martin & Wool (1968). Protein synthesis was measured as described in the Materials and Methods section; incubation mixtures contained ribosomes (20–30 μ g of rRNA), [3 H]threonine-labelled aminoacyl-tRNA (22000 c.p.m./100 μ g) and 10 mM-MgCl₂. Results are expressed as c.p.m./30 μ g of rRNA.

Ribosomes or subunits used and treatment of donor rats	Incorporation		Stimulation by TYMV RNA
	Without TYMV RNA	With TYMV RNA	
Ribosomes			
Normal	1127	1558	431
Diabetic	258	312	54
KCl-treated ribosomes			
Normal	598	747	149
Diabetic	130	147	17
Subunits			
Normal 40S	1	0	0
Diabetic 40S	28	20	0
Normal 60S	37	66	29
Diabetic 60S	16	42	26
Hybrid ribosomes containing			
Normal 40S and normal 60S	77	217	140
Diabetic 40S and diabetic 60S	6	36	30
Normal 40S and diabetic 60S	10	48	38
Diabetic 40S and normal 60S	27	147	120

TYMV RNA (Table 3). However, there was no further loss on separation of subunits by centrifugation followed by reassociation of the subunits by dialysis. Reassociated ribosomes in which the diabetic-muscle 60S subunit was combined with either normal- or diabetic-muscle 40S subunits were less active in amino acid incorporation in the presence of TYMV RNA than those containing the normal-muscle 60S subunit. The diabetic defect in translation of TYMV RNA is therefore in the 60S subunit.

DISCUSSION

Ribosomes from skeletal muscle of diabetic rats were less active than normal ribosomes in protein synthesis directed by TYMV RNA. Moreover, the difference between normal- and diabetic-muscle ribosomes was retained by particles that had been dissociated into subunits and then reassociated. The reassociated ribosomes had little endogenous protein-synthetic activity, possibly because ribosomes active in endogenous protein synthesis are not dissociated into subunits under our conditions (Martin *et al.* 1969). Thus the decrease in translation of TYMV RNA by ribosomes from diabetic rats is not due to the smaller number of ribosomes active in endogenous protein synthesis.

A larger fraction of the population of ribosomes from muscle of diabetic rats than of those from normal rats could bind TYMV RNA; however, the

equilibrium constants of the binding reaction were the same. Therefore, the diminished ability of diabetic-muscle ribosomes to translate TYMV RNA is apparently not related to binding of the template to the ribosomes. This conclusion is supported by the observation that the diabetic defect in translation of TYMV RNA is associated with the 60S subunit whereas mRNA is bound preferentially to the 40S subunit.

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