Short Communications

Concentration of Elongation Factor 2 in Rat Skeletal Muscle During Protein Depletion and Re-Feeding

SUNNEY D. ALEXIS, VERNON R. YOUNG

*Department of Nutrition and Food Science, Massachusetts Institute of Technology,
Cambridge, Mass. 02139, U.S.A.*

and D. MICHAEL GILL

Department of Biology, Harvard University, Cambridge, Mass. 02138, U.S.A.

(Received 16 April 1974)

Rat skeletal-muscle elongation factor 2 was assayed by causing it to react with NAD⁺ by using fragment A of diphtheria toxin as the catalyst. Dietary protein restriction decreased the concentration of elongation factor 2 in homogenates of whole muscle. These decreases paralleled a decline in muscle RNA so that the number of molecules of elongation factor 2 per ribosome appeared to be independent of the diet. We conclude that elongation factor 2 is probably not the factor limiting the rate of muscle protein synthesis and is not responsible for the fall in the protein-synthetic rate *in vivo* observed in the muscles of animals whose dietary protein intake is inadequate.

Henshaw *et al.* (1971) have suggested, from studies of ribosome-distribution profiles and ¹⁴C-labelled amino acid incorporation *in vivo* and *in vitro*, that the decreased rate of protein synthesis in livers of starved rats may be due to a limitation of the availability of elongation factors. van Venrooij *et al.* (1970) concluded that nutritional factors modulate the rate of protein synthesis in Ehrlich ascites cells at the stage of both peptide-chain initiation and elongation. Further, studies on mouse spleen (Willis & Starr, 1971) and rat kidney (Girgis & Nicholls, 1971, 1972) have indicated that the concentration of elongation factor 1 may be rate-limiting for protein synthesis under certain conditions.

The rate of muscle protein synthesis is decreased by as much as 50% when rats are starved (Millward, 1970) or fed on a diet low in protein (Waterlow & Stephen, 1966, 1968; Young *et al.*, 1971). This diminished protein synthesis is associated with a decrease in the number of ribosomes (Young & Alexis, 1968) and in the capacity of crude ribosome (Young & Alexis, 1968; Alexis *et al.*, 1971; Alexis & Young, 1973; von der Decken & Omstedt, 1970; Omstedt & von der Decken, 1972) and soluble enzyme (Alexis *et al.*, 1971; Migliorini & Manchester, 1971) fractions of skeletal muscle to catalyse amino acid incorporation *in vitro*.

Whether the availability of one of the elongation factors in muscle becomes rate-limiting for polypeptide synthesis *in vivo* under unfavourable nutritional conditions has not previously been explored. Thus we have measured the concentration of elongation factor 2 in the hind-leg muscles of rats given either an adequate- or low-protein diet, and during re-

covery from dietary protein restriction. The concentration of elongation factor 2 was decreased during dietary protein deprivation, but this decline paralleled a decrease in muscle RNA. From these observations, it appears that elongation factor 2 is probably not a limiting factor for muscle protein synthesis under the dietary conditions studied.

Methods

Animals and diets. Young male rats (C. D. Strain, Charles River Breeding Laboratories, Wilmington, Mass., U.S.A.) weighing 90–100g were fed on a fully adequate (18% lactalbumin) diet for 3 days after their arrival. They were then randomly divided into two dietary groups, one continuing on the adequate-protein diet, the other receiving a low-protein (0.5% lactalbumin) diet (Young *et al.*, 1971). After consuming the low-protein diet for 15 or 19 days, some rats were re-fed with the adequate diet. Food and water were offered *ad libitum*. Lights were on in the animal quarters between 07:00 and 19:00h. Rats were taken for tissue preparation at about 09:00h having had access to food throughout the night.

Preparation of muscle homogenates. For each time-point in the experiments, two or three rats from each dietary group were decapitated and the mixed muscle of both hind legs was quickly removed.

Visible fat and nerves were trimmed from the hind-leg muscles, and muscles from each group were pooled, weighed, minced and homogenized with a Polytron homogenizer (Chen & Young, 1968) in 2 volumes of buffer [0.25M-KCl–0.1M-Tris-HCl (pH 7.6)–0.01M-MgCl₂–1mM-dithiothreitol–10% gly-

cerol]. We then diluted the homogenates to 4g of original muscle/50ml in the same buffer.

Extraction and assay of elongation factor 2. Total muscle elongation factor 2 was assayed by a modification of a procedure described previously (Gill & Dinius, 1973). Portions of each muscle homogenate (3g) were shaken at 4°C for 15 min with 1 ml of 2M-NaCl, 0.5g of activated charcoal and 20 µmol of dithiothreitol. The homogenates were then centrifuged at 100000g for 1 h. The NAD⁺-free supernatants, containing all the elongation factor 2 of the muscle, were removed and 0.1 ml portions were incubated with 0.3 ml of the following mixture: 10 µCi of [³²P]NAD⁺ of accurately known specific radioactivity (between 150 and 250 c.p.m./pmol), 10 µg of fragment A of diphtheria toxin/ml, 1 mg of serum albumin/ml, 0.05 M-dithiothreitol and 0.1 M-Tris-HCl (pH 8.0). Trichloroacetic acid-insoluble material was collected by filtration and counted for radioactivity by liquid scintillation. From each homogenate two extracts were made and assayed in duplicate. The average of the four values was corrected for counts recovered after incubation without toxin protein. We found it unnecessary to incubate in the presence of an imidazole derivative (Gill & Dinius, 1973) because interference from poly-(ADP-ribose) formation was negligible. Control experiments similar to those described earlier (Gill & Dinius, 1973) established that the difference in counts obtained with and without toxin was a true measure of the amount of elongation factor 2 present in the extracts.

RNA was measured by the method of Munro & Fleck (1966) on diluted samples of the homogenate,

which had been frozen at -70°C immediately after preparation.

Results

The content of elongation factor 2 of rat hind-leg muscles was measured in rats fed at different intervals on the low-protein diet and in control rats of the same age and starting weight and maintained on the adequate diet. Muscle is a poor source of elongation factor 2 and the variability in enzyme activities in different muscle samples was correspondingly greater than that found with tissues providing a richer source of the enzyme (Gill & Dinius, 1973). The results, summarized in Table 1, show that in the well-nourished animals there was no consistent change ($P > 0.1$) in the content of muscle elongation factor 2 with time on the diet. However, the concentration of elongation factor 2 was significantly lowered ($P < 0.001$) within 6 to 10 days of dietary protein restriction. Within 2 to 5 days of re-feeding the poorly nourished animals, muscle concentrations of elongation factor 2 returned to those maintained in the well-nourished controls.

Muscle total RNA declined with dietary protein restriction (Table 1), and within 5 days of re-feeding it had returned to the value observed in control rats. In tissues of well-nourished mammals, there appears to be approximately constant ratio of elongation factor 2 to ribosome number, as determined by RNA determination (Gill & Dinius, 1973). From Table 1, it is also evident that for skeletal muscle an approximately constant elongation factor 2/RNA ratio is maintained regardless of the animal's nutritional status.

Table 1. Concentration of RNA and elongation factor 2 in skeletal muscle of rats given adequate- or low-protein diet, and during re-feeding

Each adequate-protein entry is based on a pooled sample from two rats. Each low-protein entry is based on a pooled sample from three rats. Concentration of elongation factor 2 (EF-2) is expressed as nmol/g of fresh muscle. RNA values are mg/g of fresh muscle. Concentration of elongation factor 2 for the low-protein group was significantly lower than for the adequate group, as determined by the Student's *t* test ($P < 0.001$). The EF-2/RNA ratio for the low-protein group was not significantly different ($P > 0.1$) from control values.

Period	Days on diet	Adequate-protein diet				Low-protein diet			
		Body weight (g)	EF-2	RNA	EF-2/RNA	Body weight (g)	EF-2	RNA	EF-2/RNA
Depletion	1	110	0.70	1.60	0.44	100	0.70	1.58	0.44
	6	131	0.74	1.42	0.52	84	0.54	1.10	0.49
	8	157	0.75	1.56	0.48	88	0.41	1.05	0.39
	10	164	0.75	1.48	0.51	82	0.57	1.00	0.57
	15	220	0.72	1.51	0.48	81	0.44	0.74	0.59
	19	262	0.68	1.33	0.51	79	0.53	0.90	0.59
Re-feeding	1	87	0.46	0.91	0.50				
	2	97	0.51	1.07	0.48				
	5	123	0.71	1.53	0.46				
	9	165	0.72	1.77	0.42				

Discussion

The initiation and elongation rates of polypeptide synthesis in liver, cardiac and skeletal muscle can be altered by nutritional factors (Henshaw *et al.*, 1971; van Venrooij *et al.*, 1970; Jefferson *et al.*, 1972). Young (1974) has reviewed this aspect of the regulation of muscle protein synthesis. However, whether the availability of one of the elongation factors is rate-limiting for polypeptide chain elongation in muscle under unfavourable nutritional conditions has not previously been determined.

In the present studies we have determined the concentration of elongation factor 2 in skeletal muscle of rats and observed that dietary protein restriction significantly lowers its concentration. This effect occurs within 6 days. However, the concentration of elongation factor 2 relative to muscle total RNA remained approximately constant and did not differ from that in muscle of well-nourished controls. Because the proportion of total RNA that is rRNA appears to be maintained at about 80%, even during dietary protein deprivation (Young, 1970), our findings suggest that the availability of elongation factor 2 to ribosomes did not change in response to dietary protein restriction. Hence, if the elongation rate of protein synthesis *in vivo* is decreased in muscle under these dietary conditions, the availability of elongation factor 2 would not appear to be the rate-limiting factor.

Our results may be compared with a number of other observations made on intact mammalian cells under normal and abnormal conditions. Earl & Hindley (1971) concluded that aminoacyl-tRNA is freely available in the liver of starved, diabetic or hypophysectomized rats, as well as in muscle of normal rats, and that neither the rate of binding of aminoacyl-tRNA nor that of translocation is rate-limiting. Similarly, it seems that elongation factor 2 cannot be the rate-limiting factor in mammalian cell protein synthesis in culture, since there is a lag between the initial elongation factor 2 depletion during diphtheria intoxication and the onset of a decreased rate of cellular protein synthesis (Gill *et al.*, 1969). A similar situation exists in *Escherichia coli*, where the ratios of both elongation factors to ribosomes stay close to unity at different rates of growth (Gordon, 1970). An apparent discrepancy is the report by Henriksen & Smulson (1972) that the amount and intracellular distribution of HeLa-cell elongation factor 2 change markedly under different growth conditions. Unfortunately, they discarded a significant proportion of total cell enzyme in the 15000g pellet and did not show quantitative recovery during fractionation of the supernatant. We conclude that ribosome-bound elongation factor 2 is underestimated by the procedure they followed (Gill & Dinius, 1973). Thus we cannot assess the significance

of their observations in relation to the control of protein synthesis.

We are, however, unable to say if the ratio of molecules of elongation factor 2 to active ribosomes was maintained at exactly the same value, especially shortly after a change in diet. Because the proportion of the total ribosomes that are active in protein synthesis is diminished early during protein depletion (Young & Alexis, 1968; Young *et al.*, 1968) it is possible that there may be relatively more elongation factor 2 available per active ribosome at this time. In any event, the importance of the present results lies in the observation that the diminished rates of polypeptide synthesis which occur in muscle of rats fed on a low-protein diet is not due to a limitation in the availability of elongation factor 2.

This study was supported by grants from the National Institutes of Health (AM 13432) and the National Science Foundation (GB 13217). It is contribution no. 2058 from the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass. 02139, U.S.A.

References

- Alexis, S. D. & Young, V. R. (1973) *Biochem. J.* **136**, 773–780
- Alexis, S. D., Basta, S. S. & Young, V. R. (1971) *Biochem. J.* **128**, 521–530
- Chen, S. C. & Young, V. R. (1968) *Biochem. J.* **106**, 61–67
- Earl, D. C. N. & Hindley, S. T. (1971) *Biochem. J.* **122**, 267–276
- Gill, D. M. & Dinius, L. L. (1973) *J. Biol. Chem.* **248**, 654–658
- Gill, D. M., Pappenheimer, A. M., Jr. & Baseman, J. B. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34**, 595–602
- Girgis, G. R. & Nicholls, D. M. (1971) *Biochim. Biophys. Acta* **247**, 335–347
- Girgis, G. R. & Nicholls, D. M. (1972) *Biochim. Biophys. Acta* **269**, 465–476
- Gordon, J. E. (1970) *Biochemistry* **9**, 912–917
- Henriksen, O. & Smulson, M. E. (1972) *Arch. Biochem. Biophys.* **150**, 175–182
- Henshaw, E. C., Hirsch, C. A., Morton, B. E. & Hiatt, H. H. (1971) *J. Biol. Chem.* **246**, 436–446
- Jefferson, L. S., Koehler, J. O. & Morgan, H. E. (1972) *Proc. Nat. Acad. Sci. U.S.A.* **69**, 816–820
- Migliorini, R. H. & Manchester, K. L. (1971) *FEBS Lett.* **13**, 140–144
- Millward, D. J. (1970) *Clin. Sci.* **39**, 591–603
- Munro, H. N. & Fleck, A. (1966) *Methods Biochem. Anal.* **14**, 113–176
- Omstedt, P. T. & von der Decken, A. (1972) *Brit. J. Nutr.* **23**, 467–474
- van Venrooij, W. J. W., Henshaw, E. C. & Hirsch, C. A. (1970) *J. Biol. Chem.* **245**, 5947–5953
- von der Decken, A. & Omstedt, P. T. (1970) *J. Nutr.* **100**, 623–630
- Waterlow, J. C. & Stephen, J. M. L. (1966) *Brit. J. Nutr.* **20**, 461–484

- Waterlow, J. C. & Stephen, J. M. L. (1968) *Clin. Sci.* **35**, 287–305
- Willis, D. B. & Starr, J. L. (1971) *J. Biol. Chem.* **246**, 2828–2834
- Young, V. R. (1970) in *Mammalian Protein Metabolism* (Munro, H. N., ed.), vol. 4, chapter 40, pp. 585–674, Academic Press, New York
- Young, V. R. (1974) *J. Anim. Sci.* in the press
- Young, V. R. & Alexis, S. D. (1968) *J. Nutr.* **96**, 255–262
- Young, V. R., Chen, S. C. & McDonald, J. (1968) *Biochem. J.* **106**, 913–919
- Young, V. R., Stothers, S. C. & Vilaire, G. (1971) *J. Nutr.* **101**, 1379–1390