possible, the conformational variation between two base-pair doublets of the same base sequence is similar to or sometimes greater than the variation between those with different base sequences. As an example (see Fig. 2, a and b), a base-pair doublet, G·C followed by A·U, has been observed twice in the crystal structure of yeast phenylalanine transfer RNA. The base-pair on-plane rotation angle in these two cases differs by about 6° and the on-plane sliding distance by 0.9 Å. Both are of the same order of magnitude as the total average values of all the base-pair sequences found in known crystal structures of double helical RNA, the average values being 7° and 0.8 Å respectively. In some cases, such as A · U followed by G · C, on-plane rotation angles of three base-pair doublets are very similar, suggesting a strong apparent correlation (Fig. 2a), but the on-plane sliding distances for the same three base-pair doublets vary widely (Fig. 2b).

Despite such wide variation in on-plane rotation angles and sliding distances of base-pair doublets, there has never been a drastic conformational difference such as was found in dsDNA crystal structures, where the purine-pyrimidine sequence appears to have a tendency toward mixed sugar conformations and, in one case, alternating glycosyl bond conformation as well.

In summary, the examination of 21 base-pair doublets found in crystal structures reveals that local conformations in general are not directly correlated to the base-pair sequence in dsRNA (of course we cannot claim this for the cases with only one example, such as A·U/A·U). This observation is in contrast to the finding that dsDNA appears to be significantly dependent on both the base-pair sequence and solution conditions. These analyses further suggest that (i) the backbone conformation of dsRNA is less flexible than that of dsDNA, (ii) although there is a large restricted variation of backbone conformation of dsRNA, all of these may be described as belonging to the A form (*11*); and (iii) the base-pair sequence, thus the base-base interaction and base-solvent interaction, can induce drastic conformational changes of the dsDNA backbone but not of the dsRNA backbone. This restricted conformational variability of dsRNA within the overall frame of the dsRNA-A form is probably one of the reasons that RNA, but not DNA, is used to build structural molecules such as transfer RNA and ribosomal RNA by all living cells. From a physical point of view, the O2′-hydroxyl group of RNA is probably playing double roles of sterically restricting the variation of backbone conformation and providing additional potential for hydrogen bond formation for structural rigidity, as was observed in the tertiary structure of yeast phenylalanine transfer RNA (*12*).

STEPHEN R. HOLBROOK
Department of Chemistry, University of California, Berkeley 94720
JOEL L. SUSSMAN
Department of Structural Chemistry, Weizmann Institute of Science, Rehovot, Israel
SUNG-HOU KIM
Department of Chemistry, University of California, Berkeley

References and Notes

1. F. M. Pohl and T. M. Jovin, *J. Mol. Biol.* **67**, 375 (1972).
2. D. J. Patel, L. L. Canuel, F. M. Pohl, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2508 (1979).
3. H. Shindo, R. T. Simpson, J. S. Cohen, *J. Biol. Chem.* **254**, 8125 (1979).
4. R. D. Wells and R. M. Wartell, *Biochem. Nucleic Acids* **6**, 41 (1974).
5. A. H. J. Wang *et al.*, *Nature (London)* **282**, 680 (1979).
6. H. Drew, T. Takano, S. Tanaka, K. Itakura, R. E. Dickerson, *ibid.* **286**, 567 (1980).
7. J. L. Crawford *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4016 (1980).
8. M. A. Viswamitra *et al.*, *Nature (London)* **273**, 687 (1978).
9. R. Wing *et al.*, *ibid.* **287**, 755 (1980).
10. R. E. Dickerson, personal communication.
11. S. Arnott, R. Chandrasekaran, E. Selsing, in *Structure and Conformation of Nucleic Acids and Protein-Nucleic Interactions*, M. Sundaralingam and S. Rao, Eds. (University Park Press, Baltimore, 1975), p. 577.
12. S.-H. Kim, in *Transfer RNA Structure, Properties and Recognition*, P. Schimmel, D. Soll, J. Abelson, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1979), p. 83.
13. J. L. Sussman, S. R. Holbrook, R. W. Warrant, S.-H. Kim, *J. Mol. Biol.* **123**, 631 (1978).
14. J. M. Rosenberg, N. C. Seeman, R. O. Day, A. Rich, *ibid.* **104**, 145 (1976).
15. N. C. Seeman, J. M. Rosenberg, F. L. Suddath, J. J. Kim, A. Rich, *ibid.*, p. 109.
16. B. Hingerty, E. Subramanian, S. D. Stellman, T. Sato, S. B. Broyde, R. Langridge, *Acta Crystallogr. Sect. B* **32**, 2998 (1976).
17. Supported by NIH grants CA 27454, NS 15174; NSF grant PCM 8019468 and in part by the United States–Israel Binational Science Program.

23 January 1981; revised 23 March 1981

Human Muscle Phosphoglycerate Mutase Deficiency: Newly Discovered Metabolic Myopathy

Abstract. *Muscle phosphoglycerate mutase activity was decreased (5.7 percent of the lowest control value) in a 52-year-old man with intolerance for strenuous exercise and recurrent pigmenturia since adolescence. All of the other enzymes of glycolysis had normal activities, and glycogen concentration was normal. Electrophoretic, heat lability, and mercury inhibition studies showed that the small residual activity in the patient's muscle was represented by the brain (BB) isoenzyme of phosphoglycerate mutase, suggesting a genetic defect of the M subunit which predominates in normal muscle. The prevalence of the BB isoenzyme in other tissues, including muscle culture, may explain why symptoms were confined to muscle.*

Cramps, myoglobinuria, and intolerance for strenuous exercise characterize the clinical picture of muscle phosphorylase and phosphofructokinase deficiencies (*1*). Phosphofructokinase deficiency is the only known genetic defect of the glycolytic pathway in human muscle. We now report a defect of muscle phosphoglycerate mutase (PGAM) (E.C. 2.7.5.3) in a patient with symptoms similar to those of phosphofructokinase deficiency. Starting in adolescence, this man noted muscle pain, "stiffness," and weakness after 15 to 30 minutes of intense exercise. The episodes were often followed by pigmenturia, but the urine was never examined for myoglobin. Despite these problems, he led a relatively normal life, served in the army, then worked as a security guard. At age 52, results of a physical examination were normal except for gouty tophi at the wrists and elbows; strength was normal. Severe atherosclerosis of the coronary arteries was present. On two occasions, ischemic exercise of the forearm was followed by an abnormally small rise of venous lactate; histochemical and ultrastructural studies of a muscle biopsy indicated a mild increase of glycogen (*2*).

The clinical syndrome and the minimal increase of lactate after ischemic exercise suggested muscle phosphorylase or phosphofructokinase deficiency, but both of these enzyme activities (*3*) were normal in a muscle extract (Table 1). Studies of anaerobic glycolysis in vitro (*3*) showed decreased lactate production by the patient's muscle, with glycogen or hexose-phosphate glycolytic intermediates from glucose 1-phosphate to fructose 1,6-diphosphate. This suggested a defect below the phosphofructokinase reaction (Fig. 1), which was confirmed by measurement of individual enzymes of glycolysis (*4*) (Table 1). All enzyme activities were normal except that of PGAM, which had a mean value of 3.6 percent of the normal mean. Although the range of PGAM activity in normal human muscle is wide (253 to 588 μmoles of 3-phosphoglycerate converted per

0036-8075/81/0612-1277$00.50/0

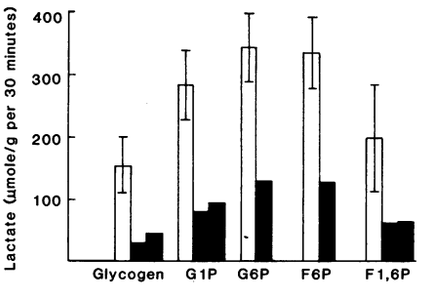

Fig. 1. Anaerobic glycolysis in vitro. (Open bars) control muscles (N = 16); (solid bars) patient's muscle. Standard deviations are indicated.

minute per gram in 27 controls), the patient's activity (14.1, 14.5, 14.5 in three different determinations) was less than 6 percent of the lowest normal value. Glycogen concentration (*5*) was not increased in the patient's muscle (12.5 mg/g compared with a mean ± standard deviation (S.D.) of 10.4 ± 1.8 for 91 controls), and the concentrations of glucose 6-phosphate (138 nmole/g) and fructose 6-phosphate (15 nmole/g) were also normal (*3*). The activities of several enzymes of lipid metabolism, including carnitine palmityl transferase, were also normal (*6*).

Starch-gel electrophoresis of human PGAM indicates that PGAM is a dimer containing, in different tissues, different proportions of a slow-migrating muscle (MM) isoenzyme, a fast-migrating brain (BB) isoenzyme, and an intermediate hybrid (MB) form. The electrophoretic pattern of normal, mature human muscle PGAM showed an overwhelming predominance of the MM band, with only faint BB and MB bands (*7*). To understand the nature of the residual PGAM activity in our patient's muscle, we compared the electrophoretic pattern (*8*) with that of normal muscle enzyme (Fig. 2). Normal muscle extracts (diluted 1 : 50 to prevent overloading) showed an intense MM band (Fig. 2, lane 6); faint BB and MB bands were seen in overloaded preparations. In contrast, undiluted preparations of the patient's muscle showed a distinct BB band, with barely discernible MM or MB bands (Fig. 2, lane 5). These data suggested a biochemical error resulting from a genetic defect of the M subunit of PGAM, with the small residual activity due mostly to the BB isoenzyme.

Additional evidence that the residual PGAM activity in the patient's muscle was represented by the BB isoenzyme was provided by studies of heat lability and inhibition by mercury. Because the MM form of PGAM is more heat-stable than the BB isoenzyme, we found that incubation of normal muscle extract at 60°C for 20 minutes caused only 30 percent inhibition of PGAM activity (*7*), as contrasted with 80 percent inhibition in our patient's muscle extract. Conversely, exposure of normal muscle extracts to 1 mM $HgCl_2$ (*9*) caused 92 to 97 percent inhibition of PGAM activity, but only 9 percent inhibition in the patient's muscle extract. The small amount of PGAM activity (3 to 6 percent of the total) that was not inhibited by mercury in normal muscle (presumably BB) was similar to the amount of residual PGAM activity in our patient's muscle.

The different proportions of MM and BB isoenzymes in various tissues may also explain why the clinical manifestations of a genetic defect of the M subunit were confined to the skeletal muscle. The electrophoretic pattern of PGAM in normal erythrocytes, leukocytes, and cultured skin fibroblasts resembled that of the brain enzyme, with an absolute predominance of the BB band (*7*). Accordingly, PGAM activity was normal in red and white blood cells from our patient. Normal human heart, like fetal heart (*7*), showed all three bands (MM, BB, and MB), with a prevalence of the MM band in the adult heart (data not shown). Therefore, a partial defect of PGAM was to be expected in the heart of our patient; although he did have signs of heart disease in recent years, these were attributed to coronary insufficiency. The residual activity of BB may be enough to support adequate glycolysis in the heart. In contrast to the marked decrease of PGAM activity in the muscle biopsy, the enzyme activity was normal in fused noninnervated muscle cultures (*10*) from the patient (594 nmole of 3-phosphoglycerate converted per minute per milligram of protein in the patient and 559 and 573 in two controls). This was not surprising, because the isoenzyme pattern of PGAM, like that of other enzymes that exist in multiple tissue-specific isoenzyme forms (*8*), changes during muscle development (*7*, *9*). The pattern of the muscle cultures (Fig. 2, lanes 1 to 3) was similar to that of immature (gestational age, 9 to 11 weeks) fetal muscle (*7*) and consisted almost exclusively of the BB isoenzyme. This finding explains the normal activity in the patient's muscle culture. In more mature (gestational age, 16 weeks) fetal muscle (Fig. 2, lane 4), the M subunit was also expressed, resulting in a three-banded pattern similar to that of the normal cardiac enzyme.

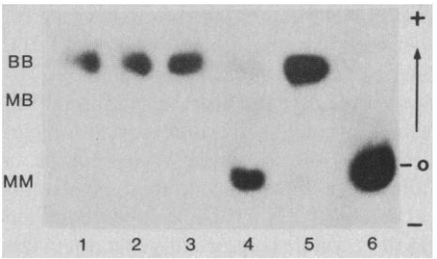

Fig. 2. Cellulose acetate electrophoresis of PGAM in human tissues (*8*): lane 1, culture of patient's muscle; lanes 2 and 3, control muscle cultures; lane 4, fetal muscle (16 weeks gestational age); lane 5, extract of patient's muscle (undiluted); lane 6, control muscle extract (1 : 50).

In strenuous exercise (at work intensities close to the maximal oxygen uptake) energy is derived almost exclusively from the breakdown of muscle glycogen and glycolysis, and glycogen depletion appears to coincide with exhaustion (*11*). In phosphorylase or phosphofructokinase deficiency, exercise intolerance and myoglobinuria have been attributed to impairment of this energy pathway, and the same pathogenetic mechanism is probably operative in PGAM deficiency. Although the severe bottleneck at the level of the PGAM reaction may cause

Table 1. Activities of phosphorylase and glycolytic enzymes in human muscle. Phosphorylase activity is expressed as micromoles of glucose 1-phosphate liberated per minute per gram of fresh tissue ± standard deviation (S.D.). All other enzyme activities are expressed as micromoles of substrate converted per minute per gram of fresh tissue (± S.D.). N is the number of controls. Control muscles were obtained by diagnostic biopsy from patients who were ultimately deemed to be free of muscle disease. All biopsies were taken from the quadriceps (vastus lateralis) muscle.

Enzyme	Enzyme activities		
	Controls	N	Patient
Phosphorylase	21.9 ± 5.5	44	21.0
Phosphoglucomutase	41.8 ± 11.0	12	50.9
Phosphohexoisomerase	133.5 ± 25.0	7	125.8
Phosphofructokinase	23.9 ± 4.7	16	31.5
Aldolase	51.6 ± 8.4	9	48.4
Glyceraldehyde phosphate dehydrogenase	69.3 ± 12.4	8	65.0
Phosphoglycerate kinase	133.5 ± 38.4	8	137.5
Phosphoglycerate mutase	401.7 ± 114.7	27	14.4
Enolase	150.4 ± 21.3	7	148.7
Pyruvate kinase	122.5 ± 41.9	10	159.7
Lactate dehydrogenase	311.0 ± 91.7	7	425.8

insufficient anaerobic energy production during strenuous exercise, PGAM is the glycolytic enzyme with highest activity in normal human muscle (Table 1). Therefore, the small residual activity of the BB isoenzyme found in the patient was still approximately half that of phosphofructokinase, the rate-limiting enzyme of glycolysis. This incomplete block of the glycolytic pathway may explain the modest rise of venous lactate after ischemic exercise, the decreased but not absent formation of lactate by muscle extracts in anaerobic conditions, and the normal concentration of glycogen and glycolytic intermediates in muscle. The frequency of this enzyme defect remains to be determined, but muscle PGAM deficiency is now added to phosphorylase, phosphofructokinase, and carnitine palmityl transferase deficiencies in the differential diagnosis of human recurrent myoglobinuria.

Note added in proof: A genetic defect of the M subunit of lactate dehydrogenase has been recently reported in an 18-year-old man with recurrent myoglobinuria induced by intense exercise (*12*).

SALVATORE DIMAURO
ARMAND F. MIRANDA
SHAHNAZ KHAN, KEVIN GITLIN
H. Houston Merritt Clinical Research Center for Muscular Dystrophy and Related Diseases, Columbia University, College of Physicians and Surgeons, New York 10032
ROBERT FRIEDMAN
Department of Medicine, New York University, New York 10016

References and Notes

1. S. DiMauro, in *Handbook of Clinical Neurology*, P. J. Vinken, G. W. Bruyn, S. P. Ringel, Eds. (North-Holland, Amsterdam, 1980), vol. 41, p. 175.
2. S. DiMauro, A. F. Miranda, A. P. Hays, R. Friedman, M. Olarte, in preparation.
3. R. B. Layzer, L. P. Rowland, H. M. Ranney, *Arch. Neurol.* **17**, 512 (1967). For determination of phosphorylase, muscle was homogenized in nine volumes of a medium composed of 40 m*M* β-glycerophosphate (*p*H 6.8), 40 m*M* NaF, 10 m*M* EDTA, and 20 m*M* mercaptoethanol, and for determination of phosphofructokinase, in nine volumes of 30 m*M* KF and 1 m*M* EDTA. The tissue was homogenized in motor-driven, all-glass homogenizers, the homogenate centrifuged at 10,000*g* for 10 minutes and the supernatant used for enzyme assays.
4. H. U. Bergmeyer, K. Gawehn, M. Grassl, in *Methods of Enzymatic Analysis*, H. U. Bergmeyer, Ed. (Academic Press, New York, 1974), vol. 1, p. 425. Aldolase activity was measured by the method of R. Wu and E. Racker [*J. Biol. Chem.* **234**, 1029 (1959)], and lactate dehydrogenase was measured by the method of A. Kornberg [*Meth. Enzymol.* **1**, 441 (1955)].
5. S. DiMauro *et al.*, *Ann. Neurol.* **5**, 422 (1979).
6. S. DiMauro and P. M. Melis DiMauro, *Science* **182**, 929 (1973).
7. G. S. Omenn and P. T. Wade Cohen, *In Vitro* **7**, 132 (1971); G. S. Omenn and S. C. Y. Cheung, *Am. J. Hum. Genet.* **26**, 393 (1974); G. S. Omenn and M. A. Hermodson, in *Isozymes III: Developmental Biology*, C. L. Markert, Ed. (Academic Press, New York, 1975), p. 1005.
8. A. F. Miranda, H. Somer, S. DiMauro, in *Muscle Regeneration*, A. Mauro *et al.*, Eds. (Raven, New York, 1979), p. 453. For electrophoresis, 0.4-μl samples of appropriately diluted tissue extracts were applied twice on cellulose acetate membranes (7.5 by 4.5 cm, Titan III–Iso-Flur; Helena Laboratories, Beaumont, Texas) that had been soaked in running buffer overnight. Electrophoresis was carried out in 0.5*M* tris-barbiturate buffer, *p*H 8.6, for 15 minutes at 4°C (Titan chamber, Helena) at 350 V. The substrate, identical in composition to that used for biochemical assay (*4*), was prepared in 20 percent sucrose and applied to a separate cellulose acetate membrane. This was pressed firmly on top of the electropherogram, starting on one side at a 30° angle to avoid air bubbles. The sandwiched membranes were incubated for 30 minutes at 37°C, air-dried, illuminated with an ultraviolet lamp (360 nm) and photographed on Polaroid 107 film through a 2E Wratten filter. Bands of activity appeared as dark areas on a fluorescent background.
9. J. Grisolia, D. Diederich, S. Grisolia, *Biochem. Biophys. Res. Commun.* **41**, 1238 (1970).
10. Muscle cultures, grown as described (*8*), were trypsinized, rinsed in phosphate-buffered saline (*p*H 7.4), and centrifuged at 2000*g* for 10 minutes. The pellets were suspended in 100 to 150 μl of 0.1*M* tris-barbiturate buffer (*p*H 8.0) alternately frozen and thawed three times, subjected to ultrasonication for 15 seconds, and centrifuged at 10,000*g* for 15 minutes in the cold. Differentiation was tested by fusion of myoblasts into multinucleated syncytia and by the appearance of muscle-specific creatine kinase isoenzymes (*8*). Control muscle cultures were obtained during diagnostic muscle biopsies from patients ultimately deemed to be free of neuromuscular disease.
11. P. Felig and J. Wahren, *N. Engl. J. Med.* **293**, 1078 (1975); B. Essen, L. Hagenfeldt, L. Kaijser, *J. Physiol. (London)* **265**, 489 (1977).
12. T. Kanno, K. Sudo, I. Takeuchi, S. Kanda, N. Honda, Y. Nishimura, K. Oyama, *Clin. Chim. Acta* **108**, 267 (1980).
13. Supported by grants NS-11766-07 from the National Institute of Neurological and Communicative Disorders and Stroke and from the Muscular Dystrophy Association, and by grant AM-25599-02 from the National Institute of Arthritis, Metabolism, and Digestive Diseases.

16 December 1980

Somatomedin-C Mediates Growth Hormone Negative Feedback by Effects on Both the Hypothalamus and the Pituitary

Abstract. *Somatomedin-C stimulates somatostatin release to a maximum of 390 percent of basal release during short-term (20-minute) incubation of rat hypothalamus. It has no effect on basal or stimulated growth hormone release from primary cultures of rat adenohypophyseal cells during a 4-hour incubation, but inhibits stimulated release by more than 90 percent after 24 hours. These findings suggest that somatomedin-C participates in the growth hormone negative feedback loop with an immediate effect on hypothalamic somatostatin and a delayed effect on the anterior pituitary.*

Homeostasis of the hypothalamic-pituitary–thyroid, –adrenal, and –gonadal axes is maintained largely by a feedback control. This control is exerted at multiple levels by the target gland hormones at the pituitary and hypothalamus and by the pituitary trophic hormones at the hypothalamus (*1*).

Central nervous system regulation of pituitary growth hormone (GH) secretion is mediated by the balance between a hypothalamic inhibiting factor—somatostatin—and an as yet uncharacterized releasing factor (*2*). We have previously demonstrated that GH acts at the hypothalamus to stimulate synthesis and release of somatostatin (*3*), suggesting a negative feedback control of this pituitary hormone at the hypothalamic level.

Although no distinct endocrine target organ for GH action is known, many of its peripheral effects, especially on tissue growth and anabolism, are mediated by the somatomedins (*4*). The somatomedins constitute a family of peptide hormones that includes insulin-like growth factors 1 and 2, with structures similar to that of proinsulin (*5*), and somatomedins SM-A (*6*), and SM-C (*7*). A feedback effect of these GH-dependent peptides on the regulation of GH might therefore be expected, but has not, to our knowledge, been described. To explore this possibility we have studied the effect of highly purified SM-C in vitro on the release of somatostatin by rat hypothalamus and of GH by dispersed rat adenohypophyseal cells in primary monolayer culture. The results indicate that SM-C stimulates hypothalamic somatostatin release during a 20-minute incubation in a dose-related manner and inhibits stimulated pituitary GH release after prolonged (24-hour) but not short-term (4-hour) exposure. These findings suggest that the acute SM-C feedback is exerted at the hypothalamic level and mediated by the release of somatostatin, whereas delayed effects occur at the anterior pituitary by direct inhibition of GH secretion.

Intact rat medial-basal hypothalamus (MBH) and septum and preoptic area (SPO) tissue blocks (*8*) were individually incubated in Krebs-Ringer bicarbonate buffer containing glucose (14 m*M*) and bacitracin (0.5 mg/ml) in an atmosphere of 95 percent O_2 : 5 percent CO_2. After a 60-minute equilibration period to achieve stable somatostatin release, a 20-minute basal incubation was performed in buffer alone (for MBH, 72 ± 5 pg of somatostatin, $N = 83$; for SPO, 129 ± 5 pg of somatostatin, $N = 104$). This was followed by a 20-minute incubation in buffer containing highly purified (*9*) SM-C (5 to 50 ng/ml) or control materials includ-

0036-8075/81/0612-1279$00.50/0