Human Hexokinase Isoenzymes of Muscle in Health and Disease

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Total hexokinase activity (ATP-α-hexose 6-phosphotransferase EC 2.7.1.1) in muscle has been measured from patients suffering from Duchenne muscular dystrophy and was found to differ little from that of normal (Ronzoni et al., 1961; Heyck et al., 1963; Davidenkova et al., 1970). Since, however, the total activity of the enzyme is a summation of the activities of its isoenzymes in that tissue, at the substrate concentration at which the assay is performed, it is possible for one or more isoenzymes to differ in their properties without necessarily affecting the total activity. We have described previously such a difference in the electrophoretic properties of hexokinase isoenzyme II in patients suffering from the X-linked Becker and Duchenne dystrophies (Strickland & Ellis, 1975).

To eliminate the possibility that the change in isoenzyme II is merely a reflection of diseased and atrophied muscle, we have examined the electrophoretic patterns in a number of other muscle diseases.

Tissue samples were homogenized in 0.1 M-Tris/HCl buffer, pH 7.4, centrifuged concentrated, and subjected to electrophoresis on Cellogel as previously described (Strickland & Ellis, 1975). Staining of protein was carried out for 5 min in a solution of 0.2 g of Ponceau S and 3 g of trichloroacetic acid in 100 ml of water. Some of the electrophoretograms were stained for hexokinase activity at three glucose concentrations, 10 mM, 500 μM and 1 μM, by the method of Katzen et al. (1970). The remainder were stained by the method of Rogers et al. (1975), but at the same three glucose concentrations above, 10 mM, 500 μM and 10 μM. There were no differences in the results of staining, except that the method of Rogers et al. (1975) stained the isoenzymes more heavily.

Bands of hexokinase activity were referred for comparison to the albumin band of the electrophoretogram stained for protein. There was no difference observed between the appearance of bands and density of staining in muscle that had been stored at −70°C with that of fresh muscle. The normal muscle was obtained during orthopaedic operations and the diseased muscle at biopsy.

Figs. 1 and 2 show the electrophoretic patterns obtained from the various muscle samples used. Fig. 1 shows patterns from male subjects, and Fig. 2 patterns from female subjects. There is no difference between the diseased samples in the relative distances travelled by isoenzymes I and II, except for the X-linked Becker and Duchenne muscular dystrophies. This suggests that the changes in electrophoretic mobility in these cases (Becker and Duchenne dystrophies) are characteristic of these two types of X-linked muscular dystrophies and not of generally diseased muscle. There was some variation in the position of isoenzyme III in most samples, but, since this seems to be random, it may be due to normal population variation.

There is a difference between the intensity of staining of the isoenzymes of normal adult male and normal adult female; males have all isoenzymes present and considerable amounts of isoenzymes I and II, whereas females have little other than isoenzyme I, with the exception of cervical muscle from normal females, which more nearly resembled the male. Otherwise all types of muscle within the normal male group, and within the normal female group were similar (these included deltoid, gastrocnemius, palmaris longus, gluteus maximus, latissimus dorsi and spinalis).

The two samples of muscle from confirmed female carriers of X-linked Duchenne muscular dystrophy appeared normal in electrophoretic pattern, although there is rather more of the type III isoenzyme than is usually seen.

Isoenzymes I and II seem most prevalent in muscle, having $K_m$ values of $5.0 \times 10^{-5} \text{M}$ and $3.0 \times 10^{-4} \text{M}$ respectively. It is probable that, if either one or both of isoenzymes I and II were abnormal, glucose metabolism would be impaired. An abnormal isoenzyme I would have effects more noticeable in tissues other than muscle, e.g. brain, since brain contains mostly isoenzyme I and little II or III. Muscle is the tissue relying most heavily...
**Fig. 1. Hexokinase isoenzymes of male human muscle in health and disease**

The Figure shows the electrophoretic patterns produced on Cellogel at pH 8.4. The degree of activity of the bands is represented by density of shading. The numbers of specimens used are given in parentheses.
Fig. 2. Hexokinase isoenzymes of female human muscle in health and disease

The Figure shows electrophoretic patterns produced on Cellogel at pH 8.4. The degree of activity of the bands is represented by density of shading. The numbers of specimens used are given in parentheses.

on isoenzyme II, therefore this tissue would probably be the first to express the abnormality. The X-linked Becker and Duchenne dystrophies appear to have an altered isoenzyme II pattern, and this may be linked directly with aetiology of the disease.

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