CLASSICAL PERSPECTIVES

When fibres go slack and cross bridges are free to run: a brilliant method to study kinetic properties of acto-myosin interaction

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Muscle contraction is brought about by independent force or movement generators, which can be identified with cross bridges, i.e. with myosin heads interacting with actin. This implies that isometric tension is dependent on the degree of filament overlap, i.e. on sarcomere length, and on activation level, whereas shortening velocity at zero load is not (Huxley, 1957, 1974). Whereas the dependence of isometric tension on the degree of filament overlap was demonstrated by the length clamp experiments published in 1966 (Gordon *et al.* 1966; see companion Classical Perspective by Allen & Westerblad, 2007), the definitive demonstration that shortening velocity at zero load is independent of sarcomere length was only given in 1979 (Edman, 1979). Actually, measurement of shortening velocity without load is not simple, as such conditions cannot be easily obtained with load clamp or with ramp shortening. In the article 'The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres' (Edman, 1979) a new method, derived from initial observations by A. V. Hill (and mentioned in his book; Hill, 1970) was designed. The reliable determination of the steady state shortening velocity at zero load (V_o) was achieved by a linear regression of the shortening amplitudes against the times elapsed from the transition from isometric contraction to zero load to the beginning of tension redevelopment (see Fig. 1). This allowed the separation of the steady state shortening from the early elastic and contractile transient response. Experiments were carried out on single muscle fibres dissected from the leg muscles of the frog *Rana temporaria* and electrically stimulated at low temperature and showed that V_o is constant over a large range of sarcomere lengths (1.65–2.7 μm, see Fig. 1*C*) and is not sensitive to changes in activation level. Both sarcomere length

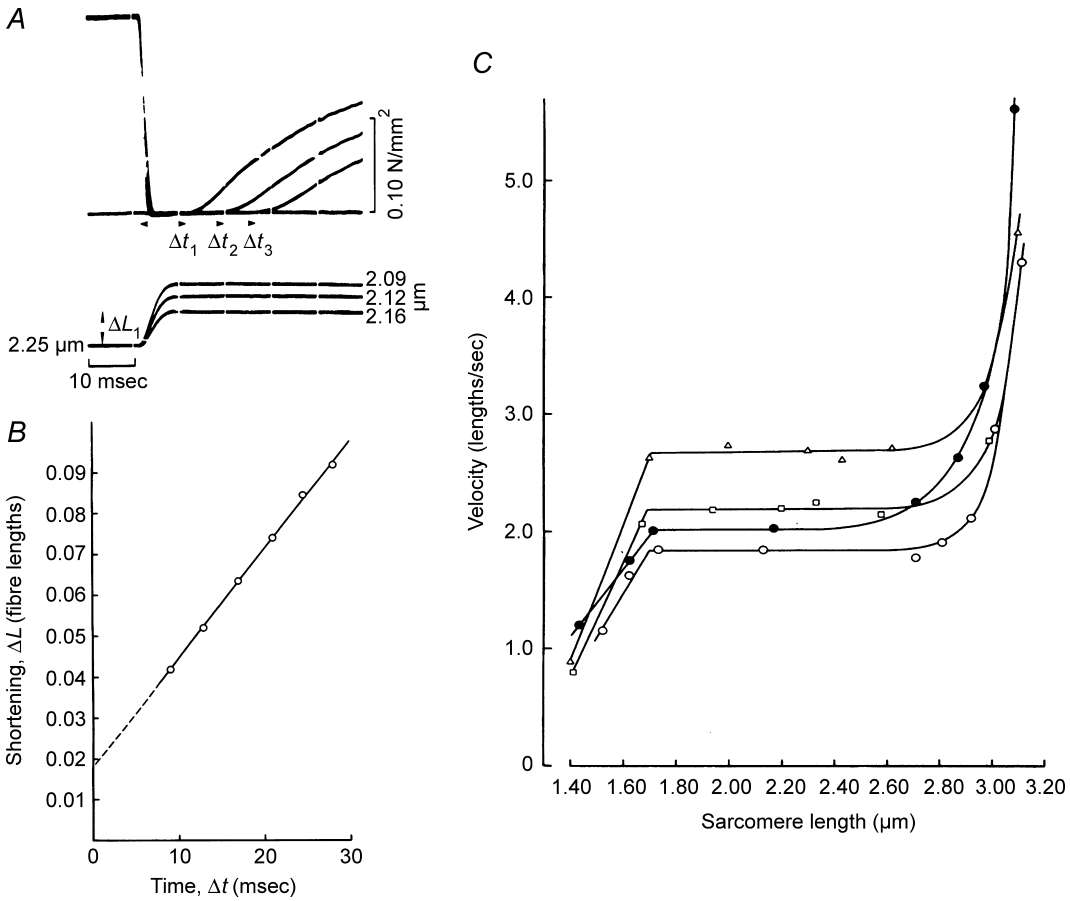

Figure 1. V_o determined with the slack test protocol is independent of sarcomere length
A, superimposed records of three releases with different amplitudes; *B*, linear regression between release amplitudes and time from release to the beginning of tension re-development; V_o is given by the slope and series elasticity by the intercept on the ordinate; *C*, V_o of four fibres is independent of sarcomere length. From Edman (1979).

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and activation level change the number of working cross bridges, but cannot change V_o which does appear to be an intrinsic property of each individual acto-myosin interaction. Very interestingly, alterations of interfilament spacing obtained by changing either sarcomere length (Edman, 1979) or osmolarity (Edman & Hwang, 1977) do not affect V_o.

Maximum shortening velocity at zero load (V_{max}) can be derived by extrapolating the force–velocity data obtained with load-clamp or with ramp shortening: in intact single muscle fibres V_o is just slightly higher than V_{max} (~7%) (Edman, 1979) or even closer according to other studies (Julian *et al.* 1986). Larger differences (V_o up to 60% greater than V_{max}) found in whole muscles (Claflin & Faulkner, 1985) are attributable to the fact that whole muscles contain different fibre types each with its own maximum speed of shortening. At finite loads, all fibre types contribute, but in the slack test, only the fastest fibres determine V_o (Hill, 1970; Claflin & Faulkner, 1985). Small, but significant, differences between V_o and V_{max} are observed in skinned fibres. A possible explanation is that the force–velocity curve deviates from a hyperbolic shape at very low loads and a linear fit on the data points below 0.05 isometric tension (P_o) leads to the identity of extrapolated V_{max} and measured V_o (Julian *et al.* 1986). In agreement with this view, the difference between V_o and V_{max} is not significant in slow fibres which have higher curvature of the force–velocity relation, whereas V_o is significantly greater than V_{max} in fast fibres (Bottinelli *et al.* 1996). Further problems in V_o determination are caused by the fact that skinned fibres do not shorten at a constant velocity during force clamps (Ferenczi *et al.* 1984; Brenner, 1986; Julian *et al.* 1986) and the curvature of the length signal is likely to be present also during shortening at zero load. A limitation of the release amplitude to less than 15% of fibre length is, however, sufficient to work in the region of linear shortening and avoid any influence of the activation level. Actually, the question of whether maximum velocity of shortening in skinned fibres is dependent on free [Ca^{2+}], and thus on the level of activation, was the object of a long-lasting controversy (see for, example, Podolsky & Teicholz, 1970; Julian, 1971). In intact frog fibres, however, the slack test protocol showed without any doubt that changes in the level of activation do not influence V_o (Edman, 1979) and, more recently, the finding was fully confirmed in intact murine single fibres comparing V_o in control conditions and in the presence of dantrolene (Westerblad *et al.* 1998). In measuring shortening velocity at zero load, special attention must be paid to the possible influence of resting tension. Actually, at sarcomere lengths longer than 2.7 μm, where resting fibres develop increasing amounts of passive tension, slack tests revealed a substantial increase in V_o (Edman, 1979) (see Fig. 1*C*). Such increase was perfectly explained by the assumption that passive forces act as a negative load on the contractile component, thus increasing the rate of filament sliding (Edman, 1979).

Once it was clearly established that V_o determined with the slack protocol was independent of sarcomere length and of activation level and was a direct expression of acto-myosin kinetics, the determination of V_o became one of the most used ways to study the kinetic properties of myosin. The method is simple, reliable and can be applied in a completely preserved sarcomeric architecture. Thus, the slack test protocol on permeabilized rabbit soleus fibres was used to demonstrate that V_o values in different fibre types are determined by MHC isoforms (Reiser *et al.* 1985) and to study the interplay between MHC and MLC isoforms in determining kinetic properties of muscle fibres (Sweeney *et al.* 1988; Bottinelli *et al.* 1994). Later on, human skeletal muscle fibres were studied using the slack test (Larsson & Moss, 1993; Bottinelli *et al.* 1996) and more recently muscle fibres of large animals such as horse (Rome *et al.* 1990), pig (Toniolo *et al.* 2004), cow (Toniolo *et al.* 2005) or of small laboratory animals such as mice and rats (Pellegrino *et al.* 2003).

The collection of V_o values measured in skeletal muscle fibres containing distinct myosin isoforms now covers a range of about 20-fold. It has thus become possible to study the correlation with the sliding filament velocity measured with *in vitro* motility assay (Pellegrino *et al.* 2003) on myosin extracted from skeletal muscle fibres. The highly significant correlation can be considered as proof that the kinetic parameters measured with the slack test and that measured with motility *in vitro* tests are the same and correspond to the speed of actin filament translocation by myosin motors. The same wide range of V_o values has formed the basis for a comparison between the speed of actin translocation and the rates of the ATPase cycle of acto-myosin. Such comparison has indicated the rate of ADP release from the catalytic side as the likely determinant of V_o (Weiss *et al.* 2001).

The application of the slack test protocol has also been extended to cardiac muscle. It is worth remembering that, in cardiac muscle, activation level and resting tension play an even more important role than in skeletal muscles. V_o of cardiac trabeculae was for the first time determined by Herland and co-workers (Herland *et al.* 1990). Since then, V_o determination based on the slack test protocol has been often adopted to characterize myosin isoforms expressed in cardiomyocytes (Pereira *et al.* 2000) and to separate the effects on myosin kinetics from changes in activation in conditions which enhance or depress cardiac contractility (Strang & Moss, 1995; Hwang *et al.* 2005).

Isolated myofibrils are presently the thinnest preparations (diameter 1–2 μm) where sarcomere architecture is preserved and myosin can interact with actin in virtually physiological conditions. The slack test protocol was first employed to measure V_o in myofibrils of skeletal muscle (Tesi *et al.* 1999), in a study on the modulation of shortening velocity by substrate (ATP) concentration. More recently (Opitz *et al.* 2003), the slack test protocol has been applied to cardiac myofibrils to study the interplay between elastic recoil due to titin and active shortening due to acto-myosin interaction. The same issue was taken up in frog muscle fibres (Edman, 1979) showing that passive tension contributes to fibre shortening causing a substantial increase in velocity. Titin-driven passive recoil is much faster than active unloaded shortening velocity suggesting that damped myofibrillar elastic recoil could accelerate active contraction speed of myocardium during early systolic shortening (Opitz *et al.* 2003).

These latter applications combine the original design of the slack test protocol together with the recent technical advances which have made experiments on myofibrils possible. Together with the fruitful applications to characterize myosin isoforms, the development of slack test-based measurements in novel experimental systems such as myofibrils clearly demonstrates the long life time of the paper by Edman published in 1979 and its long lasting impact on muscle biophysical and biological literature.

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