Physiology

The *ACE* gene and muscle performance

Angiotensin-converting enzyme in human skeletal muscle[1] can be encoded by either of two variants of the *ACE* gene[2], one of which carries an insertion of 287 base pairs. This longer allele gives rise to lower enzyme activity[2], and is associated with enhanced endurance performance[3] and an anabolic response to intense exercise training[4]. Here we examine training-related changes in the mechanical efficiency of human skeletal muscle (energy used per unit power output) and find that the presence of this *ACE* allele confers an enhanced mechanical efficiency in trained muscle.

Subjects and staff were blind to genotype during experimentation and data analysis; genotypes are represented as 'I' for the *ACE* allele carrying the 287-base-pair insertion, and 'D' for the allele that does not. Caucasian army recruits (58 men: 35 II and 23 DD) were studied before and after an 11-week programme of (primarily aerobic) physical training. Subjects pedalled on a bicycle ergometer at a constant 60 r.p.m. for 3 min at each of three successive external power outputs (40, 60 and 80 W). Steady-state oxygen uptake ($\dot{V}O_2$ in ml min^{-1}) and the respiratory exchange ratio were measured and used to calculate the energy expended per minute at each stage.

The delta efficiency (percentage ratio of the change in work performed per min to the change in energy expended per min), which represents the efficiency of muscular contraction[5], was calculated. We assessed changes in delta efficiency with training by using paired *t*-tests, and comparisons were made between genotype groups by independent *t*-test. Values of $P<0.05$ were considered statistically significant.

Before training, the delta efficiency was independent of genotype (24.5 and 24.9% respectively, $P=0.59$), but the response to training was strongly genotype-dependent, with delta efficiency rising significantly only among those of II genotype (absolute change of −0.26% for those of DD genotype ($P>0.05$) and 1.87% for those of II genotype ($P<0.01$): $P<0.025$ for II compared with DD; changes were independent of all pretraining characteristics (Fig. 1)). These differences represent a proportional increase in efficiency of 8.62% relative to baseline for those of II genotype and −0.39% for DD, which may have more biological impact than this value would suggest[6].

We do not know how the II genotype helps to improve the mechanical efficiency of trained muscle, but it may be related to an increase in slow-twitch rather than fast-twitch muscle fibres, which are more efficient in slow contraction[7]. The lower ACE enzyme activity associated with the II genotype may also raise local concentrations of nitric oxide, which in turn may improve the efficiency of mitochondrial respiration and hence contractile function in both cardiac and skeletal muscle[8]. The number of mitochondrial uncoupling proteins in skeletal and cardiac muscle drops during endurance exercise training[9], and this reduction might be ACE-genotype dependent.

Our results have implications beyond sporting activity. The mechanical and metabolic efficiency of skeletal muscle is increased in situations in which achieving more external work for less energy utilization might be advantageous (such as lactation[10] and dietary-induced energy deficiency[11]). Congestive heart failure, for example[12], interferes with the delivery of oxygen and metabolic substrates to the whole body, so improved skeletal muscle mechanical efficiency would be beneficial. During a heart attack, the limited blood supply to the myocardium means that there is a sudden drop in the uptake of metabolic substrates and oxygen, when enhanced cardiac muscle metabolic efficiency would be an advantage.

Such benefits could be associated with lower ACE activity, an idea that may partly explain the beneficial effects of ACE inhibitors on myocardial cell survival during ischaemia[13] and on the survival of patients with cardiac dysfunction[14].

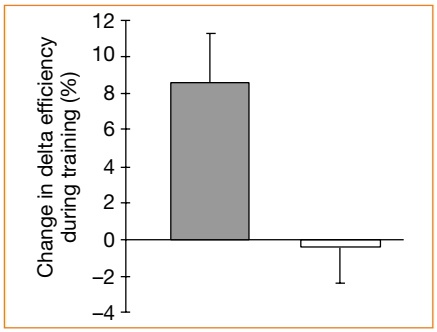

Figure 1 Change in delta efficiency (mean ± s.e.m.) during training in subjects of ACE genotypes II (shaded) and DD (white). ACE genotype was determined in 579 male army recruits as before[15]. At entry, 108 randomly selected homozyotes (59 II, 49 DD) exercised on an electronically braked cycle ergometer (Lode Rehcor, Netherlands) at 60 r.p.m. At external power outputs of 40, 60 and 80 watts (3-min stages), the rate of energy expenditure (in watts) was calculated from oxygen uptake (ml min^{-1}) and respiratory exchange ratio (Cardiokinetics metabolic measurement cart, Medical Graphics Corp.)[16]. After an 11-week training period, 58 subjects (35 II: 19.4 ± 0.3 yr, 1.78 ± 0.01 m, 71.3 ± 1.2 kg: all baseline characteristics independent of genotype) were retested. Those completing the study did not differ in any measured respect from those who did not. Asterisk, $P<0.025$. This study was approved by the appropriate ethics committee and written, informed consent was obtained from each participant.

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Structural biology

A new model for protein stereospecificity

The ability of proteins to discriminate between optical isomers is vital for living systems and is exploited in drug design. Here we present a new model to explain this stereospecificity of proteins on the basis of crystallographic data.

Examples of the importance of stereospecificity are that only one of the two mirror images of the amino acid serine can activate a key receptor in the brain[1], and stereoisomers of pollutant molecules are selectively degraded in different soils[2].

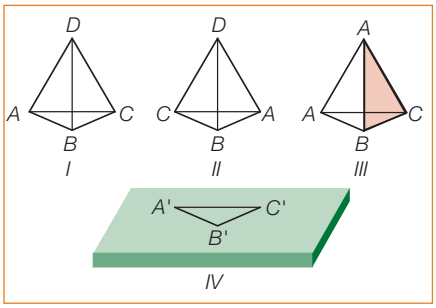

Figure 1 The original three-point attachment model[7,8]. The ligand binds so that groups *A*, *B*, and *C* of one enantiomer (*I*) bind to protein sites *A′*, *B′* and *C′* of the enzyme, respectively. It is easily seen that the other enantiomer (*II*) cannot yield an equivalent coincidence of groups *A*, *B* and *C* with *A′*, *B′* and *C′*. If there are two *A* groups to produce a prochiral molecule (*III*), the model can distinguish between the two identical groups. The protein surface to which the chiral molecules bind is shown in *IV*.

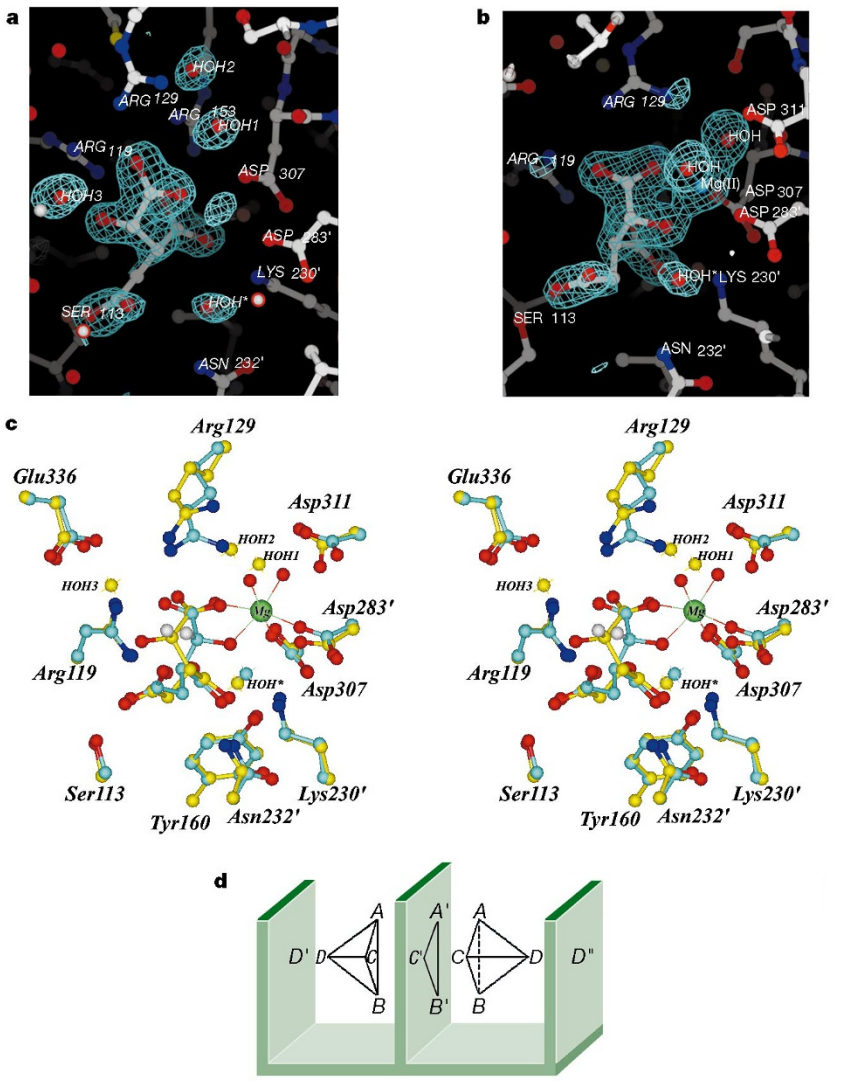

Figure 2 Protein specificity. **a,b,** Final omit maps of positive $(F_o\text{-}F_c)\alpha_{calc}$ difference electron density contoured at 3.0σ in the active site of complexed isocitrate dehydrogenase. **a,** L-isocitrate, and **b,** D-isocitrate plus Mg^{2+}. The structures were determined as described[9]. **c,** Stereoview of the superposition of the X-ray structures for L-isocitrate (yellow) and D-isocitrate plus Mg^{2+} (blue). Only the residues involved in enantiomeric discrimination are shown. **d,** Four-point location model for stereoselectivity of a protein, showing how a protein might provide two sites (*D′* and *D″*) in either of two locations for interaction with group *D* on a chiral carbon atom: *D′* would bind one enantiomer and *D″* would bind its mirror image.

The accepted explanation[3–6] for this stereospecificity is based on a three-point attachment model[7,8] for enzymes and drug receptors. In this model, when three groups (*A*, *B* and *C*) of the tetrahedral carbon atom bind to a protein surface at specific sites *A′*, *B′* and *C′* (Fig. 1), it is impossible to bind the equivalent groups *A*, *B* and *C* of its mirror image (enantiomer) at the same three sites. We find that this model does not always hold, and use the enzyme isocitrate dehydrogenase (IDH) as an example to show that the 3-point model needs to be revised to provide a more general mechanism for stereospecificity.

When metal-free crystals of IDH are presented with a racemic mixture of isocitrate, only the L-isomer (2S,3R) binds to the enzyme, as revealed by electron-density maps of the crystal structure at 1.7 Å resolution (Fig. 2a). The exclusive presence of L-isocitrate in the active site of IDH indicates that the D-isomer must bind only weakly to the metal-free (apo) enzyme. But when enzyme crystals are presented with a racemic mixture of isocitrate in the presence of Mg^{2+}, only the D-isomer is seen in the active site of the 1.85 Å crystal structure (Fig. 2b).

Superposition of the structures of the two complexes (Fig. 2c) shows that three of the four groups attached to the tetrahedral C2 atom of D- and L-isocitrate bind to the same three locations in the IDH active site. The difference is the fourth group, the hydroxyl of the C2 carbon. The -OH group of L-isocitrate associates with an arginine residue at position 119 in the metal-free enzyme, in contrast to the -OH group of D-isocitrate, which associates with the metal and with two aspartate residues at positions 283′ and 307 in the active enzyme. Thus, it is the position of the fourth group, *D* in Fig. 1, that makes it possible for IDH to distinguish between the two enantiomers.

When this finding is generalized against published X-ray structures, the three-point attachment hypothesis only works if it is assumed that the ligand can approach a flat protein surface only from the top. In other words, a fourth location, whether a binding site or a direction, is essential to distinguish between enantiomers in an actual protein structure — so if the binding sites on the protein are in a cleft or on protruding residues, a three-point attachment will not be sufficient to discriminate between isomers. This is illustrated in Fig. 2d, where groups *A*, *B* and *C* of different isomers occupy the same protein locations (*A′*, *B′* and *C′*), whereas the *D* groups, which point in different directions, interact at different positions (*D′* and *D″*), as they do in IDH.

The model in Fig. 2d is called a 'four-location' model because it is not necessary for there to be four binding sites. If, for instance, there are three sites and a fourth location so that the ligand can bind in only one direction, then enantiomers can be distinguished. For example, substrates for the enzyme chymotrypsin are L-amino acids, whereas D-amino acids act as inhibitors. Our four-location model explains how D-isomers can occupy the three binding sites in an orientation that prevents cleavage. Crystal structures determined for mandelate racemase indicate that this protein also fits the four-location model.

We conclude that a four-location model is needed to explain a protein's ability to discriminate between L- and D-isomers. The four locations can, for example, be four attachment sites or three attachment sites and a direction, but a minimum of four designated locations are needed.

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