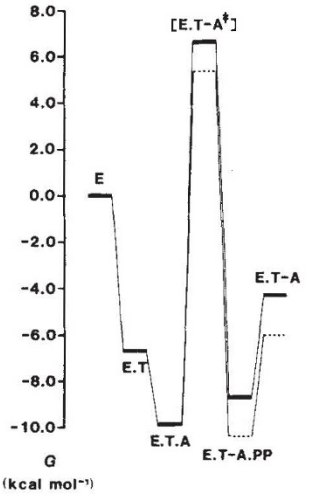

Fig. 2 Gibbs free-energy profiles for the formation of tyrosyl adenylate (E.T-A) by wild-type enzyme (- - - -) and the mutant Cys → Gly 35 (——) (equation (3)). The rate constants for the formation of E.T-A and the transition state E.T−A‡ are taken from Table 1.

$$E \underset{\pm T}{\overset{K_S}{\rightleftharpoons}} E\cdot T \underset{\pm A}{\overset{K'_S}{\rightleftharpoons}} E\cdot T\cdot A \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E\cdot T\text{-}A\cdot PP \underset{\pm PP}{\overset{K_{PP}}{\rightleftharpoons}} E\cdot T-A \quad (3)$$

The reverse rate constant k_{-3} (16 s^{-1} for wild type, 33 s^{-1} for mutant) and dissociation constant for pyrophosphate, K_{PP}(0.61 mM for wild type, 0.63 mM for mutant) were measured by stopped-flow fluorimetry[7]. Free energies were calculated from the standard equations using a standard state of 1 M for tyrosine (T), ATP (A) and pyrophosphate.

values containing rate and equilibrium constants for the pyrophosphorolysis reaction. These data thus gave no conclusive evidence on the interconversion of binding and chemical activation energies. Values for k_3 and the dissociation constants (K_S and K'_S) in equation (2) can, however, be measured

$$E \underset{K_S}{\overset{Tyr}{\rightleftharpoons}} E\cdot Tyr \underset{K'_S}{\overset{ATP}{\rightleftharpoons}} E\cdot Tyr\cdot ATP \xrightarrow{k_3} E\cdot Tyr\text{—}AMP+PP \quad (2)$$

directly by stopped-flow fluorimetry[5] and equilibrium dialysis (Table 1). From these values, free-energy contributions to binding and catalysis for individual groups can be calculated using the standard equations[3] (Table 2).

The residues that were mutated are all in the binding site of the enzyme, but are not directly involved in bond making and breaking (Fig. 1). Removal of side chains that bind to tyrosine (Tyr → Phe 34 and Tyr → Phe 169) does not significantly alter k_3 but increases the dissociation constant of tyrosine from the E · Tyr · ATP complex (Table 1). The hydroxyl moieties of Tyr 34 and Tyr 169, therefore, can be assumed to interact equally well with tyrosine in its unreacted form and in the transition state of the reaction. The binding energies of the −OH groups of Tyr 34 and Tyr 169 with the substrate and transition state are readily calculated[3] and are seen to be nearly identical (Table 2).

In contrast, removal of side chains that interact with ATP causes significant changes in k_3 but, apart from His → Gly 48, hardly affects the value of K'_S for ATP. Mutation of Cys 35 to Gly 35 or Ser 35 leads to a 10-fold lowering of k_3 with no significant change in K'_S. Changing His → Gly 48 results in a mixture of effects, including a fourfold lowering of k_3 and a twofold increase in K'_S. Mutation of Thr → Ala 51 leads to a twofold increase in k_3 with no discernible effect on K'_S for ATP. This result is entirely consistent with the earlier proposal from structural observations and steady-state kinetic measurements that the bond between Thr 51 and the ribose ring oxygen is long and weak and contributes no binding energy[5,6]. As the value of k_3 is increased on deletion of this bond, it must be even longer and weaker in the transition state. (The presence of an alanine at position 51 gives a more active enzyme, and it is intriguing that the tyrosyl-tRNA synthetase from *Bacillus caldotenax*, which is 99% homologous with that from *Bacillus stearothermophilus*, does have alanine at position 51; ref. 7).

Thus, the side chains of residues 35, 48 and 51 probably have different interaction energies with ATP depending on whether it is in its unreacted form or its transition-state structure. The differences could arise from two effects, or a mixture of the two: (1) an unfavourable interaction in the enzyme–substrate complex that is relieved on formation of the transition state (substrate destabilization); and (2) an interaction that is not properly made in the enzyme–substrate but is realized in the enzyme–transition state complex (transition-state stabilization). We have shown by construction of the full free-energy profile for the formation of tyrosyl adenylate (Fig. 2) that the side chain of Cys 35 does not function purely by substrate destabilization. Removal of the side chain of Cys 35 (Cys → Gly 35) lowers the affinity of the enzyme for tyrosyl adenylate by the full amount expected for the loss of a hydrogen bond[4], showing that it makes favourable interactions with the adenosine moiety of the products as well as with the transition state.

It seems unlikely from model building that the mutation of residues in this study will cause any structural artefact. Further, the double-mutant test has been applied to residues 35 and 48 and no change of structure was detected[6].

We have concluded, therefore, that the interactions of important side chains of the enzyme with tyrosine remain unaltered but that those with ATP are optimized as the enzyme–substrate complex reaches the transition state. Thus we have shown that the binding energy of the enzyme and substrate can be used to enhance the catalytic rate and we have quantified its importance.

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Corrigenda

The nucleotide sequences of *copia* and *copia*-related RNA in *Drosophila* virus-like particles

T. Emori, T. Shiba, S. Kanaya, S. Inouye, S. Yuki & K. Saigo
Nature **315**, 773–776 (1985)

ALL U3s and U5s on page 776, lines 16–17 and in Fig. 2 should be U5s and U3s respectively.

Analytical solution for the effect of increasing CO_2 on global mean temperature

T. M. L. Wigley & M. E. Schlesinger
Nature **315**, 649–652 (1985)

IN Fig. 1 legend, the lower part of the figure should be that for $\kappa = 3\ cm^2\ s^{-1}$ and the upper part for $\kappa = 1\ cm^2\ s^{-1}$ and not vice versa. In the acknowledgements the NSF/DOE grant number should be ATM 8205-992.

Erratum

X-linkage of steroid sulphatase in the mouse is evidence for a functional Y-linked allele

E. Keitges, M. Rivest, M. Siniscalco & S. M. Gartler
Nature **315**, 226–227 (1985)

The first line of the first column on p. 227 was printed in the wrong position and should be read as the last line of the first column of that page.