TABLE 1 Steady-state kinetic parameters of wild-type and mutant PFK

	Forward reaction					Reverse reaction			
	k_{cat} (s^{-1})	$K_{m[Fru6P]}$ (μM)	$K_{m[ATP]}$ (μM)	$S_{1/2}$ (μM)	n_H	k_{cat} (s^{-1})	$K_{m[ADP]}$ (μM)	$S_{1/2[Fru1,6P_2]}$ (mM)	n_H
Wild type	134	30	63	540	4	22	52	1.9	h
RS162	95	4,950	42	3,700	2.1	5	124	28.1	2.0
RS243	186	1,600	26	16,000	2.7	8.9	41	13.0	2.0
RS72	4	96	75	700	2.2	1.5	56	39.5	h

PFK catalyses the phosphorylation of Fru6P to Fru1,6P_2 by ATP. The enzyme from *Escherichia coli* (PFK-1; EC 2.7.1.11) is a tetramer and shows positive cooperativity with respect to Fru6P, allosteric activation by ADP or GDP, and allosteric inhibition by phosphoenopyruvate (PEP). $S_{1/2}$, Fru6P concentration at half-maximal velocity in the absence of GDP; n_H, Hill constant determined from fitting kinetic data to $V = V_{max}(S^n_{1/2} + S^n)/S^n$; k_{cat} values were measured at saturating concentrations of both substrates and in the presence of 1 mM GDP. Catalysis in the wild-type enzyme is probably rate-limited by the phosphoryl transfer step[8], although this has not been determined. $K_{m[Fru6P]}$ values were measured in the presence of 1 mM GDP. $K_{m[ATP]}$ values were obtained at saturating values of Fru6P. GDP was not included for these measurements as it acts as a competitive inhibitor for ATP. Reverse reaction kinetics for both wild type and RS72 are hyperbolic (*h*). Their $K_{m[Fru1,6P_2]}$ is equal to $S_{1/2}$. Arg-to-Ser mutations were chosen to eliminate the electrostatic interactions of these residues while still maintaining the polar character of the region. Mutants were produced using oligonucléotide-directed mutagenesis[9]. Wild-type and mutant enzymes were expressed from the plasmid pHL1[10] in *E. coli* strain DF1020, which is deleted for both PFK genes[11]. Enzymes were purified using a blue A column (Amicon)[12] and Sephacryl S-200 gel filtration chromatography (Pharmacia)[8]. In the forward direction, enzyme activity was measured at 25 °C, pH 8.0, in 100 mM Tris-HCl, 10 mM dithiothreitol, 10 mM $MgCl_2$, 10 mM NH_4Cl by coupling Fru1,6P_2 production to the oxidation of NADH[8]. Reverse reaction activity was measured as for the forward reaction, except the production of Fru6P was coupled to the reduction of NADP as described[12]. Coupling enzymes and substrates were from Boehringer Mannheim. Kinetic parameters were obtained by fitting data to either Michaelis–Menten or Hill equations using the Enzfitter program (R. Leatherbarrow, personal communication). Measurements are the averages of at least three determinations.

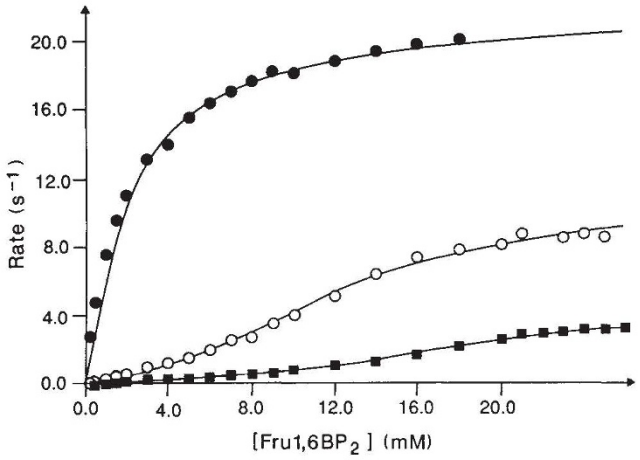

FIG. 2 Reverse reaction kinetics for wild-type, RS162 and RS243 enzymes. ●, Wild type; ○, RS243; ■, RS162. Curves represent best fits to Michaelis–Menten (wild type) or Hill (RS162 and RS243) equations, the parameters of which are given in Table 1.

Arg 72 and Glu 241 in the R state of these mutants may be responsible for this, but structural analysis will be required to verify this hypothesis.

In summary, electrostatic interactions between Arg 162, Arg 243 and the 6-phosphate of Fru6P bound to the neighbouring subunit stabilize the R state of PFK, whereas in the absence of Fru6P, a salt bridge between Arg 72 and Glu 241 of the neighbouring subunit stabilizes the T-state conformation. These active-site arginines therefore play critical roles in the communication of cooperative and allosteric signals between subunits, particularly Arg 162, which performs the largest movement between the T- and R-state configurations. □

Received 22 August; accepted 21 December 1989.

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ACKNOWLEDGEMENTS. We thank M. F. Perutz, T. Schirmer and C. E. Kundrot for discussions. S.A.B. was supported by a fellowship from the M.R.C. of Canada.

ERRATUM

Volatiles in submarine glasses as a discriminant of tectonic origin: application to the Troodos ophiolite

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Nature **343,** 159–161 (1990)

THE figure legend to Fig. 1 of this letter was omitted and a legend from another paper used in error. The figure is shown below with the correct legend.

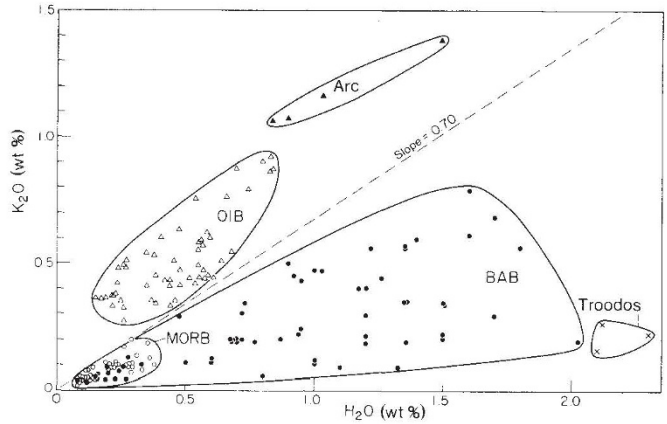

FIG. 1 K_2O plotted against H_2O for unaltered submarine volcanic glasses from known tectonic environments. MORB-field (○) (Mid-Atlantic Ridge[13], East Pacific Rise[14], Galapagos[15,16]); BAB-field (●) (Mariana Trough[12], Scotia Sea[17], Fiji/Lau[18] and Woodlark basins[19]); OIB field (△) (Hawaii[9–11]); Arc field (▲) (Mariana island arc[12]); Troodos (×) (this study). Note that MORB, BAB and Troodos fields have $K_2O/H_2O < 0.70$.