aqueous domains. These are manifested as extensive bulk-swelling properties and abnormal viscosity–concentration relationships. This is what happens here because the polymer leaves the cell and enters the aqueous medium when the released mucopolysaccharide vesicles can actually be seen in micrographs swelling and bursting and when viscosity measurements on newly released mucin show large increases in reduced viscosity accompanying dilution[5,6]. But why does the polymer remain relatively unswollen within the cell? The environment is still aqueous and a rapid and catastrophic domain expansion might be expected, but this patently does not occur. The finding that the polyanion exists intracellularly as the potassium salt, coupled with the observation that this salt is somehow different in conformation or charge distribution from other cation forms, may explain the ability of the cell to keep the polyanion packaged. This may be achieved by maintaining specific inward membrane-mediated pumping of potassium while specific exclusion of other cations at the polyanion vesicle membranes may explain the failure of the magnesium–calcium salt to form.

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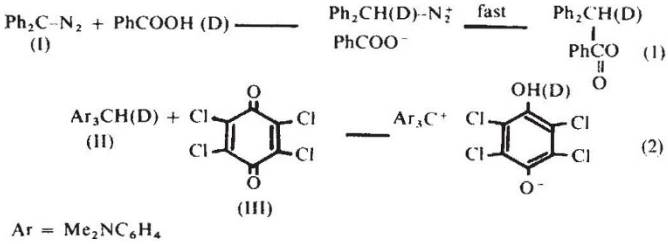
Pressure effects on proton tunnelling

We report here the first measurement of the effect of pressure on primary kinetic isotope effects of reactions in the liquid phase. We have determined rates of two reactions in solution by a sampling technique at pressures up to 2 kbar using both normal and deuterium-labelled reagents as indicated. Reaction (1) is between benzoic acid and diphenyldiazomethane (I) which has been shown to proceed by a rate-determining proton transfer[1,2]. Reaction (2) is between leuco crystal violet (II) and chloranil (III) in which the slow step is a hydride transfer[3].



In the butyl ether solvent used, reaction (1) exhibits an isotope effect which is in the normal region ($k_H/k_D = 4.5$), that is, accountable in terms of differences in zero-point energy and vibrational frequencies of reagents and transition state[4]. The abnormally large isotope effect ($k_H/k_D = 11.2$) in reaction (2) has been assumed to be due to a tunnelling contribution.

We find that the behaviour of the two isotope effects under pressure is quite different. That of reaction (1) remains almost invariant while that of (2) smoothly decreases until at 2 kbar it levels off at a value near the maximum 'normal' ratio of about

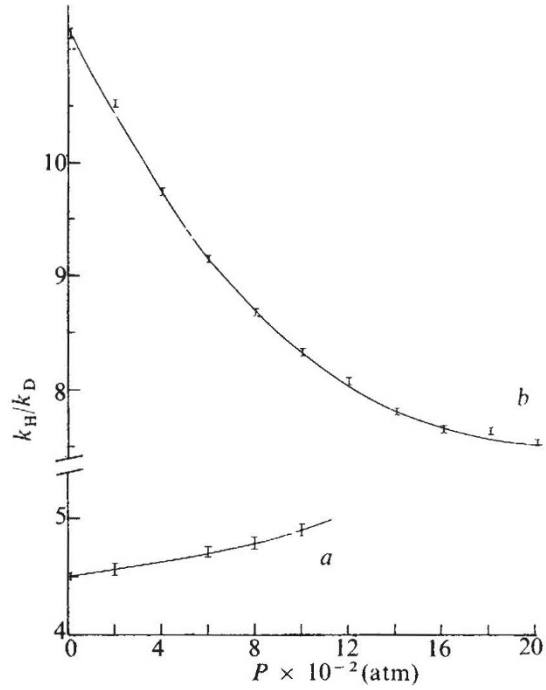


Fig. 1 Effect of pressure on the kinetic isotope effect of hydrogen transfer reactions. *a*, Reaction of diphenyldiazomethane in dibutyl ether at 26.5 °C; *b*, reaction of leuco crystal violet in acetonitrile at 29.5 °C.

7.5 (Fig. 1). It follows that the volumes of activation, $\Delta V‡$, calculated (using the regression equation $\ln k = A - \Delta V‡/RT + CP^2$)[5] for reactions (1) (H) and (1) (D) are the same (-12.8 cm^3 mol^{-1}) while those of reactions (2) (H) and (2) (D) are very divergent, being -25.5 and -35.8 cm^3 mol^{-1} respectively.

It is assumed with good justification that isotopic substitution does not alter the reaction pathway or position of the transition state along the reaction coordinate[6]; therefore it seems likely that the value of $\Delta V‡$ for reaction (2) (H) is anomalous because of a tunnelling component. In this reaction external pressure serves to lower the potential barrier to the activation process. Consequently a higher proportion of reagents will be capable of surmounting the barrier normally and the relative importance of tunnelling will diminish with increasing pressure analogous to the effect of an increase in temperature[7]. This analysis suggests that the tunnelling contribution of a reaction with a positive volume of activation (that is, which has a rate decreasing with pressure) and consequently the isotope effect, should increase with pressure. In general, where tunnelling is important, $\Delta V‡(H) < \Delta V‡(D)$.

Although more data are needed to give confidence to this interpretation the result suggests that pressure dependence of the isotope effect may serve as a further criterion of the occurrence of tunnelling in hydrogen-transfer reactions.

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