Isotope rate effects with D_2O in several enzyme systems

Several recent papers have reported effects observed when single enzymes or more complex multi-enzyme systems are studied in D_2O (refs. 1–3). No corrections were made for the differences in D^+ concentration in D_2O as compared with H^+ concentrations in H_2O when the solutions have identical pH_m values as determined using a glass electrode. LUMRY *et al.*[4] have shown that D^+ concentration in D_2O solutions containing low concentrations of ordinary water and its ions could be secured from the glass-electrode pH_m value through the formula (at room temperature)

$$pD = pH_m + 0.4.$$

Appropriate values for mixed D_2O–H_2O solutions can also be derived. Recent experimental confirmation and discussion of the formula has been given by GLASCOE AND LONG[5] and by MIKKELSEN AND NIELSEN[6].

SELTZER *et al.*[7] have discussed the question of comparison of measured rates in the two kinds of water at pD = pH *versus* the comparison where the buffer ratios are equal or where the rate constants are at maximum values. To some extent this is at present a matter of preference but the utility of the comparison at equal buffer ratios is limited unless the buffer characteristics in D_2O are known. The ion product of D_2O and its temperature dependence are different from H_2O values. pD, on the other hand, has a firm and simple significance as the activity of the cationic form of the hydrogen isotope so that if emphasis is on acid–base behavior, comparison at pD = pH will be the more useful. We[8] have studied the citrate-condensing enzyme in D_2O and have found striking changes in k_H/k_D which are strongly dependent on pH and pD. Table I compares k_H/k_D for several enzymes with and without the use of the formula.

TABLE I

ISOTOPE RATE EFFECTS OF SEVERAL ENZYMES

Citrate-condensing enzyme was assayed spectrophotometrically at 233 mμ as described by OCHOA[9]. Fumarase was assayed in phosphate buffer in the direction of hydration[10]. Malate dehydrogenase was assayed in tris(hydroxymethyl)aminomethane–acetate buffers in the direction of oxaloacetate reduction[11].

Enzyme	*pH*	k_H/k_D* (pH H_2O = pH D_2O)	k_H/k_D** (pH = pD)
Citrate condensing Enzyme	7.0	3.0	3.9
	8.0	2.8	2.7
	9.0	1.5	1.5
	10.0	1.2	0.8
Fumarase***	7.8	2.1	1.4
	8.0	1.9	1.3
	8.2	1.7	1.2
Malate dehydrogenase	7.5	4.3	5.6
	8.5	5.7	8.4
	9.5	2.8	3.8

* The ratio of initial velocities in H_2O and D_2O (> 99%) when the glass-electrode reading in D_2O is equal to the reading in H_2O.

** Initial velocities ratio where pH = pD.

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It is readily noted that errors can be made not only in the value of the isotope effect but it is even possible that the direction of the isotope effect will be incorrectly given. Other choices for comparison conditions will, of course, give similar quantitative and qualitative differences.

It should also be pointed out that isotope effects on reaction rates in enzyme systems may be an unreliable source of information about such reactions. In the first place it is seldom possible to know the composition of the apparent rate constants in terms of the true rate constants of the elementary steps which are actually involved[12]. It is necessary to know which elementary rate constants are directly influenced if isotope effects are to be unequivocally interpreted and indeed even to know the conditions under which rates in the two kinds of water are to be compared. In the second place, even in apparently simple systems the secondary isotope effects may be of comparable magnitude to the primary effects. An interesting case of such an ambiguity in the interpretation of isotope effects has appeared in the study of electron-exchange reactions of relatively simple inorganic ions[13]. In the third place, it is usually unwise to draw conclusions from isotope effects without temperature studies which show whether or not the effects appear in the activation energy or apparent entropy or both since the implications in terms of mechanism may be quite different in the three cases[14]. In the fourth place, it must be remembered that protein hydration varies with the isotopic composition of the water and that the conformational details of some proteins may also be so changed as to influence their catalytic function. There are additional less important dangers but these would appear to be the major complications.

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Department of Biological Chemistry,
The University of Michigan, Ann Arbor, Michigan and School of Chemistry, University of Minnesota, Minneapolis, Minn. (U.S.A.)

PAUL A. SRERE
G. W. KOSICKI*
RUFUS LUMRY

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