Acid and base catalysis in a non-enzymic transfer reaction A possible enzyme model*

A possible mechanism for the catalytic powers of transfer enzyme is a concerted acid base catalysis with activation of both the donor and the acceptor molecules[1,2]. Non-enzymic reactions exist in which intramolecular catalysis activates the donor[3,4,5] or in which intermolecular catalysis activates a ring opening[1] but heretofore no model for a transfer reaction has been observed in which both acceptor and donor are synchronously activated. It is believed that such a model has been uncovered in studies on the properties of thiophosphates.

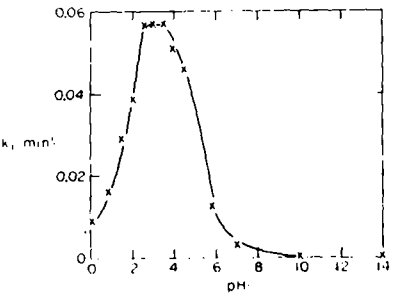
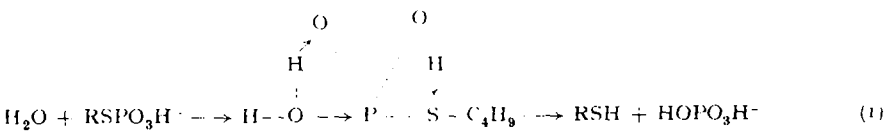


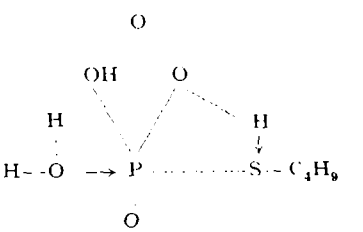
Fig. 1. Hydrolysis of $C_4H_9SPO_3H_2$ at varying pH. $1.8–2.0 \cdot 10^{-3} M$ ester, $\mu = 1.0$, $T = 37°$ C.

Butyl thiophosphate was prepared by reaction of $POCl_3$ with sodium butyl mercaptide in pyridine. Purification and analysis were performed via the barium and the cyclohexylammonium salts which gave analytical values showing a purity of 98%. The kinetics of hydrolysis over the range of 1 N OH^- to 1 N H^+ are shown in Fig. 1. The rate remains the same from 1 N H^+ to 4 N H^+. The salient features are the lack of acid catalysis and the pH maximum in the region (pH 2–4) corresponding to the presence of the monoanion, $RSPO_3H^-$. The enhanced reactivity of the monoanion, which has also been found for other phosphate esters[4,6,7,8], has been puzzling because explanations, such as electrostatic repulsion, which account for the increase in rate on going from the dianion to the monoanion fail to explain the decrease on going from the monoanion to the uncharged acid.

A mechanism which satisfactorily explains this and the lack of acid catalysis is shown in equation 1 in which the bonds being broken are shown by a dotted line and those being formed by an arrow. The proton of the acceptor, water, is transferred to the negative oxygen, thereby increasing the electron sharing tendencies of the water oxygen and thus increasing its reactivity towards the phosphorus. The proton of the hydroxyl is synchronously transferred to the thiomercaptide group, thereby aiding the breaking of the P–S bond. This mechanism explains the negligible rate in alkaline solutions, where the donor activating OH group is absent, and the decreased rate in acid where the acceptor activating O^- is absent. At about pH 0, the water is either not activated (equation 2) or weakly activated by transfer to the doubly bonded oxygen, but the intramolecular transfer of a proton is still so efficient that no added catalysis is obtained by the protons in the solution. Lack of acid catalysis has been observed in other cases in which an intramolecular proton transfer has been postulated[5].



(1)



(2)

Other alternatives have been examined and found to reveal serious deficiencies. For example, attack by OH^- on the uncharged ester can be excluded on the basis of observed rates for the reaction of alkali with trimethylphosphate, and an ionization (SN_1) type mechanism was excluded on the basis of the varying MeOH to water reactivities of a number of phosphate esters[9].

It should be noted that electron shifts will all be in directions which aid the movements

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of protons and atoms as shown in equation 1. Thus, a synchronous shifting of protons should occur with the breaking of the P–S and formation of the P–O bonds. A system which combines the acid and base in a single unit capable of such synchronous action may well be the reason for the efficiency of enzyme catalysis.

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Isolation of a quinone from beef heart mitochondria*

From lipid extracts of beef heart mitochondria we have isolated a new compound capable of undergoing reversible oxidation and reduction. The absorption spectrum of the oxidized and reduced forms are shown in Fig. 1. The oxidation-reduction behaviour as well as the infrared spectrum indicate that the compound is a quinone. For convenience it will be referred to as Q-275. A compound with similar spectral properties has been observed also in beef liver mitochondria. This yellow-orange, crystalline material has been recrystallized from several solvents to a constant melting point (48–49° C) and to a constant extinction coefficient. The purity of the compound is being investigated by chromatographic procedures. Q-275 is insoluble in water, but is soluble in most lipid solvents.

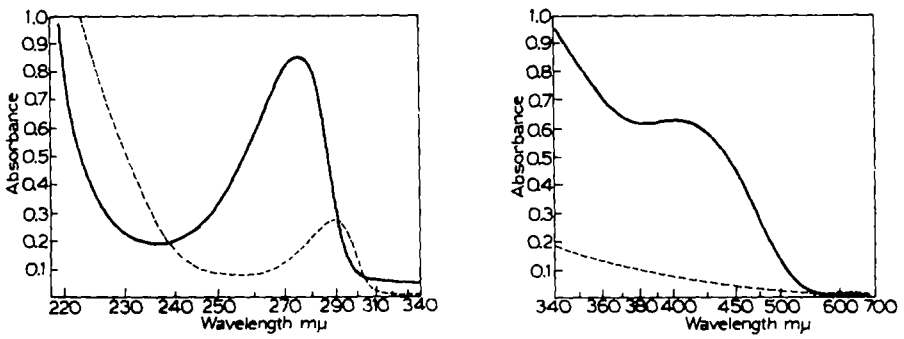

Fig. 1. Absorption spectrum of Q-275 in absolute ethanol. Solid line represents oxidized form, and the dotted line represents the spectrum obtained after shaking with a few grains of KBH_4. Concentrations used for 1 cm path in mg/ml: Ultraviolet range, 0.0425; visible range, 0.75.

In addition to beef heart mitochondria, Q-275 has been found in various electron transporting particles derived from these mitochondria. The concentration of Q-275 (mg/g protein) was found to be as follows: Mitochondria[1], 2.5; ETP[1], 2.7; SDC[2], 6.0; green fraction[3], 0.5. The presence of Q-275 appears to be correlated with succinate oxidizing capacity.

That Q-275 is involved in the electron transport activities of the aforementioned particles is indicated by several lines of evidence.

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