From the equilibrium constants the relative oxidation-reduction potential of the 15 thiol-disulphide couples involved in the 10 systems studied, can be calculated. Of particular interest is the fact that the above equilibrium constants can be used for the calculation of the oxidation-reduction potential of cysteine, relative to that of glutathione. It is clear from Table 1 that the calculations, based on 5 independent sets of data, show good agreement. The results seem to present a solution to this controversial question.

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Enzyme Kinetics in Univalent Buffers

J. S. McKINLEY MCKEE

Biochemical Institute, University of Oslo, Blindern, Oslo

Many ions have different effects on the kinetics of the fumarase-catalyzed reaction¹:

With buffer ions specific effects arise due to the binding of the different buffer ions. Kinetic studies in phosphate buffers² proved unsatisfactory due to the presence of HPO_4^{--} and H_2PO_4^- ions, each with its own effect on the enzyme and the relative concentration of which varied with pH. As the kinetic constants are unaffected by the buffer cation, while being very sensitive to the anion concentration and type, univalent buffers of the uncharged base type (e. g. imidazole, tris, amidol-chloride, fluoride or perchlorate) have been used. Thus specific buffer effects can be

studied as there is a single anion, the concentration of which can be held constant over a wide pH range.

The experiments have consisted of determining the pH variation of the maximum velocities (V_M , V_F) and Michaelis constants (K_M , K_F) for the forward and reverse reactions. These were obtained from Liveweaver-Burk plots. It was important to use substrate concentrations (range $4K-K/4$) which gave neither substrate activation or inhibition³, as otherwise erroneous kinetic constants resulted. The enzyme solutions were prepared from crystalline fumarase, and the kinetics were followed on the 80—100 % transmission expanded scale of a Beckman DUR spectrophotometer². The Michaelis constants for malate and fumarate in 5 mM chloride, perchlorate or acetate buffers rise markedly above pH 8.0. With malate, the K_M values are approximately constant below pH 7.0, while with fumarate this represents a minimum. Plots of V_M , V_F , V_M/K_M , and $V_F/4.4K_F$ vs. pH are symmetrical and bell shaped. For each buffer the data were consistent with the ionisation of two groups in the enzymatic site, having different ionisation constants in the free enzyme and the fumarase and L-malate complexes. In the various buffers the pK values vary markedly. Good agreement was found between the experimental points and theoretical curves calculated from pH independent kinetic constants and the pK values². In interpreting the data the secondary ionisations of the substrates have to be taken into account at the lowest pH values. A comparison of the kinetic constants in phosphate (approximate values) with those in the univalent buffers shows that while neither buffer type or concentration markedly affect the pH independent maximum velocity or turnover number, yet the corresponding Michaelis constants are much greater in phosphate, presumably due to the divalent ions present. As both substrates give the same ionisation constants for two essential groups in the free enzyme (V_M/K_M and $V_F/K_{eq}K_F$ superimposable) it seems that it is the same enzymatic site that is involved in the proton transfer reactions resulting in hydration or dehydration.

The author wishes to thank professor R. A. Alberty for many helpful discussions.

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