8. The Influence of Hydrogen-Ion Concentration upon the Equilibrium State in Phosphorylase Systems

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Observations have been reported in earlier communications [Hanes, 1940, 1, 2, 3] on the reversible transformation of glucose-1-phosphate into starch and inorganic orthophosphate, catalysed by an enzyme (phosphorylase) which is present in many higher plants. The reaction may be represented as follows:

$$\text{Starch} + \underset{\text{(free-P)}}{\text{inorganic phosphate}} \rightleftharpoons \underset{\text{(ester-P)}}{\text{glucose-1-phosphate}}.$$

Experiments with purified phosphorylase from potatoes showed that the equilibrium state, defined by the values of the ratio free-P/(free-P + ester-P), or by the ratio free-P/ester-P, was not significantly affected by alterations within wide limits in the gross concentration of starch or in the concentration of total phosphate (free-P + ester-P). It was observed, however, that the equilibrium was markedly affected by alterations in the concentration of hydrogen ions, larger proportions of the reactants existing in the form of starch and inorganic phosphate under more acid conditions. Thus, as the *p*H value was varied from 5·0 to 7·0, the values of the ratio free-P/ester-P decreased progressively from about 10·8 to 3·1 [Hanes, 1940, 1, 2].

It is of interest that a similar effect has been reported recently by Cori & Cori [1940] in connexion with the action of the corresponding enzyme (glycogen-phosphorylase) from animal tissues (brain, liver, and muscle). The average values which they give for the equilibrium ratio free-P/ester-P are 5·7 at *p*H 6·0 and 2·9 at *p*H 7·55. Also they show that this ratio is unaffected by wide variations in the concentration of polysaccharide (glycogen) present.

The purpose of the present note is to compare the effects of H^+ concentration upon the equilibria attained in these reversible reactions catalysed by the plant and animal phosphorylases and at the same time to extend and modify the interpretation of this effect which has already been advanced.

It was concluded earlier [Hanes, 1940, 3] that alterations in the hydrogen-ion concentration affected the equilibrium owing to the differential effects upon the dissociation of orthophosphoric acid and glucose-1-phosphoric acid. Whereas the values of the ratio free-P/ester-P, i.e. total orthophosphate/total glucose-1-phosphate, decreased from about 10·8 to 3·1 over the range of *p*H from 5·0 to 7·0, calculations showed that the ratio of the divalent ions of the two acids remained approximately constant. This ratio $[HOPO_3^=]/[ROPO_3^=]$ (R representing the glucose molecule substituted at carbon atom 1) had a value of about 2·2. It was thus clear that an equilibrium, independent of H^+, existed between similarly charged ions of the opposing reactants. This preliminary analysis was, however, incomplete, and misleading to the extent that emphasis was placed on the divalent ions to the exclusion of the other species of the two reactants. It should have been made clear that a constant ratio for the divalent ions implies a constant ratio also for the undissociated acids and for the monovalent ions. For, given the two acids $HOPO_3H_2$ (with dissociation constants $k_1 = 1{\cdot}07 \times 10^{-2}$ and $k_2 = 1{\cdot}51 \times 10^{-7}$) and $ROPO_3H_2$ (with dissociation constants $k_1 = 7{\cdot}8 \times 10^{-2}$ and $k_2 = 7{\cdot}4 \times 10^{-7}$) the following identities will hold, whatever the $[H^+]$:

$$\frac{[HOPO_3H_2]}{[ROPO_3H_2]} = \frac{7{\cdot}8}{1{\cdot}07} \times \frac{[HOPO_3H^-]}{[ROPO_3H^-]} \text{ and } \frac{[HOPO_3H^-]}{[ROPO_3H^-]} = \frac{7{\cdot}4}{1{\cdot}51} \times \frac{[HOPO_3^=]}{[ROPO_3^=]}.$$

With the ratio of the divalent ions approximately constant at a value of about 2·2 it follows that the ratios of the monovalent ions and of the undissociated acids will be approximately constant at values of about 10·7 and 77 respectively.

For the purpose of a close comparison of the equilibria attained over a range of *p*H values in the presence of the plant and animal enzymes respectively, use may be made of the gross ratio free-P/ester-P or of the ratio free-P/ester-P for *any* pair of similarly charged ions. For convenience in plotting the results we have chosen the ratio of the univalent ions. These ratios are, of course, derived from the estimated free-P/ester-P values by multiplying by a factor, changing with *p*H, calculated from the dissociation constants of the acids and the [H+]; so that a given percentage error in the estimate of free-P/ester-P will produce the same percentage error in the estimate of the ratio of corresponding ions.

The values calculated are shown in Figs. 1 and 2, but before dealing with these it seems desirable to consider the effect on them of experimental errors in the primary analytical determinations.

Experimental error in defining equilibrium states. The primary data consist of analytical determinations of free-P and of (free-P + ester-P) on samples of reaction mixtures in which it is judged that the equilibrium state has been attained. These values are obtained by measuring the amounts of inorganic orthophosphate present in suitable aliquots before and after they have been subjected to a treatment which causes the complete hydrolysis of glucose-1-phosphate (7 min. heating at 100° in the presence of *N* H_2SO_4 or $HClO_4$). The determined values for free-P, however, require a correction owing to the fact that a small proportion of the glucose-1-phosphate present is hydrolysed during contact with the acid reagents used to precipitate protein and to determine inorganic phosphate. Thus, in the technique used by Hanes, about 0·4 % of the ester present is hydrolysed. On this account it is probable that the corrected values for free-P are subject to a slightly greater error than the values for (free-P + ester-P). A small error in making this correction will, however, have only a small effect on the derived value free-P/ester-P. An over- (or an under-) estimate by as much as 2 % in the amount of ester-P assumed to be hydrolysed will, over the range of *p*H from 5 to 7·5, decrease (or increase) the value free-P/ester-P by less than 3 %. An error of the same magnitude in the ratio of the two determinations free-P/(free-P + ester-P) will on the other hand have, especially at low *p*H values, a considerable effect on the derived data. An overestimate of 2 % in the ratio free-P/(free-P + ester-P) produces in the ratio free-P/ester-P an overestimate of about 27 % at *p*H 5·0 and of about 8 % at *p*H 7·5. An underestimate of 2 % in the ratio free-P/(free-P + ester-P) produces an underestimate in the ratio free-P/ester-P of about 18 % at *p*H 5·0 and of about 7 % at *p*H 7·5. Considerable scatter in the derived ratios, especially at lower *p*H values, might therefore be expected. Moreover, a small *systematic* error in the estimate free-P/(free-P + ester-P) may produce a marked drift with *p*H in the derived ratios. A systematic overestimate will produce a downward drift as *p*H increases, and a systematic underestimate an upward drift.

Equilibrium states in plant and animal phosphorylase systems

The data for potato phosphorylase, described in detail earlier [Hanes, 1940, 3], consist of 17 evaluations of the composition of systems at equilibrium at different *p*H values and attained by the reaction proceeding from both sides. Data for three animal phosphorylases (from muscle, liver, and brain tissue) were presented graphically by Cori & Cori [1940, Fig. 9], values of free-P/ester-P being plotted against *p*H value. Numerical values corresponding to each plotted point have been determined from the graph with precautions against the introduction of significant error. The observed free-P/ester-P ratios from the two sets of data are shown plotted against *p*H in Fig. 1. The upper horizontal lines, extending over the *p*H ranges used with the plant and animal systems respectively, indicate the average values of the ionic ratios (monovalent free-P)/(monovalent ester-P)

for the two sets of data. Similar horizontal lines below indicate the average values of the ratios for the divalent ions. The continuous curves, calculated separately for the plant

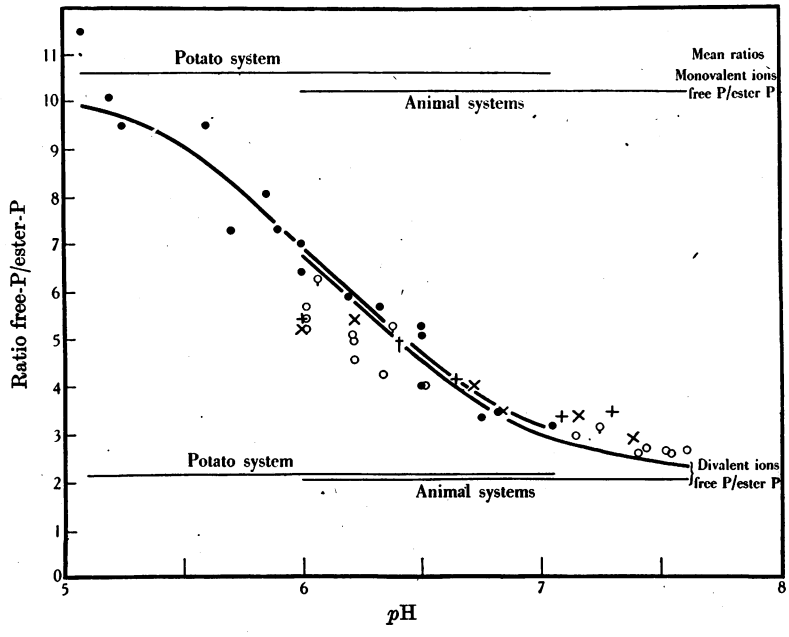

Fig. 1. Animal systems (Cori & Cori). ♀, o, muscle; ×, brain; †, +, liver. ♀ and †, equilibria approached from the free-P side. o, × and +, equilibria approached from the ester-P side. Plant system (Hanes). •, potato (equilibria approached from either side).

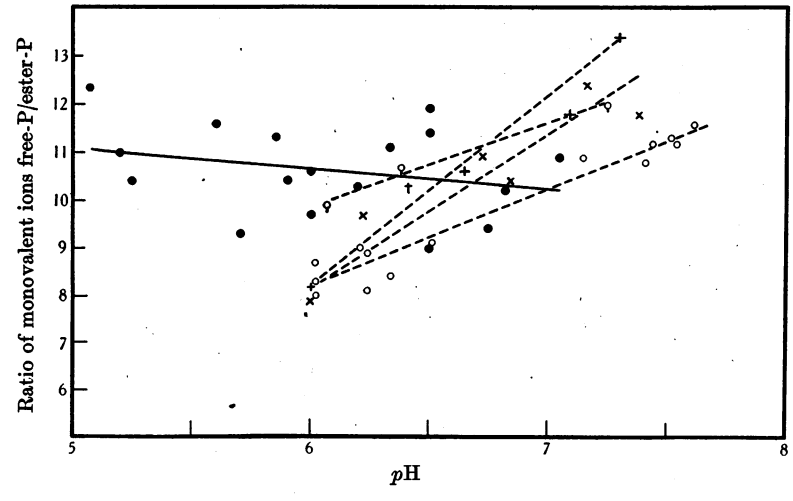

Fig. 2. Notation as in Fig. 1. Solid line—regression line for the plant system. Broken lines—separate regression lines for the animal systems as follows: ♀, muscle, approached from the free-P side; o, muscle, approached from the ester-P side; ×, brain, approached from the ester-P side; †, +, liver, one point approached from the free-P side, the rest from the ester-P side.

and for the animal systems, show the free-P/ester-P ratios expected if the ratios of the corresponding ions remain constant at their mean values. It will be seen that as the *p*H changes from the region *p*H 5 where the two acids are present mainly as monovalent ions,

to *p*H 7 where the acids are present mainly as divalent ions, so the free-P/ester-P ratios for the potato system drift from a level approaching the mean calculated ratio for monovalent ions to a level approaching the mean ratio for divalent ions. Also, although there is some scatter the observed values lie fairly closely about the expected curve.

The free-P/ester-P values for the animal enzyme systems follow a very similar course and show roughly the same percentage scatter about the expected curve. It will be noticed however that below about *p*H 6·7 the observed values tend to fall below the theoretical curve while above *p*H 6·7 they rise above it.

There is thus a close similarity in the equilibria in the plant and animal systems and in the relation to [H+]. The somewhat divergent drift of the values for the animal systems suggests however that identity of the equilibria is not definitely established. The differences become rather clearer when one compares, as in Fig. 2, the values for the ratios of corresponding ions. (The values plotted are those for the univalent ions.) The mean ratio for the animal systems, viz. 10·2 (standard deviation of mean 0·29) is only slightly below and does not differ significantly from the mean ratio for the plant system, viz. 10·6 (standard deviation of mean 0·23). But whereas the plant values show no significant drift with *p*H (the calculated regression is only −0·44 per *p*H unit and is not statistically significant) all the sets of animal values show more or less pronounced upward drifts (the regression coefficients range from +1·7 to +3·6 per *p*H unit and are all statistically significant).

From this analysis of the available data it may be concluded that the ratio of corresponding free-P and ester-P ions does remain constant at equilibrium in the case of the plant system, but the same conclusion cannot at present be drawn for the animal systems. It is, of course, possible that the constancy observed in the plant systems is fictitious, the discrepancy between this and the animal systems arising from some systematic error in the primary determinations for the plant system. Assuming however that, at equilibrium, both in animal and plant systems, the ratios of corresponding ions are really constant, independent of *p*H, it is calculated that the average upward drift in the observed values for the animal systems would be accounted for if there had been a *systematic* underestimate of the order of about 7 % in the determinations of the free-P/(free-P + ester-P) ratios. This would however imply a value for the constant ratio (monovalent free-P)/(monovalent ester-P) of about 17 for the animal data, taken all together, as against a value of 10·6 for the plant system, so that the equilibria, although related in the same way to [H+], would not be identical. Moreover, an underestimation of this order in the primary data for the animal systems seems very unlikely, and it is therefore more probable that the discrepancy reflects some real difference in the reactions occurring in the plant and animal systems, the drift shown by the animal systems suggesting the existence in these of some complexity that is absent from the plant system.

To conclude, it would appear that there is a closely similar basis for the effect of [H+] on the equilibria in the plant and animal phosphorylase systems, but that a direct experimental comparison, using, if possible, more precise methods of estimation, is required to determine either the identity of the equilibria in the two systems or the points of detail in which they differ.

So far as one of us (C.S.H.) is concerned, the work formed part of the programme of the Food Investigation Board and is published by permission of the Department of Scientific and Industrial Research.

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