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QUANTITATIVE ANALYSIS OF THE MIXED ACTIVATING EFFECTS OF THE ALKALI METAL IONS ON INTESTINAL BRUSH-BORDER SUCRASE AT pH 5.2

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Summary

The activation of rabbit brush-border sucrase by the alkali metal ions, Li^+, Na^+ and K^+, was analyzed using the equations of the random-order allosteric model previously proposed for sucrase (Mahmood, A. and Alvarado, F. (1975) Arch. Biochem. Biophys. 168, 585). The alkali metals have mixed activating effects in *tert*-butylamine buffers at pH 5.2, including:

1. Affinity-type activation, where the apparent K_m decreases as a hyperbolic function of the metal concentration.

2. Capacity-type activation, where the apparent V increases with the metal concentration.

These two effects were analyzed quantitatively: firstly, by using linear transformations that allowed us to solve each partial equation separately and secondly, by iteration of the general equation, which permits treating the mixed effects as a whole. Results are consistent with the interpretation that a single metal-binding (activator) site suffices to explain the simultaneous occurrence of the two types of kinetic effect. Nevertheless, complicating factors exist that may require the postulation of additional sites for monovalent cations. In particular, the *tert*-butylammonium ion appears to interfere with the effects of the alkali metals, especially Li^+.

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Introduction

We have shown previously [1,2] that the alkali metal ions have complex effects on intestinal brush-border sucrase (sucrose α-D-glucohydrolase, EC 3.2.1.48). Below pH 7, only enzyme activation occurs, whereas above pH 7, activation is followed by inhibition at higher metal concentrations, resulting in biphasic curves. In the rabbit, activation in the pH range 5—7 is mixed and involves: (1) Affinity-type (K-type) activation kinetics, where the apparent K_m decreases as a hyperbolic function of metal concentration and (2) Capacity-type (V-type) activation kinetics, where the apparent V increases with the metal concentration. According to allosteric theory [3,4], a single activator site (distinct from the substrate-binding site) can exert a double activation (K-type plus V-type), but the evidence obtained with rabbit sucrase [2] was not enough to eliminate a second possible interpretation that the kinetically distinguishable effects on either K_m or V may involve separate metal-binding (activator) sites. Two observations favored this second interpretation. Firstly, at pH 5.2, the alkali metal ions, Li^+, Na^+ and K^+, have strong mixed activating effects, but at pH values around 7, only Na^+ is effective in causing a small (40—50%) but consistent increase in V without any effect on K_m. Secondly, in lithium buffers at pH 5.2, the capacity component was saturated when the affinity component was not. In effect, in the presence of the Li^+ (20 mM) contained in the buffers, the apparent V seemed to be at its maximum limit. Here, the addition of Na^+ did not change V but caused a 4-fold decrease in apparent K_m, thus giving a strong affinity-type effect [2].

In the present work we have quantitatively analyzed the kinetic effects of the alkali metal ions Li^+, Na^+ and K^+, on rabbit brush-border sucrase at pH 5.2 to ascertain whether one or more metal-binding sites are involved in enzyme activation. The results seem compatible with the interpretation that a single metal-binding activator site is responsible for the mixed kinetics observed. However, the situation appears to be complicated by additional, still unclear effects of the monovalent cations on the enzyme, including the *tert*-butylammonium ion used to prepare the metal-free buffers.

Materials and Methods

Reagents and buffers. All reagents were of AR grade. All buffers were prepared using distilled, then deionized, water and were checked with an Eppendorf flame photometer to ensure the absence of contaminating cations. Further details for the preparation of the buffers are given in Ref. 2.

Determination of sucrase activity. Sucrase activity was measured using a two-step procedure involving the determination of the D-glucose product with glucose oxidase and peroxidase after stopping the reaction with Tris, as previously described [5]. Protein was determined according to the method of Lowry et al. [6].

Calculation and expression of results. Velocities are given in units per mg of protein: 1 unit = 1 μmol/min under standard conditions [5]. For the calculation of the kinetic parameters, V and K_m, the $[S]/v = f[S]$ transformation was used [7]. Straight lines were calculated using the 'raw' experimental data,

without weighting. Six values of [S] were used for each curve (range, 8—40 mM), each experimental point involving at least two or three determinations. Correlation coefficients for the regression lines ranged from 0.980 to greater than 0.999, indicating high significance ($P < 0.005$ for six data points). Calculations were performed using a programmable desk calculator (Hewlett-Packard 9815A), except for the iteration analysis (see text), where the computer facilities of the C.I.R.C.E., Orsay, were used.

Enzyme preparation. The sucrase-isomaltase complex was solubilized with papain from brush-border membrane vesicles and purified to homogeneity using standard procedures [2,8].

Results and Discussion

To determine whether or not a single metal-binding activator site can account for the coexistence of activating effects of the alkali metal ions on both K_m and V at pH 5.2, we carried out sucrose saturation experiments in the presence and absence of fixed concentrations of Li^+, Na^+ or K^+. The results (Table I) include: (a) one series using Na^+ as the activator in the presence of a

TABLE I

RABBIT BRUSH-BORDER SUCRASE: ACTIVATING EFFECTS OF THE ALKALI-METAL IONS AT pH 5.2

Kinetic analysis at constant [Na^+] and variable [S]. Kinetic constants calculated according to the $[S]/v = f[S]$ transformation, where n is the number of experiments per series. The buffers were either lithium maleate with 22.4 mM Li^+ or a citric acid/maleic acid/phosphoric acid/glycine (2 : 2 : 2 : 2, mM) universal buffer with 22.4 mM *tert*-butylamine as the base.

n Activator	mM	V^{app} units/mg protein	K^{app} mM
(a) Lithium buffer			
1 —	—	10.8	23.8
1 Na^+	0.2	11.3	23.1
1 Na^+	1.0	10.0	11.9
1 Na^+	5.0	11.1	10.2
1 Na^+	20.0	11.5	8.3
1 Na^+	100.0	11.4	7.2
	(mean = 11.0)		
(b) *tert*-Butylamine buffer			
10 —	—	4.5	41.0
4 Na^+	1.0	7.4	35.8
2 Na^+	5.0	8.0	15.9
2 Na^+	20.0	10.2	13.6
2 Na^+	100.0	11.0	10.7
3 K^+	1.0	6.9	13.1
2 K^+	5.0	7.0	11.4
2 K^+	20.0	7.7	11.1
2 K^+	100.0	8.4	10.4
4 Li^+	1.0	3.8	33.8
4 Li^+	5.0	4.7	30.8
4 Li^+	20.0	6.2	28.9
4 Li^+	100.0	6.0	19.0

maleate buffer containing 22.4 mM Li^+ and (b) three series involving Li^+, Na^+ or K^+ as the activator in a universal buffer [2] containing 22.4 mM *tert*-butylamine as the base. In the lithium buffer Na^+ exerts a pure affinity-type effect, since K_m falls as $[Na^+]$ increases, whereas V remains essentially unchanged. On the other hand, in the *tert*-butylamine buffer, all three metals have mixed activating effects that involve both K_m and V. Our aim was to evaluate these results in terms of the allosteric model we have been using to explain the mechanism of non-obligatory activation of intestinal brush-border sucrase by the alkali metal ions [2,4,5] (We use the term 'allosteric' in its most ample sense: indirect interactions between distinct sites that do not necessarily occur in separate monomers). We shall begin by describing briefly the most salient features of this model.

The allosteric model

The model illustrated in Fig. 1 is a composite of previously published forms (i.e., Fig. 1 in Refs. 4 and 5): it defines the nomenclature used. Also, it illustrates quite clearly the possibility of explaining, with a single activator site, the simultaneous existence of both K-type and V-type allosteric effects of the alkali metals on intestinal brush-border sucrase. The model contains six different kinetic constants. We wanted to ascertain whether our experimental results can be adapted to a single set of such constants. A lack of fit would mean that the model does not describe the entire situation, thus requiring the postulation of additional metal-binding activator sites.

The overall rate equation which can be deduced from the definitions given in Fig. 1 is presented here in a particularly useful form, equivalent to Eqns. 14

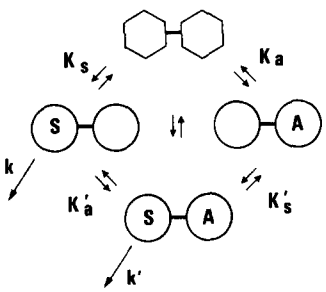

Fig. 1. Schematic representation of the allosteric two-site model for mixed K-type plus V-type activation (or inhibition) kinetics in brush-border sucrase.

The left and right hexagons represent the empty enzyme sites in the sucrase monomer: they are specific, respectively, for the substrate, S, or for the allosteric modifier, A. Each of these two species can bind independently. Their binding induces a conformational change or allosteric transition in the enzyme, represented by the transformation of the hexagons into circles. K'_S, K_S, K_a and K'_a indicate the dissociation constants characteristic of each partial reaction. Ignoring the unlikely triple collision (S + E + A $\rightleftharpoons$ S-E-A), indicated by the vertical arrows, the formation of the ternary complex, S-E-A, can occur through either one of two different pathways with identical overall probability, involving either one of the two possible binary complexes, S-E or E-A. In the absence of additional restrictions, therefore, the model is by definition random-ordered or non-compulsory. k' and k are rate constants governing the two possible product-forming reactions, the overall reaction rate being: $v = k(\text{S-E}) + k'(\text{S-E-A})$. If both k and k' are significantly greater than zero, activation (or inhibition) by A is by definition non-obligatory.

and 15 in Ref. 4, to which we refer:

$$v = \frac{V}{\left(1 + \frac{K_s}{[S]}\left(\frac{K_a + [A]}{K_a + [A]R}\right)\right)\left(\frac{K_a + [A]R}{K_a + [A]RQ}\right)} \quad (1)$$

where R is a constant relating the four dissociation constants:

$$R = K_s/K'_s = K_a/K'_a \quad (2)$$

and Q is another constant relating the two rate coefficients:

$$Q = K'/K \quad (3)$$

Since the absolute values of these two rate coefficients are unknown, Eqn. 3 can be given in another, more useful form:

$$Q = k'/k = V'/V \quad (4)$$

in which Q relates the two operational coefficients, V and V', where $V = k\ (E_t)$, the limiting value of the apparent V in the absence of A, and $V' = k'\ (E_t)$, the limiting value of the apparent V at saturating concentrations of A (E_t being the total amount of enzyme available: see Ref. 4 for further details).

Eqn. 1 is useful because it shows that K-type and V-type effects, even though resulting from the interaction of the same modifier molecule with a single metal-binding site, do vary and can be studied independently. In effect, Eqn. 1 can be regarded as a form of the classical Michaelis-Menten equation where each kinetic parameter, K_m and V, is affected independently by the binding of the allosteric modifier, A, to a site close to, but separate from, the substrate-binding site. The first and most obvious consequence of this relationship is that in the absence of A, Eqn. 1 reverts to the simple Michaelis equation, which is another way of saying that A is a non-obligatory activator (or inhibitor). Brush-border sucrase is therefore different to many other enzymes where the alkali metals act as obligatory activators (e.g., pyruvate kinase [9]).

Thus, we analyzed the rabbit sucrase data in Table I, firstly considering the effects of the alkali metals separately on either K_m or V, according to the definitions contained in Eqn. 1. Later, we re-analyzed the same data according to the whole of Eqn. 1 by iterating the entire set of experimental data and looking for the best fit to the K and V components of this equation.

Affinity effects

From Eqn. 1 we can extract the expression defining the change in apparent K_m as a function of [A]:

$$K_m^{app} = K_s\left(\frac{K_a + [A]}{K_a + [A]R}\right) \quad (5)$$

This is a rectangular hyperbola with a positive intercept which, for our particular case of activation kinetics, is concave upwards (see Fig. 2). The apparent K_m varies between two limits: K_s when [A] = 0 and K'_s when [A] = ∞. A method for solving Eqn. 5 has been given elsewhere and need not be repeated here. This method involves the use of a linear transformation of the type:

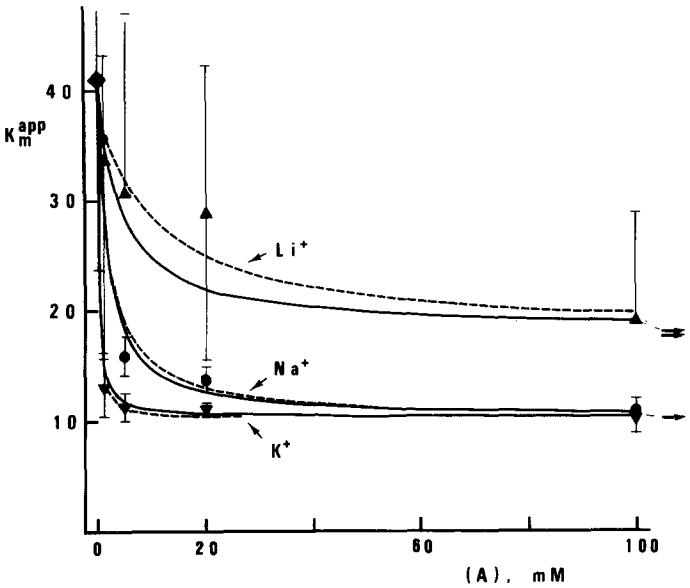

Fig. 2. Variation of the apparent K_m as a function of the alkali metal concentration in *tert*-butylamine buffers, pH 5.2. Data from Table I, where [A] is the activator: Li^+ (▲), Na^+ (●) or K^+ (▼). Vertical bars indicate standard errors. The theoretical curves shown were computed using Eqn. 5 and the constants listed in Table III (solid lines, Method 1a, dotted lines, Method 2), as explained in the text. Arrows indicate the limiting values of K_m (K'_s) as [A] approaches ∞.

$[A]/\Delta K = f[A]$ (where $\Delta K = K_s - K_m$), from which all the constants contained in Eqn. 2 can be computed (see Ref. 4 for details). An application of this treatment to the activation of a membrane-bound rabbit brush-border sucrase preparation in lithium buffer (pH 5.2) has already been illustrated (Fig. 1 in Ref. 2). We obtained a similar straight line from the purified sucrase data in Table I. The quantitative results, listed in Table II, demonstrate that essentially identical values are obtained for the dissociation constants in Eqn. 2, with either the membrane-bound or the solubilized sucrase preparations, thus confirming that these two forms of the enzyme are kinetically indistinguishable [2].

For the data obtained in *tert*-butylamine buffer (Table I), we also computed

TABLE II

KINETIC CONSTANTS INVOLVED IN THE AFFINITY-TYPE ACTIVATION OF RABBIT BRUSH-BORDER SUCRASE BY THE SODIUM ION IN LITHIUM BUFFERS AT pH 5.2

Dissociation constans (mM) were calculated by applying the $[A]/\Delta K = f[A]$ transformation [4] to the lithium buffer data in Table I (maleate buffer with 22.4 mM Li^+). The membrane-bound data come from Ref. 2 and were obtained using a succinic acid-glycine buffer with 20 mM Li^+. Soluble means the solubilized, then purified sucrase-isomaltase complex.

Sucrase preparation	K_s	K'_s	K_a	K'_a	R
Membrane-bound	26.3	8.6	3.9	1.3	3.1
Soluble	23.8	7.1	3.4	1.0	3.4

TABLE III

KINETIC CONSTANTS INVOLVED IN THE MIXED-TYPE ACTIVATION OR RABBIT BRUSH-BORDER SUCRASE BY THE ALKALI METAL IONS IN *tert*-BUTYLAMINE BUFFERS, pH 5.2

The constants were calculated from the data in Table I, using either linear transformations that permit separate calculations of the affinity-type (Method 1a) and the capacity-type (Method 1b) components, or by iteration of the overall results to fit Eqn. 1 (Method 2). This last procedure permits calculation of standard errors (±S.E.), which are given in parentheses.

Activator	Method	K_s	K'_s	K_a	K'_a	R	V	V'	Q
Sodium	1a	41.0	10.2	7.03	1.75	4.02	—	—	—
	1b	—	—	—	3.07	—	4.50	11.19	2.49
	2	39.07	10.09	8.92	2.30	3.87	4.45	11.00	2.47
		(9.73)	(3.30)	(1.70)	(0.47)	(0.56)	(0.67)	(0.53)	(0.43)
Potassium	1a	41.0	10.3	0.98	0.25	3.97	—	—	—
	1b	—	—	—	2.71	—	4.50	8.49	1.86
	2	36.17	10.22	0.76	0.21	3.54	4.18	7.77	1.86
		(13.04)	(4.25)	(0.24)	(0.07)	(0.51)	(0.89)	(0.38)	(0.43)
Lithium	1a	41.0	18.1	9.42	4.16	2.26	—	—	—
	1b	—	—	—	24.52	—	4.50	6.42	1.43
	2	38.34	17.63	23.94	11.01	2.17	4.18	6.37	1.53
		(14.85)	(11.30)	(12.06)	(5.64)	(0.86)	(0.98)	(1.11)	(0.51)

the complete solution of Eqns. 2 and 5 using the transformation $[A]/\Delta K = f[A]$: the results are listed in Table III (Method 1a).

Using the constants in Table III, we constructed theoretical curves to fit the data in Table I to Eqn. 5 (solid lines, Fig. 2). The reasonable agreement between experiment and theory supports the conclusion that the affinity-type activating effects of the alkali metal ions on rabbit sucrase at pH 5.2 involve a single metal-binding site, according to the model illustrated in Fig. 1.

The apparent value of K_s, obtained using the lithium buffer (approx. 24 mM, Table II), is considerably lower than that found using the *tert*-butylamine buffer (41 mM, Table III). This agrees fully with the proposition that Li^+ is not inert but behaves as an activator of rabbit sucrase [2]: i.e., the apparent K_s found using the lithium buffer corresponds to a point down the hyperbola relating the apparent K_m to the metal ion concentration (Eqn. 5 and Fig. 2). Accordingly, Na^+ induces a 4-fold decrease in K_m in the presence of the *tert*-butylamine buffer (a 400% affinity-type activation effect) but only a 3-fold decrease when the lithium buffer was used.

In theory, the lower limit of the apparent K_m (given by K'_s, Tables II and III, or indicated by the arrows, Fig. 2) should be the same regardless of the activator and buffer used. This expectation was confirmed for both Na^+ and K^+ in *tert*-butylamine buffer. However, the results reveal an anomaly: in the *tert*-butylamine buffer, Li^+ causes K'_s to attain a much higher level (approx. 18 mM) than that given by either Na^+ or K^+ (10 mM). Possible reasons for this result will be discussed later in this paper.

Capacity effects

It is striking that no activating V-effect of Na^+ at pH 5.2 occurs in lithium buffer but that it does in *tert*-butylamine buffer. However, in both cases an

identical limiting value of $V = 11$ is attained (Table I).

Interpreting capacity effects is always difficult because at least two entirely different mechanisms may coexist: (1) the metal may participate directly in the catalysis or at least act as a bridge between the substrate and certain groups in the active center of the enzyme, (2) the metal may have a true allosteric effect and exert its action through specific sites separate from the active center. Brush-border sucrase is active in metal-free buffers and after exhaustive dialysis against EDTA in the pH range 5.2—8.3 [2]. This observation strongly suggests that the alkali metal ions are non-obligatory activators and justifies our using the working hypothesis that true allosteric mechanisms are involved in sucrase activation. The problem is then simplified as to whether the allosteric site responsible for the observed V effects is the same as that implicated in the affinity effects described in the preceding section.

According to our model, allosteric capacity effects may vary independently of any possible simultaneous effects on K_m. From Eqn. 1 we define the apparent V:

$$V^{app} = V\left(\frac{K_a + [A]RQ}{K_a + [A]R}\right) \tag{6}$$

which shows this capacity parameter to be a hyperbolic function of [A] and also to be affected by the values of constants, K_a, R and Q. Interestingly enough, the affinity parameter described above (see Eqn. 5) is not affected by Q, whereas the capacity parameter (Eqn. 6) is affected by R. This is because R defines reversible interactions between the enzyme, S and A, which are independent of any possible ensuing catalysis. On the contrary, catalysis, which is governed by the rate coefficients, k and k', cannot take place in the absence of S binding, hence the presence of R in Eqn. 6. Nevertheless, R can be eliminated using Eqn. 2, thus yielding:

$$V^{app} = V\left(\frac{K'_a + [A]Q}{K'_a + [A]}\right) \tag{7}$$

It seems clear that capacity effects are influenced only by Q and by the value of K'_a, the dissociation constant for the interaction between A and E-S complex (Fig. 1). When there are no concomitant K-type effects (pure V-type activation), then $K'_a = K_a$, a simplification that in no way affects the above equation.

Eqn. 7 gives a rectangular hyperbola with a positive intercept which, for the case of activation applying to rabbit sucrase, is concave downwards (see Fig. 4). The equation contains three unknown constants and, therefore, has no simple solution. However, by analogy with the ΔK treatment mentioned above, it can be solved by introducing the kinetic device, ΔV, which is defined as the change in V^{app} that takes place when a given concentration of the activator is added, such that $\Delta V = V^{app} - V$. By substituting V^{app} (Eqn. 7), we obtain a hyperbola passing through the origin:

$$\Delta V = \frac{(V' - V)}{1 + \frac{K'_a}{A}} \tag{8}$$

This equation can be solved readily, e.g. using a linear transformation of the

Woolf [7] type:

$$\frac{[A]}{\Delta V} = \frac{K'_a}{(V' - V)} + \frac{1}{(V' - V)}[A] \quad (9)$$

from which V' and K'_a can be derived.

This means that plots of $[A]/\Delta V = f[A]$ should give straight lines if the model in Fig. 1 applies. This prediction was confirmed by plotting the data in Table I according to Eqn. 9, as illustrated for Na^+ and K^+ in Fig. 3. A similar analysis was also applied for Li^+ (not illustrated). Although the results with Li^+ are not as good, and the point at the lowest concentration used does not fall on the curve, the results obtained with this ion are also compatible with the same interpretation. From these linear transformations (see legend of Fig. 3) the relevant kinetic constants were calculated (listed in Table III, Method 1b). Furthermore, using these constants and Eqn. 7, we calculated the theoretical fit for the V data in Table I (solid lines, Fig. 4). Again, the agreement between experiment and theory seems reasonable, upholding the validity of the kinetic analysis used. Therefore, the capacity-type activating effects of the alkali metals on rabbit brush-border sucrase at pH 5.2 conform to the model in Fig. 1 and involve a single metal-binding site. The next question, then, was: are the sites involved in capacity-type activation coincident with those involved in affinity-type activation, as defined in the preceding section?

If we consider the values of K'_a calculated from the V data according to the procedure described in this section (Method 1b, Table III), we see that they disagree with the K'_a obtained from the K_m data (Method 1a, Table III). For Na^+ the error is relatively small: K'_a changes from 3.07 to 1.75, representing only a 1.7-fold difference. However, for Li^+ and K^+ the K'_a data differ by 6- and

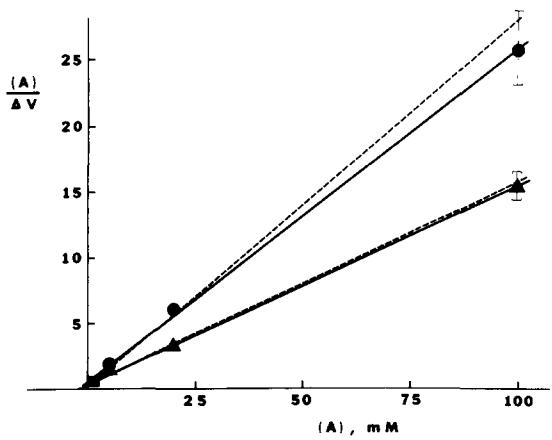

Fig. 3. Use of the ΔV transformation method to solve Eqn. 7, the variation of apparent V as a function of activator concentration. Solid lines, direct application of Eqn. 9 to the V^{app} data in Table I, giving: for Na^+ (▲), $y = 0.458 + 0.149\ x$; for K^+ (●), $y = 0.680 + 0.251\ x$ (both lines yielding linear correlation coefficients equal to or greater than 0.999). Using the intercept and slope of these lines and applying the equations as shown in text, we computed the constants listed in Table III (Method 1b). Dotted lines, here, we used the inverse procedure of calculating the intercept and slope for Eqn. 9, starting with the kinetic constants obtained by iteration of Eqn. 1 (listed in Table III, Method 2). The resulting lines are: for Na^+, $y = 0.351 + 0.153\ x$; for K^+, $y = 0.058 + 0.279\ x$.

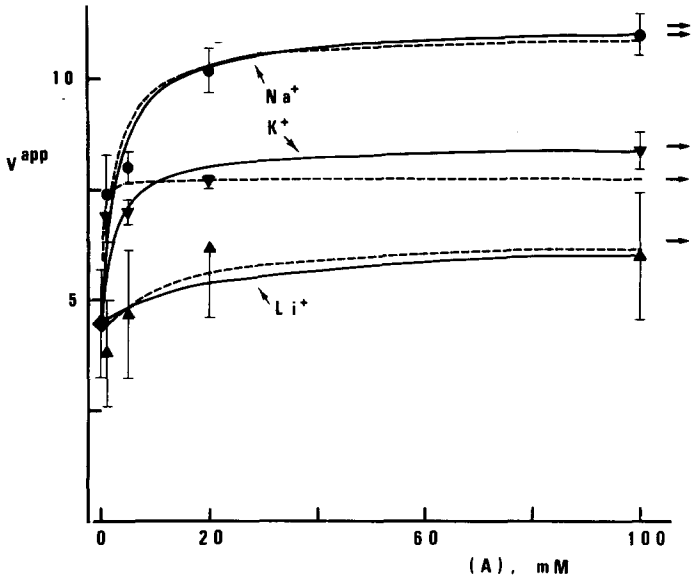

Fig. 4. Variation of the apparent V as a function of the alkali metal concentration in metal-free buffers, pH 5.2. Data from Table I, where (A) indicates the activator: Li^+ (▲), Na^+ (●) or K^+ (▼). Vertical bars indicate standard errors. The theoretical curves were computed using Eqn. 7 and the constants listed in Table III (solid lines, Method 1b; dotted lines, Method 2), as explained in the text. Arrows show the limiting values of V^{app} (V') at (A) = ∞.

10-fold, respectively. Since identical K'_a values should be obtained from either method if the single metal-binding site in Fig. 1 holds true, a 10-fold difference for K^+ might be considered as proof that more than one metal-binding activator site is involved and, therefore, that Eqn. 1 does not apply.

However, experimental data are never free of errors. The linear transformations described above depend heavily on the values calculated for either K_s or V, the kinetic parameters observed in the absence of A. But by definition, these values must be obtained from experiments performed in the absence of activation, i.e., under the most unfavorable conditions. Therefore, experimental errors will most strongly affect those kinetic parameters used as the basis for calculating other parameters: here resides one weakness of the method.

Nevertheless, we should not attach too much importance to the lack of consistency of a single constant (K'_a) in the set of six constants constituting the model. To compensate for this inconsistency, we recalculated the data as a whole, to see whether a single set of six constants could be found, capable of fitting the entire data simultaneously to Eqns. 5 and 7.

Overall treatment of the results

Following the guidelines given by Cleland [10], we prepared a computer program (available on request from C. Tellier) to calculate the best simultaneous fit to Eqns. 5 and 7 of the entire set of experimental data obtained using the *tert*-butylamine buffer. The program involves two steps. Firstly, it fits each set of curves at constant [A] to a hyperbola according to the $v = f[S]$ Michaelis equation [10]. The V and K_m data thus obtained did not significantly differ

from those calculated from linear transformations and listed in Table I. However, this time we calculated the standard errors that are incorporated into Figs. 2 and 4. Secondly, still using the entire body of V, [S] and [A] data, we calculated the overall fit to Eqns. 5 and 7 by iteration. The new set of kinetic constants thus calculated is listed in Table III (Method 2). The kinetic constants calculated in this way do not differ greatly from those found using Methods 1a and 1b. However, certain constants, particularly K'_a, are noticeably different but fall between the values calculated using Methods 1a and 1b, e.g., for Na^+ and Li^+. With K^+, however, the data approach the value obtained using Method 1a: K'_a is 0.25 and 0.21 for Methods 1a and 2, respectively, contrasting with the value of 2.71 from Method 1b. Nevertheless, all the differences between constants can be accommodated reasonably well if we take into account the fluctuation of the data, indicated by the standard errors shown in Figs. 2—4. Thus, for instance, for K^+ we see that a plot of $[K^+]/\Delta V = f[K^+]$, calculated using the values supplied by Method 2, gives a straight line that separates appreciably from that obtained using Method 1b (see Fig. 3). But, both lines fall within the limits of error of the data and therefore cannot be considered as significantly different. The reason why K^+ gives greater errors than Na^+ may have to do, at least in part, with the exceedingly high affinity of the former ion for the metal-binding site. In effect, for K^+ the relevant dissociation constants are: $K_a = 0.76$ and $K'_a = 0.21$, which are both smaller than the lowest K^+ concentration used to build the metal-saturation curves shown in Figs. 2 and 4.

Using the data obtained with Method 2, we calculated theoretical curves to fit Eqns. 5 and 7 (dotted lines, Figs. 2 and 4, respectively). The fit of the data using these theoretical curves is somewhat different to that obtained using Method 1a and b, but nearly all curves fall within the limits set by the standard errors calculated for these data. The greatest difference is obtained, once again, with K^+ (V^{app} curve, Fig. 4), where Methods 1b and 2 gave different weighting to the points in the curve. However, the discrepancies observed using these two procedures are not large enough to justify any conclusion other than that, even for K^+, one single metal-binding activator site is involved simultaneously in the observed mixed activations of brush-border sucrase by the alkali metals at pH 5.2. Therefore, we can tentatively conclude that the model illustrated in Fig. 1 is sufficient to explain the results and that a single metal-binding site is responsible for the mixed activating effects observed at pH 5.2.

Concluding remarks

The results with rabbit sucrase described here reveal similarities to and differences from those obtained using guinea-pig sucrase [5]. In the rabbit, Li^+ acts as an activator, whereas in the guinea-pig, it is essentially inert. In the guinea-pig the alkali metals behave as essentially pure affinity-type activators, but in the rabbit they have mixed effects implicating both K_m and V. Nevertheless, in both species all results, thus far available, can be explained by the existence of a single metal-binding activator site, as shown in Fig. 1. However, some of our present and previous results [2] indicate additional complications which have not yet been explained but which suggest a role for other monovalent cations, e.g., the *tert*-butylammonium ion. More work is needed (and is in fact under

way in our laboratory) to explain the following observations.

For affinity-type effects, both Na^+ and K^+ give the same limiting value of K'_s (approx. 10 mM), whereas Li^+ gives K'_s = 18 mM, suggesting that the latter ion is less effective as an activator, or that *tert*-butylamine somehow interferes with Li^+ activation. Affinity-type activation of sucrase by the alkali metals has been shown to be linked to the metal-induced deprotonation of the enzyme below pH 7: here, the deprotonated enzyme has its maximal affinity for the substrate since it has attained its lowest apparent K_m value, K'_s [2,11]. But, we still need to explain why this K'_s value is not reached in the presence of Li^+ and *tert*-butylamine (in fact, experiments now in progress indicate that in the absence of *tert*-butylamine, Li^+ gives the same limiting value for K'_s as that reached in the presence of either Na^+ or K^+ (unpublished data)). A similar anomaly exists for guinea-pig sucrase. Here, both Na^+ and K^+ behave as affinity-type activators. However, even though K^+ exhibits an affinity for the metal-binding site approx. 20 times greater than that of Na^+ (K_a is 0.08 and 1.75 mM for K^+ and Na^+, respectively [5]), the limiting value of the apparent K_m is lower when Na^+ is used as the activator (K'_s is 6.7 and 4 mM for K^+ and Na^+, respectively [5]).

With regard to capacity-type activation effects, the efficiency of the alkali metals is unequal when a metal-free, *tert*-butylamine buffer is used, as indicated by the difference in the limiting values of the apparent V, namely: V' = 11, 7.8, and 6.4 for Na^+, K^+ and Li^+, respectively (see Table III). This could be explained by saying that each metal has a different effect on the rate coefficient, k', which may vary according to the ion present. Still another observation remains unexplained. When a lithium buffer is used (containing 20 mM Li^+ but no *tert*-butylamine), V appears to be at its maximum value (V' = 11, see Table I) and the addition of Na^+ does not change this value. Perhaps *tert*-butylamine has a non-competitive effect, leading to lower (apparent) V' values for Li^+ and K^+. However, if the effect of *tert*-butylamine is non-competitive, why can Na^+ overcome it? Further work will be required to ascertain whether additional sites for monovalent cations (including the *tert*-butylammonium ion) exist in brush-border sucrase.

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