Biochimica et Biophysica Acta 821 (1985) 377–383
Elsevier

BBA 72845

Direct evidence for an ADP-sensitive phosphointermediate of $(K^+ + H^+)$-ATPase

M L Helmich-de Jong, S E van Emst-de Vries, J J H H M De Pont, F M A H Schuurmans Stekhoven and S L Bonting

Department of Biochemistry, University of Nijmegen P O Box 9101 6500 HB Nijmegen (The Netherlands)

(Received June 19th 1985)

Key words $(K^+ + H^+)$-ATPase Phosphorylation, Dephosphorylation ADP sensitivity, (Gastric mucosa)

Direct evidence for the occurrence of an ADP-sensitive phosphoenzyme of $(K^+ + H^+)$-ATPase, the proton-pumping system of the gastric parietal cell is presented. The enzyme is phosphorylated with 5 μM [γ-^{32}P]ATP in 50 mM imidazole-HCl (pH 7.0) and in the presence of 7–15 μM Mg^{2+}. Addition of 5 mM ADP to this preparation greatly accelerates its hydrolysis We have been able to establish this by stopping the phosphorylation with radioactive ATP, by adding 1 mM non-radioactive ATP, which leads to a slow monoexponential process of dephosphorylation of ^{32}P-labeled enzyme. The relative proportion of the ADP-sensitive phosphoenzyme is 22% of the total phosphoenzyme Values for the rate constants of breakdown and interconversion of the two phosphoenzyme forms have been determined.

Introduction

The $(K^+ + H^+)$-ATPase system of the gastric parietal cell generates the acid secretion in the stomach by means of an electroneutral, transmembrane exchange transport of H^+ and K^+ [1] In many respects, the enzyme resembles the $(Na^+ + K^+)$-ATPase Na^+-transport system of the animal cell membrane In one respect there was still a discrepancy direct evidence was lacking for the existence of two forms of the phosphoenzyme intermediate of $(K^+ + H^+)$-ATPase, an ADP-sensitive, K^+-insensitive one (E_1P), and an ADP-insensitive, K^+-sensitive one (E_2P) There were only suggestive findings (1) the occurrence of a K^+-insensitive fraction of the phosphoenzyme [2], (2) the occurrence of an ATP-ADP exchange reaction [3,4], (3) the formation of phosphoenzyme from the DTNB modified enzyme which is less sensitive to K^+ [5] and more sensitive to ADP [3] than the normal phosphoenzyme

In a study of the effects of Mg^{2+} on the mechanism of the enzyme, we have obtained direct evidence for the occurrence of an ADP-sensitive phosphoenzyme Subsequently, we have determined the rate constants for interconversion and breakdown of the two phosphointermediates by means of a model, employed by Klodos et al [6] for $(Na^+ + K^+)$-ATPase, which described the time dependence of E_1P and E_2P

Materials and Methods

Isolation of a membranous $(K^+ + H^+)$-ATPase preparation $(K^+ + H^+)$-ATPase is purified from porcine gastric mucosa according to Schrijen et al [7] and is stored at −20°C in 0 25 M sucrose The specific activity of the preparations ranges from 70 to 110 μmol per mg protein per h The steady-state level of phosphorylation with 5 μM ATP ranges

Abbreviations Pipes, 1 4-piperazinediethanesulfonic acid CDTA *trans*-1 2-diaminocyclohexane tetraacetic acid

from 1.0 to 1.4 nmol $^{32}P_i$ incorporated per mg protein. This level is not further increased when the ATP concentration is raised to 50 μM, suggesting that 5 μM is a saturating concentration to get maximal phosphorylation.

Formation and determination of the phosphoenzymes. Phosphorylation with ATP is carried out by adding 50 μl membrane suspension (10 μg protein) to 50 μl phosphorylation medium. The final mixture contains 50 mM imidazole-HCl (pH 7.0), 7–15 μM Mg^{2+} (including 5 μM added Mg^{2+}) and 5 μM [γ-^{32}P]ATP. After 10 s at 20°C the reaction is stopped by adding 5 ml 5% trichloroacetic acid/0.1 M H_3PO_4. Further processing is carried out as described by Schuurmans Stekhoven et al. [8].

The steady-state phosphorylation level is reached within 3 s and remains constant for at least 30 s. The endogenous concentration of Mg^{2+} in the enzyme preparation (20–100 nmol per mg protein) is sufficient to obtain maximal phosphorylation with 5 μM ATP, but we added routinely 5 μM Mg^{2+} to the incubation mixture. Dephosphorylation of the ^{32}P-labeled enzyme preparation is initiated by adding 11 μl of a non radioactive 10 mM ATP solution to the phosphorylation mixture. The residual amount of radioactive phosphoenzyme is determined in all cases by adding trichloroacetic acid as described above. Other conditions are mentioned in the text, with the final concentrations indicated. Phosphorylation blanks are prepared by adding the enzyme to the stopping reagent, after which the phosphorylation medium is added. The steady-state level of phosphorylation that is reached after dephosphorylation is determined as follows: enzyme is added to a mixture of 50 μl phosphorylation medium and 10 μl dephosphorylation medium. After 20 s reaction the phosphorylation is terminated as described above.

Reagents. [γ-^{32}P]ATP (specific activity 3 Ci per mmol) is purchased from the Radiochemical Centre (Amersham, U.K.). ATP and ADP are purchased from Boehringer (Mannheim, F.R.G.). All other chemicals are of reagent grade. The maximal concentrations of contaminating ADP and of K^+ during dephosphorylation are 7 μM and 5 μM respectively.

Kinetic calculations. Rate constants and concentrations of the phosphoenzymes are calculated from the experimental data by means of a mathematic model for the dephosphorylation of the $(Na^+ + K^+)$-ATPase, elaborated by Klodos et al. [6]. The model is described by the following scheme for dephosphorylation and interconversion of the two phosphoenzymes.

$$\overset{k_{-1}}{\leftarrow} E_1P \underset{k}{\overset{k_2}{\rightleftharpoons}} E_2P \overset{k_3}{\rightarrow}$$

Scheme 1

In the presence of ADP, k_{-1} would be increased, whereas K^+ would lead to enhancement of k_3. The scheme assumes that there is no phosphorylation from P_i. At a K_m value of 60 μM for P_i in the presence of 1 mM Mg^{2+} [9] and an ATP concentration of only 5 μM, phosphorylation by P_i could not occur to a significant degree. The rate of formation of EP from [γ-^{32}P]ATP after dilution with ATP is not zero, but Klodos et al. [6] show that this does not prohibit the application of the model.

The breakdown of phosphorylated intermediate can be followed as a function of time. Taking the symbols as used by Klodos et al. [6], the breakdown and interconversion of E_1P and E_2P can be described as

$$[EP] = [E_1P] + [E_2P] = H\ e^{-\alpha t} + G\ e^{-\beta t}$$

H, G, α and β are experimental parameters describing the observed biphasic time-dependence of [EP], H and α the slow phase of dephosphorylation and G and β the fast phase. The four parameters correlate in a certain way with the kinetic constants k_{-1}, k_2, k_{-2}, k_3, and the phosphoenzyme concentrations $[E_1P]_o$ and $[E_2P]_o$ (see Appendix). Only under special conditions are H and G equal to $[E_1P]_o$ and $[E_2P]_o$. [EP] is defined as the total concentration of acid-stable radioactive phosphoenzyme, corrected for the blank. The sum of G and H is equal to $[EP]_o$. Since in our definition $\alpha < \beta$ (see Appendix), H can be obtained as the intercept on the ordinate, by extrapolating the slow phase of dephosphorylation to $t = 0$. In order to make H and G dimensionless, the relative phosphoenzyme concentration $[EP]_{rel} = [EP]/[EP]_o$ is used. When the decomposition of phosphoenzyme is monoexponential, H is equal to 1 and G is zero.

Results

Termination of phosphorylation

Exact measurement of the dephosphorylation rate requires fast and complete termination of phosphorylation, either by removing Mg^{2+} through chelation with EDTA or CDTA [2], or by diluting the remaining radioactive ATP with an excess of non-radioactive ATP [10] Dephosphorylation can then be studied by measuring the amount of remaining radioactive phosphoenzyme at various times In the present experiment we have studied the dephosphorylation after addition of 1 mM ATP, 1 mM ATP + 5 mM ADP, 10 mM CDTA, and 10 mM CDTA + 5 mM ADP

Effect of ADP on dephosphorylation

Fig 1 shows our initial observation of a stimulating effect of ADP on dephosphorylation In this experiment phosphoenzyme is generated at a micromolar Mg^{2+} concentration When the Mg^{2+} concentration is kept low during subsequent dephosphorylation and this process is initiated by the addition of 1 mM non-radioactive ATP, then the presence of 5 mM ADP clearly stimulates the dephosphorylation process (Fig 1) This is the first direct evidence of the existence of an ADP-sensitive phosphoenzyme of $(K^+ + H^+)$-ATPase

This observation has led us to investigate in more detail the kinetics of the dephosphorylation process in the absence and presence of ADP (Fig 2) This has permitted us to calculate the relative concentrations of the two phosphoenzyme forms ($[E_1P]$ and $[E_2P]$) and the reaction rates for their dephosphorylation (k_{-1} and k_3) and their interconversion (k_2 and k_{-2}) Furthermore we have

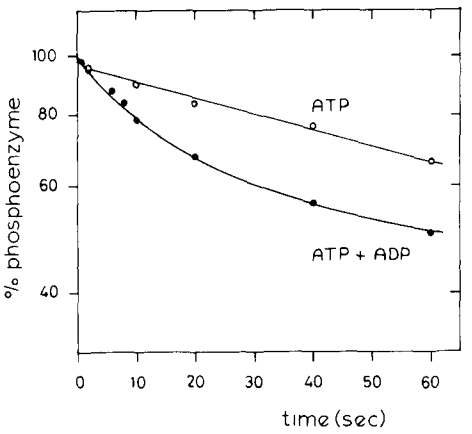


Fig 1 Effects of ATP and ADP on the phosphorylation rate Steady-state phosphorylation levels are generated by treating the enzyme preparation for 10 s at 20°C in a medium containing 5 μM Mg^{2+}, 5 μM [γ-^{32}P]ATP and 50 mM imidazole-HCl (pH 7 0) Dephosphorylation is started by addition of 1 mM ATP (○——○) or 1 mM ATP plus 5 mM ADP (●——●) After the desired length of time (2–60 s) dephosphorylation is terminated by the addition of the stopping solution Typical experiment out of three

tried to elucidate why other investigators have not been able to observe an effect of ADP on dephosphorylation

Dephosphorylation after addition of excess unlabelled ATP

Addition of 1 mM ATP (final concentration) decreases the specific radioactivity of ATP by a factor 200 The new steady-state level of radioactive phosphoenzyme, normally reached after approx 15 min (Fig 2), is approx 0 5% of that before ATP addition and is indistinguishable from that of the blanks prepared with acid-denatured enzyme

TABLE I

PARAMETERS FOR THE SLOW MONOEXPONENTIAL DEPHOSPHORYLATION OF THE PHOSPHOENZYME

Average values for the ordinate intercept H_{rel}, the rate constants α, and the calculated value of G_{rel} are derived as shown in Fig 2 by means of the equation $[EP]_{rel} = H_{rel}\ e^{-\alpha t}$ Average values with S E and number of experiments (n) between parentheses are given

	H_{rel}	α (min^{-1})	G_{rel}
1 mM ATP	0 94 ± 0 006 (5)	0 35 ± 0 006 (5)	–
1 mM ATP + 1 mM EDTA	0 94 ± 0 01 (4)	0 40 ± 0 07 (4)	–
1 mM ATP + 5 mM ADP	0 78 ± 0 005 (3)	0 36 ± 0 003 (3)	0 22 ± 0 005 (3)

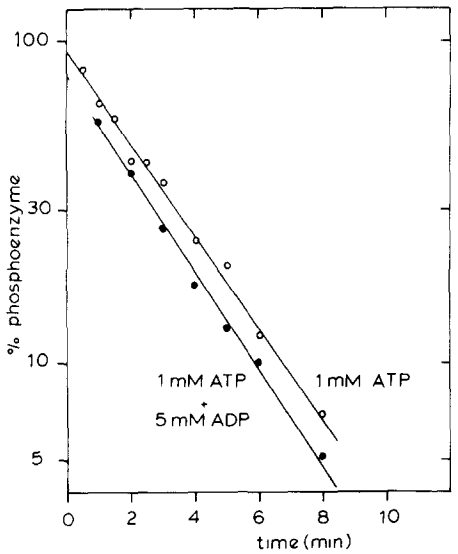


Fig 2 Effects of ATP and ADP on the dephosphorylation rate Steady-state phosphorylation levels are generated as described in the legend of Fig 1 Dephosphorylation is started by addition of 1 mM ATP (○——○) or 1 mM ATP plus 5 mM ADP (●——●) After the desired length of time (0 5–8 min) dephosphorylation is terminated by the addition of stopping solution Typical experiment out of three Lines are calculated by means of unweighted linear regression analysis

Fig 2 shows a representative experiment, in which 1 mM ATP is added to initiate the dephosphorylation process After a small initial decrease of about 6% (see also Fig 1), $[EP]_{rel}$ declines in a monoexponential way according to the function $[EP]_{rel} = H\ e^{-\alpha t}$ We calculate average values of $\alpha = 0\ 35\ min^{-1}$ (S E = 0 006, $n = 5$) and $H_{rel} = 0\ 94$ (S E = 0 006, $n = 5$) The same kinetic behaviour is observed when 1 mM ATP plus 1 mM EDTA are added (Table I) A monoexponential dephosphorylation curve is compatible with the model in Scheme 1 when $k_{-1} = k_3$ [6] The model is then equivalent to a one-pool model with a net rate constant $\alpha = k_3$, irrespective of the proportions of $[E_1P]$ and $[E_2P]$ Under these conditions the dephosphorylation of the two phosphoenzymes would be equal As $H_{rel} = 0\ 94$, the model is not exactly a one-pool model and therefore k_{-1} and k_3 are not exactly equal

Dephosphorylation after addition of ATP + ADP

When 5 mM ADP is included in the dephosphorylation mixture, a biphasic dephosphorylation curve is obtained (Fig 1) There is now a much larger initial rapid decrease in $[EP]_{rel}$, followed by a slow further decrease This indicates that part of the phosphoenzyme pool (approx 20%) is ADP-sensitive The slow phase in the decomposition of $[EP]_{rel}$ is nearly parallel to the dephosphorylation curve with 1 mM ATP alone Values of $H_{rel} = 0\ 78$ and of $\alpha = 0\ 36\ min^{-1}$ are obtained (Table I) Due to the small quantity of E_1P, it is not possible to establish unequivocally whether the initial fast drop in $[EP]_{rel}$, calculated as $G_{rel} = 1 - H_{rel}$, is a function of the ADP concentration The concentration of 5 mM ADP appears to be saturating, since increasing it to 10 mM does not change the values for H_{rel} and α This is understandable as the K_m for ADP in the ATP-ADP exchange reaction is 0 55 mM [4]

Estimation of the rate constants

In the absence of ADP, $[EP]_{rel}$ is a monoexponential function of the reaction time virtually from $t = 0$ (Fig 1), i e H_{rel} is close to 1 (Table I) When ADP is added, the dephosphorylation curve becomes biphasic, and H_{rel} decreases from 0 94 to 0 78 (Table I) Addition of ADP increases the rate of the exchange reaction, hence will increase k_{-1} An analysis of the expression for H (see Appendix) shows that H_{rel} always decreases with increasing k_{-1} and that dH_{rel}/dk_{-1} approaches zero when $k_{-1} \to \infty$ With increasing k_{-1}, H_{rel} will approach a plateau value, which is equal to the initial concentration of the second phosphoenzyme $[E_2P]_{o\,rel}$, as has been pointed out by Klodos et al [6] As 5 mM ADP appears to be saturating, the value of 0 22 for G_{rel} (Table I) must be maximal

TABLE II

RATE CONSTANTS FOR THE PHOSPHOENZYME CONVERSIONS

The kinetic model and the methods used in the calculation are described in the text

Constant	Value (min^{-1})
k'_{-1}	0 35
k''_{-1}	–
k_2	1 28
k_{-2}	0 01
k_3	0 35

and equal to $[E_1P]_{o,rel}$ This gives us the value for the ratio $[E_1P]_{o\,rel}/[E_2P]_{o,rel} = (k_{-2} + k_3)/k_2 =$ 0 28 When k_{-1} is increased in the presence of ADP, α changes very little When k_{-1} is much larger than k_2, k_{-2} and k_{-3}, it is easily understandable that $\alpha = k_{-2} + k_3$

These considerations lead to the following values for α and the rate constants

(1) $\alpha = 0\,35$ min^{-1} in the dephosphorylation experiment with ATP alone (Fig 1) and is very close to k_3, as in this case $H_{rel} = 0\,94$, which is close to 1 [6]

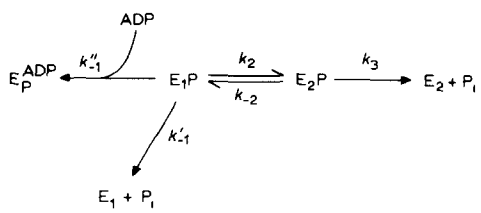
(2) From the paired difference between $\alpha = k_3$ and $\alpha_{max} = k_{-2} + k_3$ (dephosphorylation with ATP and ADP), a maximal value of $k_{-2} = 0\,01$ min^{-1} can be estimated

(3) k_2 can be calculated from the equation

$$\frac{[E_1P]_o}{[E_2P]_o} = \frac{k_{-2} + k_3}{k_2} = 0\,28, \text{ giving } k_2 = 1\,28\ min^{-1}$$

The values for the rate constants thus obtained are listed in Table II

In the experiments in which ATP and no ADP is added, there is a contaminating concentration of ADP and K^+ of 7 μM and 5 μM, respectively, which could explain the small initial drop in $[EP]_{rel}$ This suggests that like for $(Na^+ + K^+)$-ATPase [6], dephosphorylation occurs in two ways (a) spontaneous hydrolysis to E_1 and P_i and (b) ADP-dependent dephosphorylation resulting in ATP formation (Scheme 2) The relationship of the rate constants would then be $k_{-1} = k'_{-1} + k''_{-1}$ [ADP]



Scheme 2

Although in principle it should be possible to obtain a value for k''_{-1} by studying dephosphorylation in the presence of various ADP concentrations this proved technically difficult Hence, we can offer no proof that Scheme 2 is valid for $(K^+ + H^+)$-ATPase

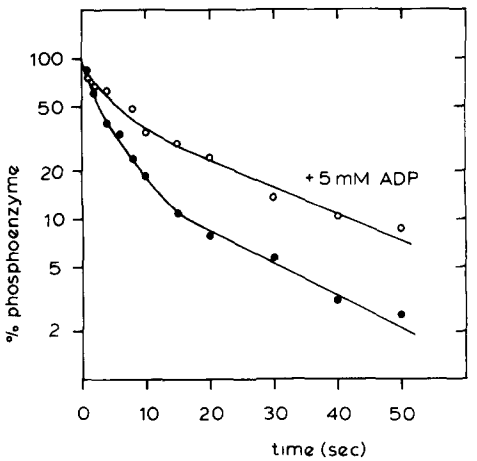


Fig 3 Effect of CDTA and ADP on the dephosphorylation rate at pH 7 5 Steady-state phosphorylation levels are generated by treated the enzyme preparation for 10 s in a medium containing 2 mM Mg^{2+}, 5 μM [γ-^{32}P]ATP and 40 mM Tris-HCl (pH 7 5) Dephosphorylation is started by addition of 10 mM CDTA (●———●) or 10 mM CDTA plus 5 mM ADP (○———○) After the desired length of time (2–50 s) dephosphorylation is terminated as described in the legend of Fig 1 Average values of three experiments are presented

Dephosphorylation after addition of CDTA

Other investigators were unable to observe an effect of ADP on the dephosphorylation of the $(K^+ + H^+)$-ATPase phosphoenzyme They used either a too low ADP concentration (0 3 mM, Ref 11), or they used another buffer (Tris-HCl or Tris-Pipes (pH 7 5)) and 1–2 mM Mg^{2+} and they terminated phosphorylation by addition of 10 mM CDTA [2,3,10] In repeating these experiments we confirm that under these conditions there is no stimulating effect of ADP on the initial phase of dephosphorylation

In Fig 3 we show an experiment in which dephosphorylation is studied using CDTA to stop phosphorylation The dephosphorylation curve is now biphasic with an initial fast phase (rate constant 14 min^{-1}) This rate constant for the fast phase, uncorrected for the subsequent slow phase corresponds to the value reported by Stewart et al [12], who do not mention the biphasic behaviour In the presence of CDTA and ADP, no significant stimulation is observed in the first 3 seconds of the dephosphorylation process In the slow phase of the dephosphorylation process we even observe an inhibition (Fig 3)

Discussion

We have been able to observe a stimulating effect of ADP on the dephosphorylation of $(K^+ + H^+)$-ATPase phosphoenzyme, which is direct evidence for the existence of an ADP-sensitive phosphoenzyme form (E_1P) However, this observation can only be made under certain conditions

When phosphorylation with [γ-^{32}P]ATP is stopped in imidazole (pH 7 0) by the addition of unlabeled ATP alone, the ensuing dephosphorylation is monoexponential We have concluded that this single-pool behaviour reflects a spontaneous dephosphorylation of the phosphoenzyme with the rate constant k_{-1} for dephosphorylation of E_1P being approximately equal to the rate constant k_3 for dephosphorylation of E_2P A monoexponential dephosphorylation would also be obtained, when there is a rapid shift in the equilibrium between E_1P and E_2P However, in that case addition of saturating ADP would dephosphorylate all EP immediately, which is clearly not the case here

After addition of ADP a fast initial decrease in $[EP]_{rel}$ is observed, followed by a slow phase which has about the same rate constant as in the absence of ADP (k'_{-1}) This leads us to the conclusion that $k_3 \gg k_{-2}$, i e the backward reaction $E_2P \rightarrow E_1P$ is very slow Although k_{-2} is small, the conversion of E_1P to E_2P cannot be negligible, when the synthesis of ATP from ADP and P_i occurs through this pathway Since we were unable to study the dependence of H_{rel} on the ADP concentration, we cannot exclude the possibility that the two intermediates are formed in parallel rather than in consecutive reactions, as assumed in the model However, dephosphorylation experiments in the presence of K^+ by Wallmark et al [2] indicate that the consecutive model is right

We have calculated values of 22% and 78% of the total phosphoenzyme for E_1P and E_2, respectively These values are also observed by us in dephosphorylation experiments in the presence of saturating K^+ (not shown in this paper), but are lower than the values reported by Wallmark et al [2] This discrepancy may be due to the way of determining the precentage of E_1P, which is done by extrapolating the slow phase of dephosphorylation to $t = 0$ This slow phase is not continued beyond 180 ms, which may be too short for a correct extrapolation Moreover, the difference may be due to different conditions during phosphorylation, since Wallmark et al [2] use millimolar Mg^{2+} during phosphorylation, which may have an effect on the ratio of E_1P and E_2P concentrations

Our findings are in contrast with results of other investigators [2,3,10,11] who found no stimulating effect by ADP on the dephosphorylation process However, they used other circumstances to study the dephosphorylation process When dephosphorylation is initiated with CDTA, we found even an inhibitory effect of ADP

It cannot easily be explained why the stimulatory effect of ADP is only observed under special circumstances One speculative explanation is that, as for $(Na^+ + K^+)$-ATPase, nucleotides have effects on the dephosphorylation process through low-affinity binding [13,14] In that way inhibitory nucleotide effects might obscure stimulatory effects of ADP

In summary we may state that we have been able to show direct evidence for an ADP-sensitive phosphoenzyme form of the $(K^+ + H^+)$-ATPase We only observe this under special conditions where the dephosphorylation process is a slow and monoexponential process This occurs in the presence of millimolar ATP and micromolar Mg^{2+} concentrations

Appendix

The following equations, defining the parameters for the dephosphorylation of E_1P and E_2P are derived from Klodos et al [6]

$$H = \frac{(h - u + 2k_2)\ [E_1P]_o + (h + u + 2k_{-2})\ [E_2P]_o}{2h} \quad \text{(A-1)}$$

$$G = \frac{(h + u - 2k_2)\ [E_1P]_o + (h - u - 2k_{-2})\ [E_2P]_o}{2h} \quad \text{(A-2)}$$

$$u = (k_{-1} + k_2) + (k_{-2} + k_3) \quad \text{(A-3)}$$

$$h = \sqrt{u^2 + 4k_2k_{-2}} \quad \text{(A-4)}$$

$$\alpha = \tfrac{1}{2}(k_{-1} + k_2 + k_{-2} + k_3 - h) \quad \text{(A-5)}$$

$$\beta = \tfrac{1}{2}(k_{-1} + k_2 + k_{-2} + k_3 + h) \Rightarrow \beta > \alpha \quad \text{(A-6)}$$

In addition we have derived from Eqns A-1, A-3 and A-4 an expression for dH_{rel}/dk_{-1},

$$H' = \frac{dH_{rel}}{dk_{-1}} = -\frac{k_2(k_3 u + 2k_{-1}k_{-2})}{k_3(k_2 + k_{-2} + k_3)} \qquad \text{(A-7)}$$

H_{rel} will decrease with increasing k_{-1}, when H' is negative, which is the case when the numerator is positive The numerator is $k_2[k_{-1}(k_3 + 2k_{-2}) + k_3(k_2 - (k_{-2} + k_3)]$ and is positive when $k_{-1} \geqslant k_3$ when $[E_1P]_o < [E_2P]_o$, as is the case in Figs 1 and 2, then $(k_{-2} + k_3) < k_2$ and thus the numerator is positive for all k_{-1} values

Acknowledgement

This investigation was financially supported by the Netherlands Organization for the Advancement of Basic Research (Z W O), through the Netherlands Biophysics Foundation

References

1 Sachs, G , Faller, L D and Rabon, E (1982) J Membrane Biol 64, 123–125
2 Wallmark, B , Stewart, H B , Rabon, E , Saccomani, G and Sachs, G (1980) J Biol Chem 255, 5313–5319
3 Rabon, E , Sachs, G and Wallmark, B (1982) Biochim Biophys Acta 688, 515–524
4 Bonting, S L , Schrijen, J J , Helmich-de Jong, M L and De Pont, J J H H M (1984) in Hydrogen Ion Transport in Epithelia (Forte, J G , Warnock, D G and Rector, F C , eds), pp 185–193, Wiley Interscience, New York
5 Schrijen, J J , Van Groningen-Luyben, W A H M , De Pont, J J H H M and Bonting, S L (1981) Biochim Biophys Acta 640, 473–486
6 Klodos, I , Nørby, J G and Plesner, I W (1981) Biochim Biophys Acta 643, 463–482
7 Schrijen, J J , Luyben, W A H M , De Pont J J H H M and Bonting, S L (1980) Biochim Biophys Acta 597, 331–344
8 Schuurmans Stekhoven, F M A H , Swarts, H G P , De Pont, J J H H M and Bonting, S L (1980) Biochim Biophys Acta 597, 100–111
9 Jackson, R J and Saccomani, G (1984) Biophys J 45, 83a
10 Wallmark, B and Mårdh, S (1979) J Biol Chem 254, 11899–11902
11 Ray, T K and Forte, J G (1976) Biochim Biophys Acta 443, 451–467
12 Stewart, H B , Wallmark, B and Sachs, G (1981) J Biol Chem 256, 2682–2690
13 Askari, A and Huang, W (1982) Biochem Biophys Res Commun 104, 1447–1453
14 Fukushima, Y , Yamada, S and Nakao, M (1984) J Biochem 95, 359–368