A Kinetic Model for Translocators in the Chloroplast Envelope as an Element of Computersimulation of the Dark Reaction of Photosynthesis

Christoph Giersch

Botanisches Institut der Universität Düsseldorf

(Z. Naturforsch. **32 c**, 263–270 [1977]; received November 22, 1976)

Chloroplast, Phosphate Translocator, Computer Simulation, Metabolite Exchange, Kinetic Model

A kinetic model for the chloroplast translocators (mediating equal and opposite exchange fluxes between the external medium and the stroma) is derived. This model is a modification of the classical Widdas model corresponding to the exchange of an arbitrary number of compounds with no contribution to net transport. It describes the rate of transport of each compound in simple terms and with a minimum number of kinetic constants. Predictions from the model agree with experimental data as recorded in the literature.

From the experimental apparent kinetic constants V_{MAX} and K_m those in terms of the model are calculated. These constants complete the model and make it applicable to the study of metabolism of photosynthesis by means of computer simulation. The set of differential equations describing the operation of the phosphate translocator is solved numerically for some illustrative examples.

Introduction

Knowledge on metabolite exchange between chloroplasts and cytoplasm is well established in qualitative terms [1]. The field appears to be prepared for a quantitative treatment which promises a better understanding of the regulation of photosynthesis. Different parameters such as light intensity metabolite levels, activities of enzymes influence metabolite transport. Ton consider them simultaneously is impossible without the aid of a computer. One possibility to follow the time course of the individual variables is to set up the corresponding differential equations and to solve them by means of a computer.

There is a large volume of literature concerning the computer simulation of enzymatic reactions and the time course in the levels of intermediates [2]. Recently a computer simulation of electron transport in photosynthesis was described [3]. In this paper a model for translocator mediated diffusion [4] is described. This model is quite general for any case of mediated diffusion with zero net flux. It is mainly applied to the phosphate translocator of the chloroplast. This carrier is situated in the inner membrane of the envelope and facilitates, in a competitive fashion, transfer of 3-phosphoglycerate, inorganic phosphate, dihydroxyacetone phosphate, and glyceraldehyde phosphate. Since CO_2 diffuses into the stroma and dihydroxyacetone phosphate is the main product of CO_2 fixation the physiological significance of the phosphate translocator is to mediate the exchange of dihydroxyacetone phosphate and inorganic phosphate, which is required for the formation of ATP [5]. It has been verified that the operation of the phosphate translocator is a strict counter exchange, mediating only equal and opposite exchange fluxes with no contribution to net transport [6]. These experimental findings are met by the assumption that the carrier is able to cross the envelope only when occupied with substrate. The model is somewhat similar to the classical Widdas model [7]. The main differences come about by the assumed counter exchange (net flux equal to zero) and by considering the exchange of N substrates (N arbitrary integer) instead of one [7] or two [8].

Requests for reprints should be sent to Dr. Christoph Giersch, Botanisches Institut II der Universität Düsseldorf, Universitätsstr. 1, *D-4000 Düsseldorf.*

Abbreviations: PGA, 3-phosphoglycerate; P_i, inorganic phosphate; DAP, dihydroxyacetone phosphate; GAP, glyceraldehyde phosphate.

The Model

The analized translocator model (Fig. 1 a) has one central TS-complex for each species. A probably more realistic model would involve two central complexes, corresponding to the diffusion process of TS across the membrane (Fig. 1 b). It is known that under steady-state conditions the final rate law does not depend on the number of TS-complexes [9]; only the definition of the model's parameters in terms of the individual microscopic rate constants changes. Hence the model of Fig. 1 a is preferred.

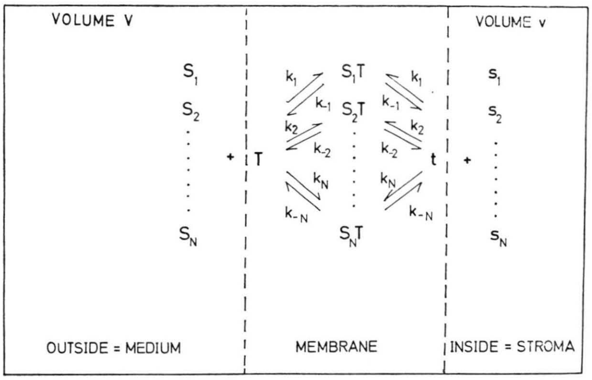

Fig. 1 a.

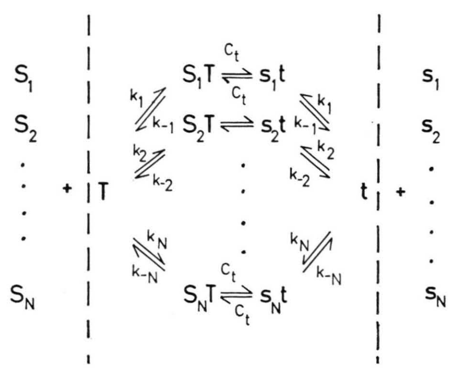

Fig. 1 b.

Fig. 1. Model for symmetrical monovalent carrier operation on the basis of counter exchange. Components present on the external face of the membrane are denoted by large letters, those on the internal face by small ones. The square brackets in denoting concentrations are omitted. Components $S_1, \ldots, S_N$ compete for the binding site of translocator T ($s_i, \ldots, s_N$ compete for t). Concentrations of S_i, s_i ($i = 1, \ldots, N$) in molarities, for the rest (T, t, $S_i\,T$, $s_i\,t$) in μmol/mg chlorophyll. The main differences to the classical Widdas model [7] are the assumptions that the translocator molecule is able to cross the envelope only when occupied ($r = \infty$ according to Stein's nomenclature, ref. 23) and that several substrates compete for the carrier. a) Model with one $S_i\,T$-complex for each species. b) Model with two $S_i\,T$-complexes. The steady state rate law (A 14) is the same for both models, only the definition of the V_i and K_i in terms of the kinetic constants k_i, k_{-i}, c_t changes (*cf.* Eqn (A 16), (A 17)).

According to the counter exchange principle for every molecule of substrate transported in one direction there will be a transport of one molecule in the other direction. Assuming that there is no change of osmotic volumes this means (see Fig. 1 a, 1 b for symbols):

$$\frac{\mathrm{d}}{\mathrm{d}t}\left(\sum_{i=1}^{N} S_i\right) = 0 = \frac{\mathrm{d}}{\mathrm{d}t}\left(\sum_{i=1}^{N} s_i\right). \qquad (1)$$

The sums of concentrations of all exchangeable intermediates are constant in time for both compartments. It is assumed that the translocator molecule is able to cross the membrane only when occupied (*cf.* Figs 1 a, 1 b). Under this condition Eqn (1) is equivalent to

$$\frac{\mathrm{d}}{\mathrm{d}t}(T) = 0 = \frac{\mathrm{d}}{\mathrm{d}t}(t) \qquad (2)$$

as is shown in the Appendix, Eqn (A 8), (A 9). The concentration of *free* translocator molecule is constant in time on the inside and on the outside of the membrane, respectively. It should be pointed out that Eqn (2), though looking like a steady-state condition, holds quite generally in any case of strict counter exchange.

The model is highly symmetrical. There is no sidedness of the membrane; the rate constants of the TS_i-complexes depend only on the substrate under consideration and not on the side of the membrane. The model is analized in terms of steady-state kinetics. As the King-Altman procedure [10] turned out to be too confusing for a model with N substrates the differential equations for the scheme of Fig. 1 a were set up and simplified according to steady-state conditions. The resulting system of equations was solved analytically. In addition, the definition of the parameters for the model involving two TS-complexes (Fig. 1 b) are given in Eqn (A 17). Details of the calculation are given in the Appendix.

Results

a) Exchange of two components

$$(S_1 = A, S_2, \ldots, S_N = 0, s_1 = 0, s_2 = b,$$
$$s_3, \ldots, s_N = 0)$$

From the general rate law (A 14) the rate of exchange of substrate A in the medium compartment for compound b in the stroma compartment in the absence of any other compound is given by

$$v_A = Q_v\, V_A \frac{\bar{A}\,\bar{b}\,V_B}{\bar{A}\,V_A + \bar{b}\,V_B + 0.5\,(\bar{A}\,\bar{b}\,V_B + \bar{A}\,\bar{b}\,V_A)}. \qquad (3)$$

v_A is the rate of transport of A from the medium to the stroma compartment (equal and opposite to the transport of b from stroma to medium). $Q_v = v/V$ is the volume quotient (*cf.* Fig. 1). It accounts for the fact that the exchange of a certain number of molecules causes different changes in concentrations in the respective compartments according to their volumes. V_A is the maximal exchange rate for compound A and corresponds to the $V_{MAX}(A)$ in terms of Michaelis-Menten kinetics. $\bar{A} = A/K_A$ is the normed dimensionless concentration of A. The V_i and K_i are given in the Appendix in terms of the microscopic rate constants of Fig. 1 (Eqn (A 15)). For $V_A = V_B$ we obtain from (3):

$$v_A = Q_v V_A \frac{A}{K_A + A(1 + 1/\bar{b})} . \tag{4}$$

For $\bar{b} \gg 1$ (this means $b \gg K_B$) the ordinary Michaelis-Menten equation is obtained: if the V_i of the two substrates are (nearly) identical and the exchange partner in the stroma is present in excess a Michaelis-Menten saturation curve results for the transport of A into the stroma. If b is comparable to K_B or even smaller ($\bar{b} \leqq 1$) it acts an uncompetitive inhibitor affecting both K_A and V_A to the same extent [11].

b) Exchange of four components

$$(S_1 = A, S_2 = B, S_3, \ldots, S_N = 0;\; s_1 = a, s_2 = b, s_3, \ldots, s_N = 0).$$

For the exchange of A for a and b in the presence of compound B we get:

$$v_A = Q_v V_A \frac{\bar{a}(V_A \bar{A} + V_B \bar{B}) - \bar{A}(V_A \bar{a} + V_B \bar{b})}{V_A(\bar{A} + \bar{a}) + V_B(\bar{B} + \bar{b}) + 0.5\{(\bar{A} + \bar{B})(V_A \bar{a} + V_B \bar{b}) + (\bar{a} + \bar{b})(V_A \bar{A} + V_B \bar{B})\}} . \tag{5}$$

From this expression it can be seen that there are two terms for the net flux of component A; the first one with the positive sign increases the concentration of A and reflects the transport from the internal to the external compartment. The second one with the negative sign loweres A and represents the transport into the internal compartment. (In Eqns (3) and (4) the minus sign was omitted to avoid confusion.) Equilibrium is reached (net flux of all components equal to zero) if the numerator vanishes. This can be rearranged to

$$\frac{a}{A} = \frac{b}{B} \tag{6 a}$$

or

$$\frac{a}{b} = \frac{A}{B} . \tag{6 b}$$

Equilibrium for the operation of the translocator is reached when the ratio of concentrations is identical for the two substrates (6 a) or if the ratio of concentrations is identical for the two compartments (6 b).

c) Exchange of 2 N components

$$(S_1, \ldots, S_N \text{ for } s_1, \ldots, s_N).$$

Having considered these special cases we can see that the general expression is a straightforward extension of Eqn (5):

$$v_P = Q_v V_P \frac{\bar{s}_p \sum_{i=1}^{N} V_i \bar{S}_i - \bar{S}_p \sum_{i=1}^{N} V_i \bar{s}_i}{\sum_{i=1}^{N} V_i(\bar{S}_i + \bar{s}_i) + \frac{1}{2}\left(\sum_{i=1}^{N} V_i \bar{s}_i \sum_{i=1}^{N} \bar{S}_i + \sum_{i=1}^{N} V_i \bar{S}_i \sum_{i=1}^{N} \bar{s}_i\right)} = \frac{\mathrm{d}}{\mathrm{d}t}(S_p) . \tag{7}$$

$$v_p = \frac{\mathrm{d}}{\mathrm{d}t}(s_p) = -\frac{1}{Q_v}\frac{\mathrm{d}}{\mathrm{d}t}(S_p), \quad p = 1, \ldots, N .$$

Eqn (7) gives the rate of exchange of S_p and s_p, respectively, for $p = 1, \ldots, N$. The transport of S_p into the internal compartment is zero if either $S_P = 0$ (trivial, no substrate available) or if $\sum_{i=1}^{N} V_i \bar{s}_i = 0$ (internal compartment empty, no exchangeable compound available). This is what we expect to occur for the operation of a counter exchange translocator. The equilibrium condition for the net flux of S_P reads in this case:

$$s_p/S_p = \sum_{\substack{i=1 \\ i \neq p}}^{N} V_i \bar{s}_i \Big/ \sum_{\substack{i=1 \\ i \neq p}}^{N} V_i \bar{S}_i . \tag{8}$$

This formula is the straightforward extension of the expression (6 a). The components b and B are replaced by the 'generalized' components $\Sigma V_i \bar{s}_i$ and $\Sigma V_i \bar{S}_i$, respectively. By summing up the flux rates V_i of all N compounds it can be shown that the sums of concentrations in the two compartments are indeed constant in time according to our assumption, Eqn (1). If the V_i are equal to each other Eqn (8) takes a very simple form:

$$v_p = Q_v V_p \frac{\bar{s}_p \cdot \mathrm{SUM} - \bar{S}_p \cdot \mathrm{sum}}{\mathrm{SUM} + \mathrm{sum} + \mathrm{SUM} \cdot \mathrm{sum}} \tag{8 a}$$

with $$\mathrm{SUM} = \sum_{i=1}^{N} \bar{S}_i \,, \quad \mathrm{sum} = \sum_{i=1}^{N} \bar{s}_i \,.$$

From this formula it is easy to get a rough semiquantitative scetch of the operation of the translocator.

Only $2N$ kinetic parameters K_i and V_i ($i = 1, \ldots, N$) are required in describing the operation of the translocator for N chemically different species. The small number of kinetic parameters is one of the advantages of this symmetrical model. It is known that with less symmetrical models there is an enormous number of terms in the rate equation (Schachter gets 90 terms in the denominator for a nonsymmetrical model corresponding to $N = 2$ in this nomenclature, ref. 12). Thus explicite summation of terms highly simplifies the handling of rate equations and drastically reduces the expense of work and paper in analytical and numerical procedures. An essential question is whether this simple model is adequate to meet the experimental facts.

Comparison Model-Experiment

Heldt and coworkers studied translocators in the chloroplast envelope [5, 6, 13–15]. They determined the apparent $V_{\mathrm{MAX}}^{\mathrm{ap}}$ and $K_{\mathrm{M}}^{\mathrm{ap}}$ of the phosphate translocator from initial rate kinetics with labelled substrates at 4 °C in the dark. Their main results which have to be met by the model are: in the Lineweaver-Burk representation of the rate of transport of labelled substrate ($1/v$ *versus* $1/S^*$) a straight line is obtained. From the intercepts one gets values for apparent $V_{\mathrm{MAX}}^{\mathrm{ap}}$ and $K_{\mathrm{M}}^{\mathrm{ap}}$ for each transported compound. A second substrate (not labelled) acts as competitive inhibitor: it alters the slope but not the intercept with the $1/v$-axis whence an apparent inhibition constant $K_{\mathrm{I}}^{\mathrm{ap}}$ is obtained. The $K_{\mathrm{M}}^{\mathrm{ap}}(\mathrm{A})$ for the transport of A is equal to the apparent inhibition constant $K_{\mathrm{I}}^{\mathrm{ap}}(\mathrm{A})$ when I is transported in the presence of A.

In deriving the Lineweaver-Burk representation from Eqn (7) we have to bear in mind that during the first few seconds there is practically no exchangeable labelled compound in the internal compartment. Hence we are allowed to abolish the first term in (7), as this term corresponds to the flux of labelled compound from the stroma to the medium. We omit the sign and neglect the ratio of volumes Q_v which does not interest in this connection. For the transport of $A^* = S_1$ in the presence of an unlabelled compound $B = S_2$ we get from (7):

$$\frac{1}{v_A} = \frac{1}{V_A}\left(\frac{1}{2} + \frac{1}{2} r_A\right) + \frac{K_A}{V_A \cdot A^*}\left(1 + \frac{B}{K_B}\left(\frac{1}{2} + \frac{1}{2} r_B\right)\right) \tag{9}$$

where

$$r_A = V_A \frac{2 + \sum_{j=1}^{N} \bar{s}_j}{\sum_{j=1}^{N} V_j \bar{s}_j} \,; \quad r_B = r_A \frac{V_B}{V_A} \,. \tag{9 a}$$

Comparing this expression (9) to the Lineweaver-Burk representation of the competitive inhibitor (*e. g.* ref. 10, p. 107)

$$\frac{1}{v_A} = \frac{1}{V_A^{\mathrm{ap}}} + \frac{K_A^{\mathrm{ap}}}{V_A^{\mathrm{ap}} \cdot A^*}\left(1 + \frac{I}{K_I^{\mathrm{ap}}}\right) \tag{10}$$

we get

$$V_A^{\mathrm{ap}} = \frac{2 V_A}{1 + r_A} \tag{11 a}$$

$$K_A^{\mathrm{ap}} = \frac{2 K_A}{1 + r_A} \tag{11 b}$$

$$K_I^{\mathrm{ap}} \; (= K_B^{\mathrm{ap}}) = \frac{2 K_B}{1 + r_B} \,. \tag{11 c}$$

This means that the Lineweaver-Burk representation of translocator mediated transport of compound A^* as calculated from the model (Eqn (9)) takes the form (10) of a simple competitive inhibition system provided the apparent parameters V_A^{ap}, K_A^{ap}, and K_I^{ap} are defined by (11 a) – (11 c), respectively. The Eqn (11 a), (11 b) constitute the link between the model parameters V_i, K_i and the experimental apparent parameters V_i^{ap}, K_i^{ap}.

From (9) it can be seen that the $1/v$ *versus* $1/A^*$ plot gives a straight line; a second substrate B acts as a competitive inhibitor with respect to A^*. From (11 b), (11 c), (10), and the analogon of (10) for the transport of B^* in the presence of A it is clear that the K_m for the transport of A^* ($= K_A$) is equal to the inhibition constant when B^* is exchanged in the presence of A. The Lineweaver-Burk plot for the transport of compound A^* in the presence and in absence of B is given in Fig. 2.

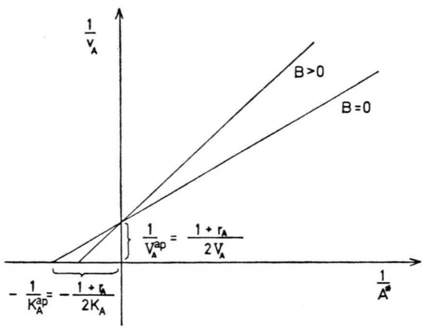

Fig. 2. Lineweaver-Burk plot of the translocator mediated transport of A^* in the presence of a second unlabelled compound B from medium to the stroma. A^* may be one of the compounds transferred by the phosphate translocator, P_i, PGA, DAP, or GAP. The relationship between the apparent kinetic constants K_A^{ap} and V_A^{ap} according to Heldt [15, 16] and the model's K_A and V_A as indicated. For further details see text.

A^* can be one of the labelled compounds PGA, P_i, DAP, or GAP or any other compound transferred by the phosphate translocator. The correlation between the apparent kinetic constants K_i^{ap}, V_i^{ap} for the four compounds PGA, P_i, DAP, and GAP as determined by Heldt and coworkers and that of the model are given by the factors r_i (Eqn (9 a)). From (11 a) we get:

$$r_i = \frac{2 \cdot V_i - V_i^{ap}}{V_i^{ap}} . \tag{12}$$

The r_i are a measure of the difference between the apparent and the model parameters. For $r_A = r_B = 1$ in (11) the apparent parameters coincide with those of the model. It can be seen from (9 a) that $r_i = 1$ is equivalent to $\Sigma \bar{s}_i \gg 2$ and $V_A = V_B = V_i$. The apparent and the model parameters are identical whenever the stroma of the chloroplasts is filled up with exchangeable substrates and all the maximal exchange rates V_i are equal to each other.

For every substrate of the phosphate translocator there is one equation (11 a) and one equation (11 b). These two sets of equations are combined with Eqn (9 a) for the r_i. We get a system of 8 equations for the 8 unknown model parameters V_{PGA}, V_{Pi}, V_{DAP}, V_{GAP}; K_{PGA}, K_{Pi}, K_{DAP}, K_{GAP}. This system can be solved provided the apparent parameters V_i^{ap}, K_i^{ap} ($i =$ PGA, P_i, DAP, GAP) and the concentrations of the four compounds in the stroma are known. The V_i^{ap}, K_i^{ap} are taken from Heldt [15, 16]. The concentrations vary from one batch to another and vary considerably in time for one experiment [17]. We choose some representative values which are supposed to be fairly realistic. Calculation is done by a FORTRAN Subroutine [18]. Table I gives the result together with the apparent constants according to Heldt [15, 16].

There are systematic differences between the r_i for the individual compounds; for stroma concentrations in the physiological range those of DAP and P_i are about one, that the for PGA definitely lower than unity. These differences reflect the competition of intermediates for the translocator molecule: as for instance the apparent rate for the transport of P_i is the highest (*cf.* Table I) though its K_m is the highest (lowest affinity) the actual capacity of the translocator for the transport of P_i (which is expressed by V_i) must be higher than the apparent capacity measured in the presence of other components with lower K_m (higher affinity for the translocator).

Except perhaps for P_i the uncertainty in the concentrations of the compounds in the stroma does not affect the V_i and K_i very much; we are able to specify a set of kinetic constants V_i, K_i which complete the translocator model and make it applicable to realistic situations.

The differential equations describing the time course of operation of the phosphate translocator are established according to (7). S_i and s_i ($i = 1, \ldots, 4$) are the concentrations of PGA, P_i, DAP, and GAP in the medium and in the stroma, respectively. The volume quotient $Q_v = v/V$ depends on the chlorophyll concentration in the experiment and on the respective volumes. With 30 μg chlorophyll/ml and 0.33 M sorbitol buffer it is about 1/1300. The constants V_i and K_i ($i = 1, \ldots. 4$) are taken from Table I. The resulting set of nonlinear first order differential equations is solved numerically by Hamming's modified predictor-corrector method [19]. Fig. 3 gives some illustrative examples of the operation of the phosphate translocator. The amount of exchanged compounds (and the time till equilibrium is reached) depends on the choosen values of concentrations. In many cases intermediates are transported against their concentration gradient (*e.g.* PGA in Fig. 3 b). This is clearly no case of active transport but an example for a situation where one gradient drives another.

From Fig. 3 a it can be seen that increasing levels of P_i diminish the extent of PGA uptake. With low medium concentrations of P_i there is a considerable concentration gradient medium/stroma for this com-

Table I. Kinetic constants V_i and K_i for the model of the phosphate translocator. The constants are calculated on the basis of Heldt's experimental values [15, 16] from Eqn (11 a), (11 b); $r_i = (2\,V_i - V_i^{ap})/V_i^{ap}$ is a measure of the difference between the model's and the apparent constants.

	Concentration stroma [mM]				V_i^{ap} or V_i [μmol mg^{-1} Chl·h^{-1}]				K_i^{ap} or K_i [μM]				r_i			
	PGA	P_i	DAP	GAP	PGA	P_i	DAP	GAP *	PGA	P_i	DAP	GAP *	PGA	P_i	DAP	GAP
Apparent values [15, 16]	—	—	—	—	32	48	45	41	110	230	80	30	—	—	—	—
Calculated	5	12	1	0.05	26.4	58.5	50.3	41.3	90	281	89	30	0.65	1.44	1.24	1.01
Values	3	14	1	0.05	25.5	54.6	47.4	39.3	87	264	84	29	0.59	1.28	1.11	0.92
Concentrations	1	16	0.5	0.025	24.7	50.8	44.5	37.3	85	243	79	27	0.54	1.12	0.98	0.82
in the physio-	0.5	16.5	2	0.1	24.5	50.0	43.9	36.9	84	239	78	27	0.53	1.08	0.95	0.80
logical range	0.5	16.5	1	0.05	24.5	50.0	43.9	36.9	84	239	78	27	0.53	1.08	0.95	0.80
Calculated	0.5	16.5	0.5	0.025	24.5	49.9	43.8	36.8	84	239	78	27	0.53	1.08	0.95	0.80
Values	0.5	16.5	0	0	24.4	49.7	43.6	36.7	84	238	78	27	0.53	1.07	0.94	0.79
Concentrations	0	17	0.5	0.025	24.2	48.9	43.1	36.3	83	234	76	26	0.51	1.04	0.92	0.77
with less	8	8	0.5	0.025	28.0	67.3	56.7	45.5	96	322	101	33	0.75	1.80	1.52	1.22
physiological	12	4	0.5	0.025	29.8	78.9	64.7	50.5	103	380	115	37	0.86	2.29	1.88	1.46
significance	16	0	1	0.025	31.1	88.7	71.2	54.4	107	425	126	40	0.94	2.70	2.16	1.65

* Preliminary data.

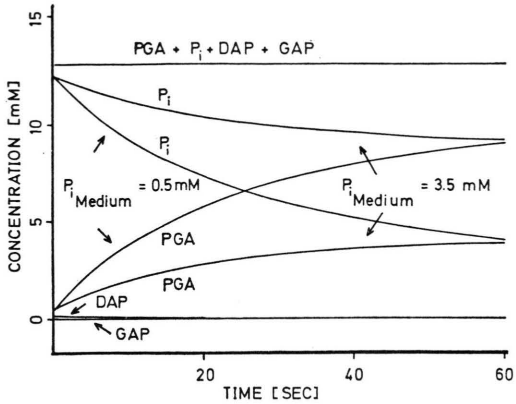

Fig. 3 a.

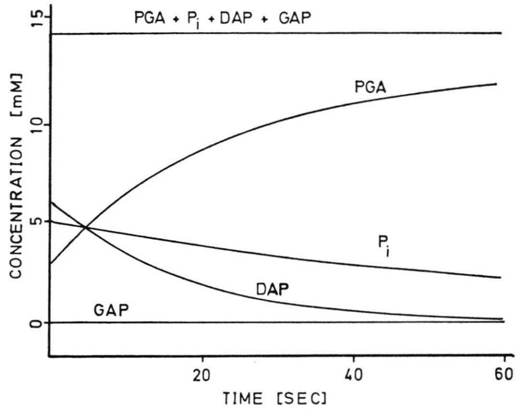

Fig. 3 b.

Fig. 3. Time course of stroma concentrations of compounds exchanged by the phosphate translocator. Due to the ratio of volumes (stroma/medium $\approx 1/1300$) the external concentrations remain practically constant in the considered time interval. Kinetic constants: V_i (μmol mg^{-1} Chl·h^{-1}) PGA, 25; P_i, 50; DAP, 45; GAP, 37; K_i (mM) PGA, 0.085; P_i, 0.25; DAP, 0.078; GAP, 0.027. a) Increase of external P_i from 0.5 to 3.5 mM loweres the rate of PGA uptake and decrease the stroma equilibrium concentration. See text for possible physiological significance. Initial concentrations in the stroma in mM: PGA, 0.5; P_i, 12; DAP, 0.1; GAP, 0.01; in the medium: PGA, 2; DAP, 0; GAP, 0. b) "Uphill" transport of PGA: the concentration gradient medium/stroma of P_i drives the uptake of PGA into the stroma against the PGA concentration gradient. Initial concentrations in the stroma in mM: PGA, 3; P_i, 5; DAP, 6; GAP, 0; in the medium: PGA, 6; P_i, 0.5; DAP, 0; GAP, 0.

pound. This gradient equilibrates, driving the uptake of PGA. Higher medium concentrations of P_i reduce the gradient; in addition, P_i competes with PGA for the binding site of the carrier molecule. Thus, the rate of PGA uptake is decreased, and the equilibrium concentration is lower (Fig. 3 a). This mechanism may play a role in the still unknown nature of phosphate-inhibition of CO_2 reduction [20].

There are some problems involved in this approach which have not yet been mentioned: most of the compounds of the phosphate translocator are exchanged as divalent anions, but there is evidence [21] that PGA is transported in its trivalent form. It can be seen from exchange experiments [6] that exchange does not occur according to the stoichiometry of charges but according to the stoichiometry of molecules. Therefore requirements for electroneutrality (cotransport of a monovalent cation or countertransport of a monovalent anion) may change the characteristics of the translocator or may even limit its operation. The apparent kinetic constants, however, are obtained from initial rate experiments and will not be affected by the differences in the valencies of exchanged compounds. This point nevertheless has to be considered in establishing the set of differential equations describing the dark reactions of photosynthesis.

Appendix: Derivation of the rate law of the translocator

From the scheme of Fig. 1 a the following set of equations is obtained:

$$\left.\begin{aligned} \dot{S}_i &= k_{-i}(S_i T) - k_i T\cdot S_i && \text{(A 1)}\\ \dot{s}_i &= \frac{V}{v} k_{-i}(S_i T) - k_i t\cdot s_i && \text{(A 2)}\\ (\dot{S_i T}) &= k_i\left(T\cdot S_i + \frac{v}{V} t\cdot s_i\right) - 2k_{-i}(S_i T) && \text{(A 3)} \end{aligned}\right\} i=1,\dots,N$$

$$\dot{T} = \sum_{i=1}^{N} k_{-i}(S_i T) - \sum_{i=1}^{N} k_i T\cdot S_i \qquad \text{(A 4)}$$

$$\dot{t} = \frac{V}{v}\sum_{i=1}^{N} k_{-i}(S_i T) - \sum_{i=1}^{N} k_i t\cdot s_i\,. \qquad \text{(A 5)}$$

The concentration t_0 of free plus occupied translocator molecules is constant in time

$$t_0 = \frac{V}{v}\left(T + \sum_{i=1}^{N}(S_i T)\right) + t\,. \qquad \text{(A 6)}$$

Conservation of each species:

$$\dot{S}_i = -\frac{v}{V}\dot{s}_i\,, \quad i=1,\dots,N\,. \qquad \text{(A 7)}$$

Comparing the sum over the N equations (A 1) to (A 4) it is concluded that

$$\sum_{i=1}^{N}\dot{S}_i = 0 \Leftrightarrow \dot{T} = 0\,. \qquad \text{(A 8)}$$

From (A 2) and (A 6) we get

$$\sum_{i=1}^{N}\dot{s}_i = 0 \Leftrightarrow \dot{t} = 0\,. \qquad \text{(A 9)}$$

From the last two equations it is evident that the concentration of *free* translocator molecules is constant in time whenever the sums of compounds in both compartments are constant in time, and vice versa. Hence we are justified in assuming the left-hand-sides of Eqn (A 4) and (A 5) to be zero.

Under steady-state conditions the left-hand-side of (A 3) is zero. The $S_i T$-complexes can be expressed by T, S_i, t, s_i:

$$(S_i T) = \frac{k_i}{2k_{-i}}\left(T\cdot S_i + \frac{v}{V} t\cdot s_i\right) \qquad \text{(A 10)}$$

From Eqn (A 10) and (A 4) one obtains after rearrangement t as a function of T, S_i, and s_i

$$t = T\sum_{i=1}^{N} k_i S_i \Big/ \frac{v}{V}\sum_{i=1}^{N} k_i s_i\,. \qquad \text{(A 11)}$$

Next (A 1) is combined with (A 10) and (A 11)

$$\dot{S}_p = \frac{k_p s_p \sum_{i=1}^{N} k_i S_i - k_p S_p \sum_{i=1}^{N} k_i s_i}{2\sum_{i=1}^{N} k_i s_i} T. \qquad \text{(A 12)}$$

Finally, the conservation of t_0 (A 6) is used to express T in terms of t_0, S_i, and s_i. From (A 6), (A 10), and (A 11)

$$T = \frac{v}{V} t_0 \frac{1}{1 + \sum_{i=1}^{N}\frac{k_i}{2k_{-i}} S_i + \frac{\sum_{i=1}^{N} k_i S_i}{\sum_{i=1}^{N} k_i s_i}\left(1 + \sum_{i=1}^{N}\frac{k_i}{2k_{-i}} s_i\right)}\,. \qquad \text{(A 13)}$$

Replacing T in (A 12) by (A 13) and rearranging terms

$$\dot{S}_p = v_p = \frac{v}{V} V_p \times \qquad \text{(A 14)}$$

$$\frac{\bar{s}_p \sum_{i=1}^{N} V_i \bar{S}_i - \bar{S}_p \sum_{i=1}^{N} V_i \bar{s}_i}{\sum_{i=1}^{N} V_i(\bar{S}_i + \bar{s}_i) + \frac{1}{2}\left(\sum_{i=1}^{N} V_i \bar{s}_i \cdot \sum_{i=1}^{N}\bar{S}_i + \sum_{i=1}^{N} V_i \bar{S}_i \cdot \sum_{i=1}^{N}\bar{s}_i\right)}$$

$$\dot{s}_p = -\frac{V}{v}\dot{S}_p$$

with

$$V_i = \frac{t_0}{2} k_{-i}; \quad \bar{S}_i = \frac{S_i}{K_i}, \quad K_i = \frac{k_{-i}}{k_i} \quad \cdot (i = 1, \ldots, p, \ldots N). \tag{A 15}$$

It is interesting to compare the definition of the V_i and K_i in terms of the microscopic rate constants (*cf.* Fig. 1 a and Eqn (A 15)) to the corresponding terms of reversible enzyme-catalized reactions [22]:

$$V_{MAX} = t_0 k_{-1}; \quad K_m = \frac{2 k_{-1}}{k_1}. \tag{A 16}$$

The V_i is just half the V_{MAX}. This makes sence since the maximal concentration of total carrier on each side of the membrane is $t_0/2$. Correspondingly, K_i is just half the K_m. The rate law for the model with two central TS-complexes (*cf.* Fig. 1 b) is identical with (A 14). Only the definition of V_i and K_i in terms of the microscopic rate constants changes:

$$V_i = \frac{t_0}{2} \frac{k_{-i} c_t}{k_{-i} + 2 c_t}; \quad K_i = \frac{k_{-i}}{2 k_i}. \tag{A 17}$$

The author wishes to thank Prof. U. Heber and Prof. H. W. Heldt for stimulating discussions and helpful suggestions. Numerical calculations were carried out on the DVA Siemens 4004/45 des Rechenzentrums der Universität Düsseldorf.

1 U. Heber, Ann. Rev. Plant Physiol. **25,** 393 [1974].

2 D. Garfinkel, M. Achs, and L. Dzubow, Proc. 14th Ann. Nutrition Conf., **Vol. 33,** 176 [1974].

3 C. Holzapfel and R. Bauer, Z. Naturforsch. **30 c,** 489 [1975].

4 A. Kotyk and K. Janacek, Cell Membrane Transport, 2nd edn. 1975, p. 65, Plenum Press, New York and London.

5 H. W. Heldt, in Horizons Biochem. Biophys. **2,** 199 (E. Quaglieriello, F. Palmieri, P. P. Singer, eds.), Addison & Wesley, Reading 1976.

6 H. W. Heldt, R. Fliege, K. Lehner, M. Milovancev, and K. Werdan, Proc. 3rd Int. Congr. on Photosynth. 1974 (M. Avron, ed.), p. 1369, Elsevier Scientific Pub. Comp., Amsterdam.

7 W. F. Widdas, J. Physiol. **118,** 23 [1952].

8 D. M. Regen and H. E. Morgan, Biochim. Biophys. Acta **79,** 151 [1964].

9 W. R. Lieb and W. D. Stein, Biochim. Biophys. Acta **143,** 178 [1974].

10 I. H. Segel, Enzyme Kinetics, pp. 506–523, John Wiley & Sons, New York 1975.

11 Ref. 10, pp. 136–138.

12 H. Schachter, in Metabolic Pathway, 3rd edn. 1972, **Vol. 6,** p. 12, Academic Press, New York.

13 H. W. Heldt and L. Rapley, FEBS Letters **10,** 143 [1970].

14 K. Werdan and H. W. Heldt, Proc. 2nd Int. Congr. on Photosynth. Res. 1971 (G. Forti, M. Avron, and A. Melandri, eds.), p. 1337, Dr. W. Junk N.V., The Hague.

15 H. W. Heldt, in Encyclopedia of Plant Physiol., New Series (A. Pirson and M. H. Zimmermann, eds.), **Vol. 3,** p. 137, Springer-Verlag, Berlin, Heidelberg, New York 1976.

16 H. W. Heldt, unpublished result.

17 R. M. C. Lilley, J. C. Chon, A. Mosbach, and H. W. Heldt, The Distribution of Metabolites between Spinach Chloroplasts and Medium during Photosynthesis in vitro, submitted to BBA.

18 Fortran Subroutine Sekant, Author Siemens.

19 IBM Scientific Subroutine Package, Version III, Fortran Subroutine HPCG.

20 W. Cockburn, C. W. Baldry, and D. A. Walker, Biochim. Biophys. Acta **143,** 614 [1967].

21 U. Heber, personal communication.

22 Ref. 10, p. 31

23 W. D. Stein, The Movement of Molecules across Cell Membranes, p. 153, Academic Press, New York and London 1967.