Biochimica et Biophysica Acta, 298 (1973) 437–445

BBA 76236

MULTIPLE TRANSPORT SYSTEMS FOR BASIC AMINO ACID TRANSPORT IN STREPTOMYCES HYDROGENANS

W. GROSS and K.-L. BURKHARDT

Chemisch-physiologisches Institut der Universität Frankfurt/Main (Germany)

(Received October 4th, 1972)

SUMMARY

In *Streptomyces hydrogenans* the uptake of basic amino acids occurs *via* several transport systems:

1. One system (System b_{Arg}) is highly specific for arginine ($K_m = 7 \cdot 10^{-6}$ M). L-Arginine influx is only inhibited by closely related analogs such as D-arginine, canavanine and arginine methyl ester, but not by lysine, ornithine, neutral and acidic amino acids.

2. A second system (System $b_{Arg/Lys}$) transports both arginine ($K_m = 0.6 \cdot 10^{-6}$ M) and lysine ($K_m = 0.9 \cdot 10^{-6}$ M). This system has a higher affinity for L-arginine than System b_{Arg}. Cells grown in synthetic media containing 12 mM L-lysine or L-arginine show a much higher transport capacity than cells grown in complete media or synthetic media with NH_4Cl as nitrogen donor. These differences in transport capacity strongly support the multi-component concept of basic amino acid transport in *Streptomyces* cells. System $b_{Arg/Lys}$ can be inhibited by analogs of arginine and lysine but not by neutral and acidic amino acids.

3. At very high extracellular concentrations of lysine or arginine both amino acids are transported *via* a system primarily mediating the uptake of neutral amino acids (System $_n$). The capacity of this system is high but its affinity to basic amino acids is very low [K_m(arginine) $= 0.7 \cdot 10^{-3}$ M; K_m(lysine) $= 1.6 \cdot 10^{-3}$ M]. This system shows exchange between neutral and basic amino acids.

INTRODUCTION

In *Streptomyces hydrogenans* amino acids are transported by several active transport systems. The uptake of acidic and neutral amino acids has been characterized in some detail[1,2]. The kinetic properties of the uptake of basic amino acids are the subject of this report. Earlier investigations[3] have shown that the transport of L-arginine and L-lysine in *S. hydrogenans* is an active carrier-mediated process: the influx of basic amino acids is saturable, can be inhibited by structurally related analogs and the uptake is completely inhibited by 1 mM 2,4-dinitrophenol. If the concentrations of L-arginine or L-lysine in the incubation medium are lower than 0.1 mM L-glutamate, L-aspartate, 2-aminoisobutyric acid, cycloleucine, L-alanine, L-methionine, L-histidine, L-phenylalanine and L-tyrosine do not inhibit the influx. Competition studies with D-arginine, L-canavanine, arginine methyl ester, D-lysine, hydroxylysine,

lysine methyl ester and L-ornithine support the hypothesis of multiple transport systems for basic amino acids. These phenomena were studied in more detail.

MATERIALS AND METHODS

Streptomyces cells were grown under various conditions with respect to the growth medium: C cells, *i.e.* cells grown in a so-called complete medium containing glucose, NaCl, peptone, beef extract and yeast extract. A cells, *i.e.* cells grown in a salt medium (3 g KH_2PO_4, 8 g Na_2HPO_4, 3 g NaCl, 0.3 g Na_2SO_4, 0.1 g $MgSO_4$, 2.5 mg $FeSO_4$, 5 g glucose, 5 g succinate, 0.2 mg thiamine, 0.2 mg riboflavin, 0.6 mg nicotinic acid, 0.4 mg pantothenate, and 0.4 μg biotin, all per l) which contained L-arginine (12 mM) as nitrogen donor. L-cells, *i.e.* cells grown in a salt medium containing L-lysine (12 mM) instead of L-arginine.

The cells were grown and harvested as described previously[4].

The density of the suspensions used for the experiments was 1.5–2.0 mg dry cell weight per ml buffer. The cells were incubated with the ^{14}C-labelled amino acids in 0.05 M sodium–potassium phosphate buffer, pH 7.1, at 30 °C. Cells and medium were rapidly separated by a filtration technique[4]. Since for the first 6 min. the uptake of arginine and lysine is linear with time, the difference between the 6-min. value and the 1-min. value was taken for further calculations. The radioactivity was determined using a scintillation counter (Tricarb). The radioactivities inside the cells and in the medium were calculated per g dry weight and per ml medium, respectively. The ratio between the impulses per g dry weight and the impulses per ml medium is a relative distribution ratio with the dimension ml/g. Knowing the extracellular concentration of amino acids, all other figures can easily be derived.

[L-^{14}C]arginine (uniformly labelled, spec. act. 10 Ci/mole), [L-^{14}C]lysine (uniformly labelled, spec.act. 10 Ci/mole), and 2-amino-[1-C^{14}]isobutyric acid (spec. act. 58 Ci/mole) were obtained from the Radiochemical Centre, Amersham; DL-guanidino-[^{14}C]canavanine (spec.act. 33 Ci/mole) from Schwarz/Mann Bioresearch, New York. The unlabelled amino acids were purchased from E. Merck, Calbiochem, and Fluka.

RESULTS

In the tested concentration range of 5–1200 μM the transport of basic amino acids is active and can be inhibited by structurally related analogs. Fig. 1 (a and b) shows the uptake velocity of L-arginine and L-lysine as a function of their extracellular concentration in an Eadie plot. The two components of the curve indicate that the uptake is mediated by at least two transport systems with very different affinities for their substrates. The steep part of the curve represents a component with a high half-saturation constant and a high transport capacity:

V(arginine) = 2.5 μmoles/g per min; $K_m = 0.7 \cdot 10^{-3}$ M

V(lysine) = 3.5 μmoles/g per min; $K_m = 1.6 \cdot 10^{-3}$ M

The flat part of the curve corresponds to systems with lower capacity but higher affinity for the transportees. The kinetic characterization of these systems will be given

later. First the transport specificities of *Streptomyces* cells exposed to high extracellular concentrations (>0.2 mM) are discussed.

If the transport of L-arginine or L-lysine reaches saturation using extracellular concentrations of 8 mM, neutral amino acids such as 2-aminoisobutyric acid or leucine inhibit the influx of labelled [^{14}C]arginine or [^{14}C]lysine even more than the addition of the corresponding amount of unlabelled arginine or lysine. This is not the

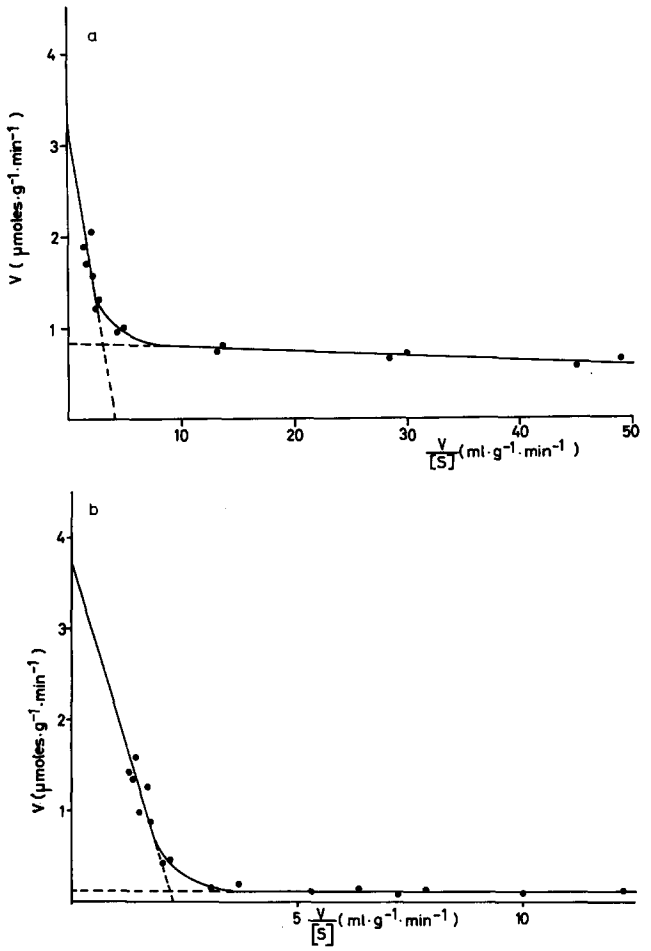

Fig. 1.(a) Eadie plot of the dependence of L-arginine influx on its extracellular concentration in C cells. Incubations were carried out in 0.05 M sodium–potassium phosphate buffer, pH 7.1, at 30 °C. 1(b) Eadie plot of the dependence of L-lysine influx on its extracellular concentration. Experimental conditions as in (a).

case if the basic amino acids are present at extracellular concentrations below 0.1 mM: in this concentration range neutral amino acids are no longer inhibitory. That arginine and lysine at high extracellular concentrations are not taken up exclusively *via* specific systems but for the greater part by the transport system for neutral amino acids, as already indicated by the competition results mentioned before, can be confirmed by

exchange experiments between neutral and basic amino acids. As indicated in Fig. 2 both lysine and arginine cause a considerable efflux of 2-aminoisobutyric acid (used representatively for neutral amino acids since it cannot be metabolized) out of the *Streptomyces* cells. Thus, the assumption seems to be valid that the steep part of the Eadie plot characterized by low affinity but high capacity represents arginine or lysine influx *via* the uptake system primarily concerned with the uptake of neutral amino acids (System n).

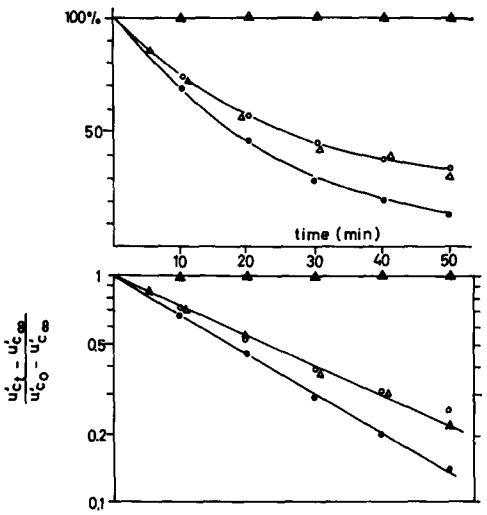

Fig. 2. Exchange between 2-aminoisobutyric acid and basic amino acids. *S. hydrogenans* cells were preloaded with 2-amino[^{14}C] isobutyric acid (extracellular concentration, 0.53 mM) in 0.05 M sodium–potassium phosphate buffer, pH 7.1, for 60 min at 30 °C. After this preincubation the cells containing 114 μmoles 2-aminoisobutyric acid per g dry weight were centrifuged and resuspended at zero time in fresh buffer containing 30 mM L-arginine (●), 30 mM L-lysine (o),0.6 mM 2-aminoisobutyric acid (△) or no extracellular amino acid as control (▲). The decrease of intracellular radioactivity was followed for 50 min. The semilogarithmic plot of the same data according to the flux equation of Heinz[5] shows a uniform course of 2-aminoisobutyric acid exit with half lifes of 17.5 min for L-arginine, 25 min for L-lysine and 25 min for 2-aminoisobutyric acid, respectively. *u'c* is the radioactivity per g dry weight and the indices 0,*t* and ∞ refer to time zero, a finite time and infinite.

On the other hand, the flat part in Fig. 1 represents the more specific uptake by a system with a high affinity. Only closely related analogs inhibit the uptake (*e.g.* canavanine inhibits arginine uptake), whereas neutral amino acids are without effect.

A kinetic analysis of the uptake of L-arginine and L-lysine in the low concentration range is shown in Fig. 3. Whereas for arginine a linear relationship between the reciprocally plotted data ($1/v$ *versus* 1/[arginine]) exists, the corresponding values for lysine transport show a curvilinear relation. These differences are caused by the very different rates at which arginine and lysine are transported *via* their specific system: whereas in the case of arginine the high-affinity system possesses a sufficiently high capacity, too, so that the part transported by System n is insignificant (less than 1% of the total transport), lysine is transported only slowly and the flux component mediated

by System n cannot be neglected. The kinetic parameters for the arginine uptake as derived from the Lineweaver–Burk plot are:

V(arginine)=0.6 μmole/g per min; K_m(arginine)=$7 \cdot 10^{-6}$ M

The evaluation of the corresponding constants for the lysine transport is much more difficult. Because of the curvilinear function shown in Fig. 3 a graphic determination of K_m and V (using the intercepts with both axes) has an inherent error. The calculated curve based on kinetic parameters determined in such a way does not fit the

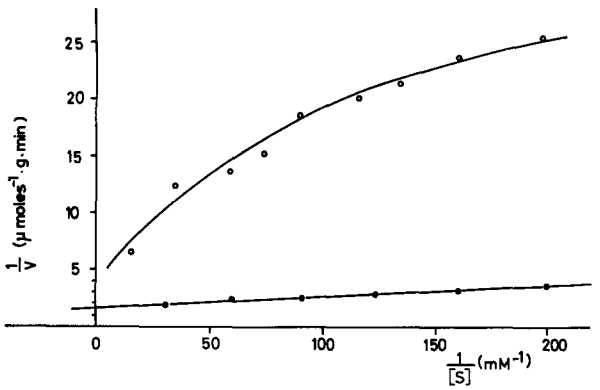

Fig. 3. Lineweaver–Burk plot of the dependence of the influx of L-arginine (●) and L-lysine (o) on low extracellular concentrations of the corresponding amino acid in wild strain cells. Experimental conditions as in Fig. 1a.

experimental values (a mathematical treatment of two-component systems is given in ref. 4). Using the graphically determined parameters as a starting point, these parameters were varied until they fitted the experimental points satisfactorily. The kinetic parameters which provide a curve in good agreement with the experimental values are:

V(lysine)=0.05 μmole/g per min; K_m(lysine)=$0.9 \cdot 10^{-6}$ M

The question whether arginine and lysine are transported by one common system or whether there are additional systems involved was tested by competition experiments. The uptake of L-[^{14}C]arginine into C cells is only inhibited by closely related analogs such as D-arginine, L-canavanine, and arginine methyl ester, but not by lysine or ornithine. In contrast, lysine analogs as well as arginine analogs reduce the influx of L-[^{14}C]lysine, the strongest inhibitor being L-arginine. The K_m and the K_i values are listed in Table I.

In C cells the inhibition constant, K_i (0.45 μM) of L-arginine for L-lysine transport is significantly lower than the K_m value (7 μM) for its own uptake since System $b_{Arg/Lys}$ cannot be detected in these cells (*cf.* Discussion).

The facts that arginine inhibits the uptake of lysine whereas lysine in reciprocal experimental conditions shows no effect on the arginine transport and that K_m and K_i for L-arginine differ widely may be explained by the assumption that arginine is transported by more than one specific system. At least two systems must exist for its uptake: one with a high transport capacity and a high specificity for arginine and a

TABLE I

(A) SUMMARY OF THE KINETIC PARAMETERS OF THE TRANSPORT SYSTEMS FOR BASIC AMINO ACIDS

Dimensions of $K_m = \mu M$ and of $V = \mu$moles/g per min. The K_m value of L-arginine for System b $_{Arg/Lys}$ can only be measured in A or L cells.

Transport substrate	*System* b_{Arg}		*System* $b_{Arg/Lys}$		*System n*	
	K_m	V	K_m	V	K_m	V
L-Arginine	7	0.6	0.6	0.01	700	2.5
L-Lysine			0.9	0.05	1600	3.5

(B) INHIBITOR CONSTANTS OF VARIOUS ANALOGS OF L-ARGININE AND L-LYSINE DETERMINED IN A SUBSTRATE CONCENTRATION RANGE BETWEEN 5–70 μM

Dimension of $K_i = \mu M$; C cells.

Labelled substrate	*Unlabelled inhibitors*				
	L-*Arginine*	D-*Arginine*	L-*Canavanine*	L-*Lysine*	L-*Ornithine*
L-Arginine	8	37	230	7000	13000
L-Lysine	0.45	97	460	0.9	54

second one mediating the transport of both arginine and lysine. As far as arginine is concerned, the second system (System b $_{Arg/Lys}$) has a much lower capacity than the specific system (System b $_{Arg}$). Lysine can be transported only by System b $_{Arg/Lys}$.

From the kinetic evidence discussed above the hypothesis of a two-component system for arginine is very likely but not proven. Further studies with the wild strain would only yield additional indirect evidence. However, if it would be possible to change the ratio of the capacities of the two systems in an appropriate way, the hypothesis becomes directly testable, *i.e.* if the capacity of System b $_{Arg/Lys}$ could be enhanced (independent of how this may be brought about), the competition pattern should change fundamentally: arginine transport should become sensitive to lysine inhibiton. Different culture media were used to select a cell type with enhanced lysine transport, since, according to the hypothesis, enhanced lysine transport would mean that the common system (System b $_{Arg/Lys}$) works more effectively. Table II shows that the influx of lysine and arginine in the low concentration range is dependent on the nitrogen donor in the culture medium. If *S. hydrogenans* cells are grown in synthetic media containing either lysine or arginine in a concentration of 12 mM as the sole N_2 source, the uptake of basic amino acids is enhanced as compared to cells grown on NH_4Cl or in a complete medium. Furthermore, the values given in Table II clearly demonstrate that in cells grown on lysine or arginine a reciprocal inhibition of influx between lysine and arginine occurs which is absent in the C cells or cells grown on NH_4Cl. In this connection special interest concerns the type of inhibition of arginine uptake by lysine. Fig. 4 shows an experiment in which the influx of L-arginine was measured together with the inhibition of this influx by two different L-lysine concentrations. The data are plotted according to Lineweaver–Burk. Under conditions of maximal inhibi-

TABLE II

UPTAKE OF L-[^{14}C]ARGININE AND L-[^{14}C]LYSINE (EXTRACELLULAR CONCENTRATION 5 μM) AND INHIBITION OF THIS UPTAKE BY UNLABELLED ARGININE AND LYSINE IN CELLS GROWN IN CULTURE MEDIA WITH DIFFERENT NITROGEN DONORS

Incubations were carried out in 0.05 M sodium–potassium phosphate buffer, pH 7.1, at 30 °C. Uptake in nmoles/g dry wt per min.

Transport substrate	*Inhibitor*	*Nitrogen donor during growth*			
		Complete medium	*NH_4Cl*	L-*Arginine*	L-*Lysine*
L-Arginine	None	165	145	204	966
L-Lysine	None	38	59	358	1028
L-Arginine	L-Lysine (0.12 mM)	165	145	41	178
L-Lysine	L-Arginine (0.012 mM)	17	0	47	138

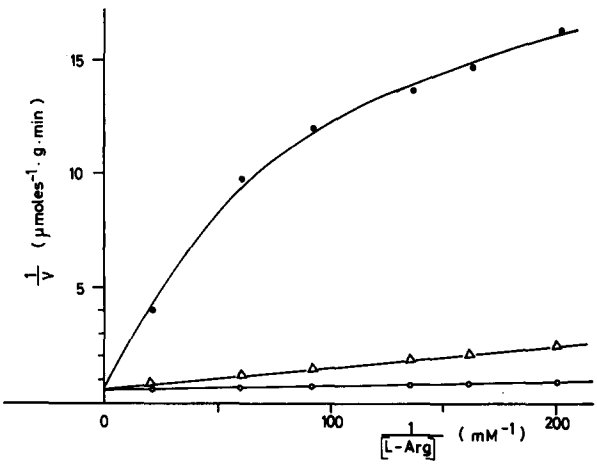

Fig. 4. Lineweaver–Burk plot of L-arginine influx in selected cells and inhibition of this influx by two different L-lysine concentrations. Cells were grown in a synthetic medium containing 12 mM L-arginine as nitrogen donor (for detail see text). The incubations were carried out in 0.05 M sodium–potassium phosphate buffer, pH 7.1, at 30 °C. ○, arginine without inhibitor; △, with 0.06 mM L-lysine as inhibitor and ●, with 6 mM L-lysine as inhibitor.

tion of arginine uptake by lysine (extracellular concentration 6 mM), a biphasic curve appears, indicating that arginine transport is partly insensitive to lysine, thus favoring the assumption of the second highly specific system (System b $_{Arg}$). A second experimental result supports the two-component concept, too: the Michaelis constants for arginine transport in C cells and cells grown in synthetic medium are not in agreement This behavior can be expected because in both cell types the dependence $1/v$ *versus* 1/[arginine] is represented by a straight line although arginine uptake is mediated by two systems. One system is veiled since the differences in transport capacity of both systems are very great. The part played by the low capacity system is negligibly small and the kinetic parameters derived from a double reciprocal plot correspond approxi-

mately to the constants of the more potent system. In C cells System b_{Arg} has the greater capacity, in A and L cells System $b_{Arg/Lys}$ is the more potent one.

DISCUSSION

Multiple transport systems for the uptake of basic amino acids have been described in several microorganisms. The assumption of two or more transport routes was based partly on incongruous competition patterns at different concentrations of the transport substrates and more convincingly established by more potent kinetic and genetic methods[6-12].

Our evidence supporting the existence of multiple forms of basic amino acid transport in *S. hydrogenans* is the following: The biphasic curves shown in Figs 1a and 1b indicate that the transport of both arginine and lysine is mediated by at least two transport systems. Free diffusion can be excluded since the fluxes of arginine and lysine are sensitive to the addition of the unlabelled species, amino acid analogs and metabolic inhibitors such as 2,4-dinitrophenol. Furthermore, the fluxes can be stimulated or inhibited by intracellular amino acids, a phenomenon described in detail previously or inhibited by intracellular amino acids, a phenomenon described in detail previously[13,14]. That the additional component with low affinity for arginine and lysine is identical with the transport system for neutral amino acids (System n) is documented in two ways: (i) the loss of specificity when going from low to high extracellular concentrations of basic amino acids (neutral amino acids become competitive inhibitors) and (ii) the exchange between neutral and basic amino acids, *e.g.* the efflux of 2-aminoisobutyric acid as a representative for neutral amino acids is greatly enhanced by high extracellular concentrations of arginine or lysine. The affinity of this system is very low but because of its high capacity it mediates at high concentrations the greatest part of the arginine (75%) and lysine (99%) influx.

Besides this less specific transport route at least two systems exist which exclusively transport basic amino acids. A single system does not explain the experimental results. In C cells the inhibition patterns of lysine and arginine uptake are different. Furthermore, the competition pattern is fundamentally changed if cells are grown in synthetic media containing L-arginine or L-lysine as N_2 donor. Whereas in the latter cells lysine inhibits the influx of arginine, this is not the case in C cells. Arginine, however, is the most potent inhibitor of lysine uptake in both cell types.

This behavior can be satisfactorily explained by the following assumption. *Streptomyces* cells possess a highly specific transport system for arginine (System b_{Arg}) and an additional common system for both, arginine and lysine (System $b_{Arg/Lys}$). At low extracellular arginine concentrations transport is nearly completely mediated by System b_{Arg}. The amount of arginine taken up *via* system $b_{Arg/Lys}$ is negligibly small since the latter system has only a small transport capacity (*cf.* maximal velocities of Table I). Therefore it is plausible that lysine cannot inhibit arginine transport significantly. On the other hand it is clear that in the Lineweaver–Burk plot a straight line is found although two systems are present. A detailed discussion of the ability to resolve multiple-component systems in the common graphic plots is given elsewhere[4].

In the A and L cells the relation between the capacities of the two systems, System $b_{Arg/Lys}$ and System $b_{Arg/Lys}$, is changed. Now the common system mediates the major part of arginine uptake. The inhibition of arginine uptake by lysine is easily

detectable since the major flux component is inhibited. The K_i value found for the inhibitory effect of arginine on lysine uptake in C cells ($0.45 \cdot 10^{-6}$ M) is in fairly good agreement with the K_m value of arginine found for the common system of A and L cells ($0.6 \cdot 10^{-6}$ M).

ACKNOWLEDGEMENTS

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (GR 424). We are grateful to S. Knöss, G. Hartmann and I. Wönne for their technical assistance.

REFERENCES

1 Gross, W. and Ring, K. (1971) *Biochim. Biophys. Acta* 233, 652–665
2 Ring, K. and Heinz, E. (1966) *Biochem. Z.* 344, 446–461
3 Gross, W. (1969) Habilitationsschrift, Frankfurt
4 Gross, W., Geck, P. Burkhardt, K.-L. and Ring, K. (1972) *Biophysik* 8, 271–279
5 Heinz, E. (1957) *J. Biol. Chem.* 225, 305–315
6 Grenson, M. (1966) *Biochim. Biophys. Acta* 127, 339–346
7 Hunter, D. R. and Segel, I. H. (1971) *Arch. Biochem. Biophys.* 144, 168–183
8 Wilson, O. H. and Holden J. T. (1969) *J. Biol. Chem.* 244, 2737–2742
9 Friede, J. D., Gilboe, P. D., Triebwasser, K. C. and Henderson, L. M. (1972) *J. Bacteriol.* 109, 179–185
10 Miller, D. L. and Rodwell, V. W. (1971) *J. Biol. Chem.* 246, 1765–1771
11 Fan, C. L., Miller, D. L. and Rodwell, V. W. (1972) *J. Biol. Chem.* 247, 2283–2288
12 Pall, M. L. (1970) *Biochim. Biophys, Acta* 203, 139–149
13 Ring, K., Gross, W. and Heinz, E. (1970) *Arch. Biochem. Biophys.* 137, 243–252
14 Gross, W., Ring, K. and Heinz, E. (1970) *Arch. Biochem. Biophys.* 137, 253–261