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# The Concentration of Glycine by Preparations of the Yeast *Saccharomyces carlsbergensis* Depleted of Adenosine Triphosphate

## EFFECTS OF PROTON GRADIENTS AND UNCOUPLING AGENTS

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1. At pH4.5 and 30°C, yeast preparations depleted of ATP in the presence of antimycin and deoxyglucose spontaneously lost $K^+$, gaining roughly an equivalent amount of $H^+$. 2. Five proton conductors including azide and 2,4-dinitrophenol accelerated this process, as did [$^{14}$C]glycine, which was absorbed with two extra equivalents of $H^+$. 3. The rate of glycine uptake at pH4.5 diminished fourfold when cellular $K^+$ fell by 20%. 4. The distribution of [$^{14}$C]propionate indicated that the intracellular pH fell from 6.2 to 5.7 when the cellular content of $K^+$ fell by 30%. 5. Glycine uptake from a 5$\mu$M solution was about 400 times faster at pH4.5 than it was at pH7.4 with 100mM-KCl present ostensibly to lower the membrane potential. 6. Yeast preparations containing 2mM-[$^{14}$C]glycine absorbed a further amount from a 0.1$\mu$M solution at pH4.5. After about 10min a net movement of [$^{14}$C]glycine out of the yeast occurred. The ratio of the cellular [$^{14}$C]glycine concentration to the concentration outside the yeast reached $4\times10^4$ in these assays, whereas at pH7.4 in the presence of 100mM-KCl it did not exceed 15 in 3h. Dinitrophenol lowered the accumulation ratio at pH4.5, apparently by causing proton conduction. 7. The observations are consistent with the notion that glycine uptake is driven by a proton symport mechanism. 8. Possible factors governing the strikingly low rate of glycine efflux as opposed to its optimum rate of influx are discussed.

Proton symport mechanisms appear to be involved in solute accumulation in various micro-organisms (Simoni & Postma, 1975; Hamilton, 1975). The energetics of these processes are, however, poorly understood. Cockburn *et al.* (1975) pointed out that, for given values of extracellular $[H^+]$ ($[H^+]_{out}$), cellular $[H^+]$ ($[H^+]_{in}$) and the membrane potential, the energy input into such transport systems increases with the number ($n$) of protons accompanying the solute molecule across the cell membrane. Previous work in these laboratories with various strains of *Saccharomyces* species indicated that $n$ is 2 for the so-called yeast general amino acid permease, whereas $n$ is 1 for certain specific amino acid permeases such as the one that absorbs proline (Eddy & Nowacki, 1971; Seaston *et al.*, 1973). Values of $n$ greater than unity have not been reported for bacterial transport systems (Hamilton, 1975), so that in the course of the present work it seemed useful to confirm that there are indeed conditions where glycine is absorbed with two equivalents of protons by one of the above yeast strains. We have further studied the effects of changing the extracellular concentrations of $H^+$ and $K^+$ on the rate and extent to which the yeast preparations concentrate glycine when their ATP content is greatly depleted (Eddy *et al.*, 1970*b*). The behaviour of the system in the presence of selected proton conductors was also examined, with the object of manipulating the size of the proton gradient acting across the plasmalemma (Mitchell, 1970).

### Materials and Methods

These were exactly as described by Seaston *et al.* (1973). The strain N.C.Y.C. no. 74 (British National Collection of Yeast Cultures, Nutfield, Surrey, U.K.) of *Saccharomyces carlsbergensis* was again used. The mineral salts/nutrient medium normally contained 2% (w/v) glucose and 0.2% (w/v) $(NH_4)_2SO_4$ (Eddy *et al.*, 1970*a*). The yeast was harvested in the exponential phase of growth, washed with water and stored at 0°C for up to 8h. At intervals portions (50mg dry wt.) were put for 20min with 5% (w/v) glucose at 30°C in 10ml of 20mM-Tris solution adjusted to pH4.5 with citric acid. The yeast was collected, washed and at once used as follows. (1) The absorption of [$^{14}$C]glycine, the efflux of $K^+$ and the accompanying proton uptake were assayed at 30°C as described by Eddy & Nowacki (1971). To study proton absorption the washed cells (50mg dry wt.) were suspended in 5mM-Tris adjusted to pH4.5 with citric acid, then 25$\mu$g of antimycin and 2.5mM-2-deoxyglucose were

quickly added in turn to give a final volume of about 4ml. The amino acid (0.5$\mu$mol) or uncoupling agent (0–200$\mu$M) were next added as required, the pH of the stirred suspension being recorded continuously. The buffering capacity of the system was later calibrated by the addition of 1$\mu$mol of HCl. $K^+$ efflux was assayed by determining the $K^+$ content of the extracellular phase at intervals. $NaN_3$ and 2,4-dinitrophenol were added in aqueous solution. The other uncoupling agents were dissolved in dimethyl sulphoxide, the separate effects of which appeared negligible. (2) The uptake and efflux of glycine in assays of several hours duration were studied by the procedures described in the Results section which involve relatively dilute cell suspensions (1mg dry wt./ml). The amino acid was added to the yeast preparations after both deoxyglucose and antimycin.

*Average cellular pH*

The yeast (50mg dry wt.) was suspended at 30°C in 20mM-Tris/citrate buffer, pH4.5, containing 0.2mM-[$^{14}$C]propionate (0.1$\mu$Ci/ml), deoxyglucose and antimycin. The uptake of propionate by the yeast reached a maximum in about 3min. The intracellular pH was computed on the basis of $pK_a = 4.87$ for propionate. Similar results were obtained with 0.2 and 1mM-propionate.

*Chemicals*

2,4-Dinitrophenol was obtained from BDH Chemicals Ltd., Poole, Dorset, U.K., $NaN_3$ from Hopkin and Williams, Romford, Essex, U.K. Pentachloronitrobenzene was a product of Fluka AG, CH-947P, Buchs, Switzerland. Carbonyl cyanide *m*-chlorophenylhydrazone was obtained from Calbiochem Ltd., London W.1, U.K., whereas 3,3',4',5-tetrachlorosalicylanilide and 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole were generous gifts from Dr. W. A. Hamilton of the University of Aberdeen, Scotland, U.K., and Dr. D. Griffiths, University of Warwick, Coventry, U.K., respectively. [1-$^{14}$C]Glycine and [2-$^{14}$C]propionate were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

## Results

*Influx of $H^+$ and the efflux of $K^+$ caused by glycine acting in yeast preparations depleted of ATP*

The uptake of glycine by the yeast preparations resulted in a roughly proportional accelerated absorption of protons (Fig. 1). A regression analysis of the 62 pairs of observations showed that 2.00±0.11 additional protons were absorbed with each equivalent of glycine. The efflux of $K^+$ was correspondingly stimulated under these conditions (Eddy & Nowacki, 1971).

*Effects of various uncoupling agents on proton uptake, glycine uptake and $K^+$ efflux*

The washed yeast (50mg dry wt.) was suspended at 30°C in 5mM-Tris buffer adjusted to pH4.5 with citric acid, together with antimycin (25$\mu$g) and 2.5mM-deoxyglucose (final vol. 4ml). A selected amount of the uncoupling agent was added at 0.5min and the rate of proton uptake assayed with the pH electrode (see the Materials and Methods section). After 1.5min, 0.5$\mu$mol of [$^{14}$C]glycine (0.5$\mu$Ci) was added.

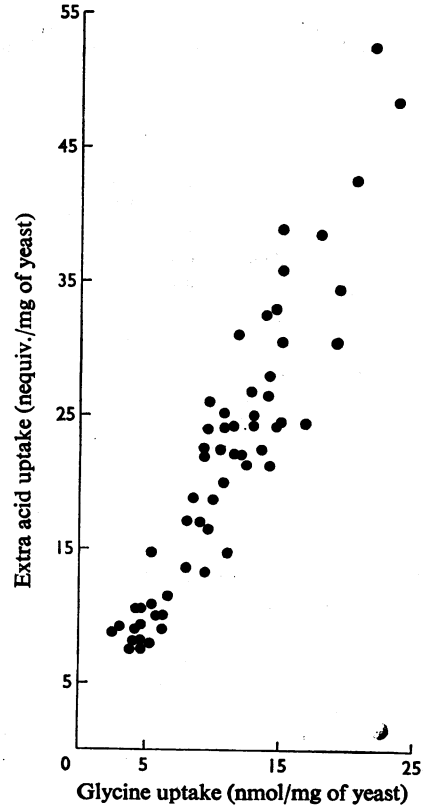



Fig. 1. *Number of extra protons absorbed with increasing amounts of glycine*

The yeast was grown with glucose and $(NH_4)_2SO_4$ and then kept at 30°C with 5% (w/v) glucose for 30min. The washed cells (50mg dry wt.) were suspended at pH4.5 with 25$\mu$g of antimycin, 5mM-2-deoxyglucose in a final volume of 4ml. In the course of 62 assays with eight preparations of the yeast the amount of [$^{14}$C]glycine added to the system was varied in seven steps from 0.25 to 2.5$\mu$mol. The amount of glycine absorbed was measured by assaying the proportion of the original $^{14}$C (0.5$\mu$Ci) left in the supernatant solution when the yeast was separated by centrifugation 2–8min later. The extra number of acid equivalents absorbed up to that time was also determined (see the Materials and Methods section).

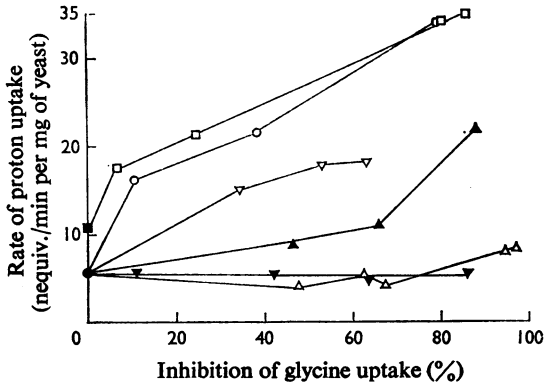



Fig. 2. *Inhibition of glycine uptake as a function of the rate of proton uptake caused by graded concentrations of various compounds*

The assays are described in the text. In each case the rate of proton uptake increased with the concentration of the uncoupling agent added. The inhibition of glycine uptake was computed as (control rate − observed rate)/(control rate), expressed as a percentage. Mean values based on at least three assays are shown. ○, Dinitrophenol (50, 100 and 200 $\mu$M); ▽, carbonyl cyanide *m*-chlorophenylhydrazone (50, 75 and 100 $\mu$M); ▲, tetrachlorotrifluoromethylbenzimidazole (25, 50 and 100 $\mu$M); △, tetrachlorosalicylanilide (10, 20, 30, 50 and 100 $\mu$M); ▼, pentachloronitrobenzene (10, 25, 50 and 100 $\mu$M); □, $NaN_3$ (50, 100, 1000 and 2000 $\mu$M); ●, control; ■, control+2mM-NaCl, for the azide series.

The amount of $^{14}C$ left in the solution after a further 1min was then assayed. The uncoupling agent was omitted from the controls.

Five presumptive proton conductors (Henderson, 1971) were studied by the above procedure, their behaviour being compared with that of pentachloronitrobenzene. This fungicide (Martin, 1971) lacks the ionizable groups on which proton conduction appears to depend (Mitchell, 1966). Each of the uncoupling agents in fact immediately accelerated the uptake of protons by the yeast to some extent (Fig. 2) and caused a roughly proportional additional efflux of $K^+$ during the 2min of observation (results not shown). The chloronitrobenzene produced no such effects. In five assays the extra acid uptake that 0.1mM-dinitrophenol caused in 20min roughly equalled the extra efflux of $K^+$ over the same period, the former being 6.4±0.5 (S.E.M.) $\mu$equiv. per 50mg dry wt. of yeast and the latter 5.5±0.4 $\mu$equiv.

Fig. 2 compares the rate of proton uptake a given concentration of the uncoupling agent caused with its effect on the absorption of glycine. In general the rate of proton uptake was a different function of the percentage inhibition of glycine uptake for each compound, except for azide and the dinitrophenol which were similar in that respect. All of the observations would have fitted the same mathematical relationship, however, if these compounds, as a group, lowered the uptake of glycine simply by increasing the permeability of the yeast plasmalemma to protons in varying degrees. Inspection of Fig. 2 suggests further that both tetrachlorosalicylanilide and pentachloronitrobenzene inhibited glycine uptake by a mechanism that appeared not to depend on an increase in proton conduction. This was in contrast with the behaviour of azide and dinitrophenol where such an increase appeared to provide a common basis for their effects on glycine absorption. Dinitrophenol was accordingly selected for further study (see below).

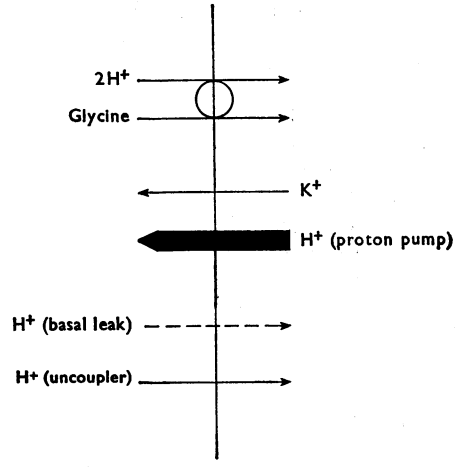
### *Proton symport model*

In Scheme 1, on the basis of the above observations and those of Pena (1975), the efflux of $K^+$ is electrically coupled to the uptake of $H^+$ through the separate pathways represented by the proton conductor, the glycine carrier and the leak. Because the natural low-proton permeability appeared to limit the efflux of $K^+$ when only the leak pathway was functioning, the cell interior would then be electrically negative relative to the exterior. The uptake of $K^+$ during energy metabolism would be caused by hyperpolarization of the cell associated with the ejection of protons through the proton pump. In a similar way protons absorbed with glycine would be recycled through the proton pump during energy metabolism. We agree with Hamilton (1975) and Eddy & Nowacki (1971) that $K^+$ probably plays no indispensable role in this process.

Conway & O'Malley (1946) and Rothstein (1960) measured the ratio $[K^+]_{in}/[K^+]_{out}$ formed in the presence of glucose both at pH4.5 and near pH2.5. We estimate from their observations that the maximum value of the product $[H^+]_{out}[K^+]_{in}/[H^+]_{in}[K^+]_{out}$ was equivalent to about 5 units of pH, or 300mV. In terms of Scheme 1 the membrane potential at pH4.5 would be about −200mV.

### *Net glycine accumulation in the presence of deoxyglucose and antimycin as a function of the ionic gradients*

Preliminary work showed that the rapid absorption of glycine from a 5 $\mu$M solution, containing 1mg dry wt. of yeast/ml, 1mM-deoxyglucose and 1 $\mu$g of antimycin/mg of yeast, all at pH4.5, depleted the glycine content of the medium by more than 95% during 5min incubation at 30°C. Eddy & Nowacki (1971) showed that the $^{14}C$ accumulating in the yeast, under conditions similar to these, first, behaved like glycine during chromatography and secondly, on the basis of ninhydrin assays of the extracellular solution, represented a net uptake rather than an exchange process.




Scheme 1. *Relations between the flow of protons, $K^+$ and glycine across the yeast plasmalemma*

When energy metabolism is inhibited by antimycin and deoxyglucose, the ejection of $H^+$ through the proton pump stops. $K^+$ then leaks out of the yeast by exchanging with $H^+$ carried in (1) with glycine, (2) by catalysed conduction in the presence of an uncoupling agent and (3) through the leak pathway. The eventual distribution of glycine will be governed both by the membrane potential and the $H^+$ gradient across the cell membrane. The following factors govern the initial rate of uptake of the solute. Let the carrier E be uncharged before protonation and assume, for simplicity, that only 1 $H^+$ is involved as co-substrate with the solute (S). Let the ordered reactions $E+S = ES$, $ES + H^+ = ESH^+$ reach equilibrium at each membrane face, with $K_1 = [E][S]/[ES]$ and $K_2 = [ES][H^+]/[ESH^+]$. Suppose further that only E and $ESH^+$ traverse the membrane, the rate constants being $k_{+1}$ and $k_{-1}$ for E and $k_{+2}$ for $ESH^+$. The initial rate of uptake of S is then proportional to:

$$k_{+2}[S]_{out}[H^+]_{out}/[K_1K_2(1+k_{+1}/k_{-1}) + [S]_{out}K_2 + [S]_{out}[H^+]_{out}(1+k_{+2}/k_{-1})]$$

where the subscript out denotes the outer solution. The significance of this equation is discussed in the text.

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The latter conclusion was confirmed in the present work by using 37 $\mu$M-glycine, the lowest concentration at which such assays were feasible (four expts.). The ratio of the average cellular concentration of the [$^{14}$C]glycine ($[Gly]_{in}$), accumulated from a 5 $\mu$M solution, to the concentration of $^{14}$C left outside the cells ($[Gly]_{out}$), eventually exceeded the factor $2\times10^3$. This direct method of assaying the magnitude of the glycine gradient ($[Gly]_{in}/[Gly]_{out}$) formed by the yeast leads to erroneous results, however, when the amount of [$^{14}$C]glycine eventually left in solution is similar to the amount of absorbed radioactive impurities that commercial preparations of [$^{14}$C]glycine appear to contain, a quantity typically about 3–4% of the $^{14}$C present (K. Indge, personal communication).

*Serial-loading technique*

The assays illustrated in Table 1 were done both to circumvent the above difficulty and to study the efflux of glycine from the yeast. The yeast was first loaded for 5 min with [$^{14}$C]glycine, at pH 4.5 in the presence of the metabolic inhibitors. The preparation was then transferred to a solution containing, initially, about 0.1 or 1 $\mu$M-glycine of the same specific activity as that used to load the cells. The system was either at pH 4.5 or at pH 7.4, with or without 100 mM-KCl present. A small amount of $^{14}$C-labelled material, which varied from 0.05 to 0.1 nmol/ml in different experiments and which was presumably glycine, had leaked from the yeast before the first sample was taken in these assays. This behaviour was taken into account in computing how the extracellular glycine concentration changed after the loaded yeast was exposed to glycine a second time. Table 1 shows that the yeast continued to absorb some glycine for up to 10 min at pH 4.5, but without exhausting the $^{14}$C content of the solution, so that errors caused by the presence of unabsorbed impurities could be neglected in computing the maximum value of the glycine gradient that the yeast formed. At pH 7.4, however, or in the presence of 100 mM-KCl, a steady net efflux rather than a temporary further uptake of glycine occurred.

We attribute the sequence of the net uptake of glycine at pH 4.5, followed by the net efflux of the amino acid, to the circumstance that the cellular pH fell as the yeast continued to absorb $H^+$ in exchange for $K^+$. Thus after 4 and 8 min respectively, in the presence of both antimycin and deoxyglucose, the distribution of propionate indicated that the average cellular pH was 6.24±0.01 (6) and 6.17±0.03 (6) S.E.M. The respective amounts of $K^+$ that had leaked from the yeast were 33 nequiv./mg and 54 nequiv./mg. When the yeast preparation had lost about 170 nequiv. of $K^+$/mg, by absorbing more $H^+$ from the solution, the cellular pH was 5.69±0.04 (6). This loss of $K^+$ occurred in about 40 min at 30°C when the yeast was kept at pH 4.5 with the two metabolic inhibitors, and in 8 min when 0.1 mM-dinitrophenol was also present. The above observations show that the pH gradient across the plasmalemma during the interval from 4 to 8 min was about 1.7 units if the cell is treated as a single compartment.

The choice of relatively low concentrations of glycine in the above assays was based on the following consideration. As noted by Eddy & Nowacki (1971), starving the yeast preparations at pH 4.5 in the absence of glycine considerably lowered the subsequent rate of uptake of that compound. We have now found that this phenomenon depends on the absorption of $H^+$ into the yeast in exchange for $K^+$. Thus the following procedures each lowered cellular $K^+$ by about 0.1 $\mu$equiv./mg and also lowered the initial rate of glycine uptake by about 75%: (1) starving the

Table 1. *Net movement of glycine after the transfer of ATP-depleted yeast already loaded with [$^{14}$C]glycine to solutions at either pH4.5 or 7.4 containing 100mM-KCl*

The washed yeast (10mg) was suspended at 30°C for 5min in 10ml of 20mM-Tris/citrate buffer solution, pH4.5, containing antimycin (10$\mu$g), 1mM-deoxyglucose and 15.6$\mu$M-[$^{14}$C]glycine (9$\mu$Ci). The yeast was recovered, washed and transferred to 20mM-Tris/citrate buffer solution (10ml) containing antimycin and deoxyglucose and the other components indicated, at 30°C. Samples (1ml) were taken at the stated times, mixed with 4ml of the appropriate buffer solution at 0°C, and the $^{14}$C content of the yeast and the extracellular solution was assayed. $[Gly]_{out}$ = [Gly] in the undiluted extracellular solution computed from its $^{14}$C content. $[Gly]_{in}$ was the similar quantity for the yeast, based on a standard cellular water content of 3.26$\mu$l/mg dry wt. of yeast. Changes in the latter quantity with time and with pH did not exceed 11% and were therefore neglected.

| Buffered yeast suspension with antimycin and deoxyglucose at | Time after resuspension (min) ... | 2 | 5 | 10 | 20 | 30 | 60 |
|---|---|---|---|---|---|---|---|
| pH4.5+0.1$\mu$M-[$^{14}$C]glycine | $[Gly]_{out}$ ($\mu$M) | 0.20 | 0.13 | 0.09 | 0.11 | 0.14 | |
| | $[Gly]_{in}$ ($\mu$M) | 2277 | 2314 | 2327 | 2379 | 2271 | |
| | $[Gly]_{in}/[Gly]_{out}$ | 11600 | 17820 | 26120 | 21560 | 16790 | |
| pH4.5+1.0$\mu$M-[$^{14}$C]glycine | $[Gly]_{out}$ ($\mu$M) | 1.02 | 0.58 | 0.31 | 0.31 | 0.30 | |
| | $[Gly]_{in}$ ($\mu$M) | 2348 | 2519 | 2569 | 2753 | 2429 | |
| | $[Gly]_{in}/[Gly]_{out}$ | 2310 | 4360 | 8330 | 8840 | 8170 | |
| pH4.5+100mM-KCl+0.1$\mu$M-[$^{14}$C]glycine | $[Gly]_{out}$ ($\mu$M) | 0.26 | 0.25 | 0.31 | 0.43 | 0.52 | 0.69 |
| | $[Gly]_{in}$ ($\mu$M) | 3595 | 3264 | 3328 | 3307 | 3232 | 3115 |
| | $[Gly]_{in}/[Gly]_{out}$ | 14010 | 13080 | 10840 | 7620 | 6270 | 4510 |
| pH4.5+0.2mM-2,4-dinitrophenol | $[Gly]_{out}$ ($\mu$M) | 0.06 | 0.08 | 0.13 | 0.24 | 0.37 | 0.75 |
| | $[Gly]_{in}$ ($\mu$M) | 2139 | 2093 | 2000 | 1967 | 1935 | 1769 |
| | $[Gly]_{in}/[Gly]_{out}$ | 33670 | 25210 | 15370 | 8300 | 5170 | 2345 |
| pH7.4+100mM-KCl | $[Gly]_{out}$ ($\mu$M) | 0.07 | 0.11 | 0.17 | 0.30 | 0.39 | 0.62 |
| | $[Gly]_{in}$ ($\mu$M) | 2070 | 2009 | 1983 | 1923 | 1901 | 1730 |
| | $[Gly]_{in}/[Gly]_{out}$ | 29490 | 18617 | 11570 | 6360 | 4860 | 2790 |

yeast (5mg/ml) for 15min in the presence of deoxyglucose and antimycin; (2) starving it for 5min in the presence also of 0.1mM-dinitrophenol, and then removing the latter by washing the cells; (3) the absorption of glycine itself, from a 0.5mM solution containing deoxyglucose and antimycin, for 4min. It seems that the amount of glycine the yeast absorbed quickly was limited by relatively small changes in the cellular pH.

*Magnitude of the glycine gradient ($[Gly]_{in}/[Gly]_{out}$)*

Table 2 summarizes a series of estimates of the maximum glycine gradient formed at pH4.5 as compared with pH7.4, in each instance with or without added KCl. The initial rate of uptake of glycine is also shown. It was about 400 times faster at pH4.5 in the solution lacking $K^+$ than at pH7.4 with 100mM-KCl present.

Table 2 shows that the magnitude of the glycine gradient formed at pH4.5 was very much larger than that formed at pH7.4 in the presence of 100mM-KCl. Indeed, in the latter circumstances the yeast concentrated the amino acid only to a small extent, the mean value of $[Gly]_{in}/[Gly]_{out}$ observed in 11 assays being 9.9±1.2 (S.E.M.). This behaviour, together with the inhibitory effect of KCl observed at both pH values, is qualitatively consistent with the notion that the yeast preparations accumulated glycine at the expense of the proton gradient acting across the plasmalemma (Scheme 1).

In five assays at pH4.5, using the serial-loading technique, the maximum value of the ratio of the cellular/extracellular glycine concentrations formed in the presence of deoxyglucose, antimycin and 0.1$\mu$M-glycine, ranged from $1.8\times10^4$ to $6.8\times10^4$ about a mean value of $4.3\times10^4$.

*Glycine efflux*

A yeast preparation containing 7nmol of glycine/mg, representing roughly a 2mM solution in the cellular water, lost about 12pmol of $^{14}$C/min per mg at pH7.4 in the presence of 100mM-KCl (Table 1). Raising the glycine content of the yeast to 0.25$\mu$mol/mg raised the efflux of glycine almost proportionately to 0.28nmol/min per mg. A similar small proportion of the total cellular content of glycine left the yeast during a 10min interval whether deoxyglucose and antimycin were both present or absent. A yeast preparation containing 18nmol of glycine/mg released 5pmol of glycine/min per mg into a solution at pH4.5 containing 1mM-[$^{12}$C]glycine. Other preliminary work showed that although the addition of 1mM-[$^{12}$C]glycine caused no marked change in the rate of efflux of $^{14}$C, it prevented the reabsorption of

Table 2. *Initial rate of glycine uptake and the maximum ratio of cellular [Gly]/extracellular [Gly] as a function of extracellular pH and [K+]*

The washed yeast (10mg dry wt.) was suspended at 30°C in the indicated solution (10ml) containing antimycin (10$\mu$g), 1mM-deoxyglucose and [$^{14}$C]glycine. Samples (1ml) were taken at intervals up to 4h. The $^{14}$C content of the yeast, expressed as the concentration in the cellular water ($[Gly]_{in}$), as well as the concentration of $^{14}$C in the suspension medium ($[Gly]_{out}$) were both then assayed. The maximum value of the ratio of these two quantities is shown below and the time at which the maximum was reached. The large accumulation ratios formed at pH4.5 were studied by loading the yeast with glycine in two stages (see Table 1 and text). The results of one of two to five assays giving similar results are shown.

| Conditions | Initial rate of glycine uptake (pmol/min per mg) | Eventual glycine distribution $[Gly]_{out}$ ($\mu$M) | Eventual glycine distribution $[Gly]_{in}$ ($\mu$M) | Eventual glycine distribution $[Gly]_{in}/[Gly]_{out}$ | Time (min) |
|---|---|---|---|---|---|
| pH4.5+5$\mu$M-glycine | 805* | <0.6 | 1310 | >2000 | 30 |
| pH4.5+100mM-KCl+5$\mu$M-glycine | 370 | 1.4 | 1065 | 770 | 60 |
| pH4.5+0.2mM-dinitrophenol+5$\mu$M-glycine | 95 | 4.0 | 250 | 62 | 120 |
| pH7.4+5$\mu$M-glycine | 90 | 3.6 | 457 | 126 | 120 |
| pH7.4+5mM-KCl+5$\mu$M-glycine | 5 | 5.2 | 76 | 15 | 180 |
| pH7.4+100mM-KCl+5$\mu$M-glycine | 2 | 5.2 | 51 | 10 | 180 |
| pH7.4+100mM-KCl+0.1$\mu$M-glycine | 0.1 | 0.1 | 1.2 | 12 | 180 |
| pH4.5 with 15$\mu$M-glycine for 5min then to 0.1$\mu$M-glycine | | 0.06 | 3046 | 49800 | 5 |

* When $[Gly]_{out}$ was saturating $V_{max.}$ was about 10nmol/min per mg.

such $^{14}$C. It seems likely, therefore, that the small amount of $^{14}$C leaking from the cells did indeed represent glycine.

*Efflux of the endogenous amino acids*

Boiling the cell preparations with water typically extracted 0.42$\mu$mol of ninhydrin-positive material/mg of yeast, computed as glycine. During assays of 30min duration with 10mg of yeast/ml at pH7.4, similar material accumulated in the suspension medium, at a steady rate of about 0.4nmol/min per mg in the presence of antimycin, deoxyglucose and 100mM-KCl. The corresponding rate at pH4.5, in the absence of KCl, was about 0.06nmol/min. Thus the relatively low rate of efflux of [$^{14}$C]glycine was matched by a relatively low rate of efflux of the endogenous amino acids. Further, the yeast retained these better at the lower pH. It is notable that the presence of such a large endogenous amino acid pool seemed to play no part in the absorption of [$^{14}$C]-glycine. The explanation appears to be that the efflux of the cellular amino acids was directly proportional to their concentrations and was kinetically independent of the influx of glycine over the range of values of extracellular [Gly], [H+] and [K+] that were studied.

*Effect of dinitrophenol on the net movements of glycine*

The observations depicted in Fig. 1 are consistent with the notion that dinitrophenol lowered the initial rate of glycine uptake by causing proton conduction. In a similar way the accumulation of glycine in the yeast during intervals of up to 3h was markedly inhibited by 0.2mM-dinitrophenol (Table 2), as would be expected if the compound virtually short-circuited the proton gradient. The efflux of glycine at pH4.5 in the presence of 0.2mM-dinitrophenol and at pH7.4 with 100mM-KCl present took place at roughly the same rate (Table 1). The proton gradient would be relatively small in both circumstances (Scheme 1).

## Discussion

The observations shown in Table 2 strongly indicate that the flow of protons into the yeast with glycine (Fig. 1) can lead to the formation of large gradients of amino acid concentration across the plasmalemma. When equilibrium is reached $[Gly]_{in}/[Gly]_{out} = ([H^+]_{out}/[H^+]_{in})^2 e^{-2VF/RT}$, where $V$ is the membrane potential and $F$, $R$ and $T$ have their usual significance. Unfortunately the magnitude of $V$ is not known, nor can it be computed simply from the distribution of $K^+$ because, in general, these ions were not at equilibrium. Studies with fluorescent carbocyanine dyes indicate that the passage of glycine into mouse ascites tumour cells depolarizes the plasmalemma (Philo & Eddy, 1975). An extension of these observations to the yeast preparations has given similar results and thus supports the notion that glycine influx is an electrogenic process as is implied in Scheme 1 (R. Brocklehurst, unpublished work). However, it remains to be seen whether the absolute

magnitude of $V$ can be determined by such procedures.

*Relative rates of influx and efflux of glycine*

The above observations confirm other reports (Swenson & Betts, 1963; Crabeel & Grenson, 1970; Kotyk *et al.*, 1971) that the efflux of amino acids from yeast, over a wide range of cellular amino acid concentrations, is very slow in comparison with the optimum rate of amino acid absorption. The latter rate was about 8nmol/min per mg of yeast at pH4.5 when $[Gly]_{out}$ was 0.2mM and the preparation was depleted of ATP. Thus $V_{max.}$ for efflux, under the conditions in which the process has been studied so far, is probably relatively small, being <0.5nmol/min per mg. Further, glycine efflux was not greatly accelerated, if at all, in the presence of dinitrophenol. Dinitrophenol nevertheless accelerated both cytosine efflux from yeast (Chevallier *et al.*, 1975) and the efflux of β-galactosides from *Escherichia coli* (Simoni & Postma, 1975). In both instances the stimulated rate of efflux approached the initial rate of solute absorption.

Boller *et al.* (1975) suggested that the main vacuoles of certain yeasts accumulate amino acids, a circumstance that might hinder their efflux across the plasmalemma. However, an insignificant proportion of the glycine accumulated at pH4.5 (Table 2) appeared to be located in the vacuoles (K. Indge, A. Seaston & A. Eddy, unpublished work). We conclude that the observed relatively low rates of efflux of the amino acids from the yeast cell reflect the kinetic properties of the amino acid permeases in the plasmalemma of this organism which evidently differ from the other systems cited above.

If the amino acid carrier behaved symmetrically, which might not strictly hold (Morville *et al.*, 1973), the influx of glycine, when $[H^+]_{out}$, $[H^+]_{in}$ and the membrane potential had given values, would equal the efflux of glycine assayed in conditions where the driving forces associated with these parameters were reversed. Indeed glycine influx was also slow (Table 2), mainly owing to a fall in $V_{max.}$ rather than to an altered $K_m$ with respect to [Gly] (Eddy *et al.*, 1970*a*), when the ionic conditions resembled those in which the efflux of glycine was studied; namely at pH7.4 in the presence of 100mM-KCl. Eddy *et al.* (1970*a*) also showed that the presence of glucose, which initiates proton ejection and would be expected to raise the membrane potential (Scheme 1), dramatically increased the rate of glycine uptake at pH7.4 in the presence of 100mM-KCl.

The above considerations imply that energy coupling in the yeast amino acid permease serves mainly to increase the $V_{max.}$ value of the entry system. This behaviour contrasts with that of the β-galactoside permease of *E. coli*, where an increase in the $K_m$ value for the exit of the galactoside solute appears to be involved as well (Simoni & Postma, 1975). Schultz & Curran (1970) have discussed some of the kinetic factors underlying this distinction.

The equation described in the legend to Scheme 1 was used to study the dependence of $V_{max.}$ on the ionic parameters and so to confirm the feasibility of the above speculations. The presence of two protons as co-substrates would be expected to increase the magnitude of the effects of an electric field on the system, but otherwise appears not to be an essential aspect of the problem. When $[S]_{out}$ is large, $V_{max.}$ is proportional to $k_{+2}[H^+]_{out}/[K_2+[H^+]_{out}(1+k_{+2}/k_{-1})]$ (Scheme 1). An electric field would either assist or hinder the movement of the charged species $ESH^+$ and so change $k_{+2}$, whereas $k_{+1}$ would remain constant and equal to $k_{-1}$. For instance, raising the membrane potential from 0 to −250mV would increase $k_{+2}$ about 10-fold (Morville *et al.*, 1973). The general characteristics of the influx and efflux of glycine, as outlined above, appear to be consistent with such a model when $k_{-1} \gg k_{+2}$ and $K_2 > [H^+]_{out}$.

Schuldiner *et al.* (1975) have suggested that the galactoside carrier of *E. coli* is negatively charged ($E^-$), in which case the protonated carrier ESH might carry no net charge and would move rapidly, $k_{+2}$ thus being large. The equation given in the legend to Scheme 1 again applies, but coupling with the electric field now takes place by acceleration of the movement of $E^-$. Thus the magnitude of $k_{-1}$ rises, that of $k_{+1}$ falls and $k_{+2}$ is constant. Such a model predicts that first, a rapid exchange between cellular and extracellular S would occur and secondly, the $K_m$ value with respect to $[S]_{out}$ would not be constant when the electric field varied unless $V_{max.}$ was also constant. Neither prediction is consistent with the behaviour of the yeast. A model in which $E^-$ associated with $2H^+$ to form $ESH^+$, or one in which E formed $ES(2H^+)$ fits the observations better. The above arguments show that the sign and the number of formal charges associated with the carrier are an important aspect of the transport kinetics.

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