RESEARCH COMMUNICATIONS

L-LYSINE UPTAKE BY RAT LIVER MITOCHONDRIA

DEPENDENCE ON L-LYSINE CONCENTRATION, pH, AND INDUCED K^+ FLUX

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The rapid accumulation of trace levels of L-[^{14}C]lysine by isolated rat liver mitochondria has been reported (King and Diwan, 1973). It had been proposed previously, on the basis of evidence that mitochondria exhibit little swelling in isosmotic L-lysine solutions, that L-lysine does not significantly enter the mitochondria (Gamble and Lehninger, 1973).

In the present studies, the distribution space of L-[^{14}C]lysine has been related to the distribution space of ^3H_2O in mitochondrial samples separated by rapid centrifugation through silicone, as in previous experiments (Johnson and Pressman, 1969; King and Diwan, 1972 and 1973). With these techniques, a $^{14}C/^3H$ distribution ratio of approximately 0.7 would indicate nonpenetration, and a ratio greater than one would be consistent with accumulation of the ^{14}C-labeled solute. Isotopic labels were obtained from New England Nuclear Corp., Boston, Mass. Nigericin was supplied by Dr. Roger Harned.

As shown in Fig. 1 A, at pH 7.4 the L-[^{14}C]lysine/^3H_2O distribution ratio decreases from a value of approximately 1.6 to a value close to one as the L-lysine concentration is increased from the trace level up to 100 mM. The nanomoles of L-lysine per gram of protein taken up in excess of the concentration in the medium, calculated from the data of Fig. 1 A, is plotted on a logarithmic scale in Fig. 1 B. The data are consistent with a maximum L-lysine uptake in excess of equilibration of approximately 10^4 nmol or 10 μmol per gram of protein.

The effect of pH on L-lysine uptake is shown in Table I. The L-lysine uptake is found to increase with increasing pH in the range from 6.8 to 8.0.

The experiment shown in Table II demonstrates the effect on L-lysine uptake of some reagents which are known (e.g. Pressman et al., 1967; Diwan, 1973) to affect

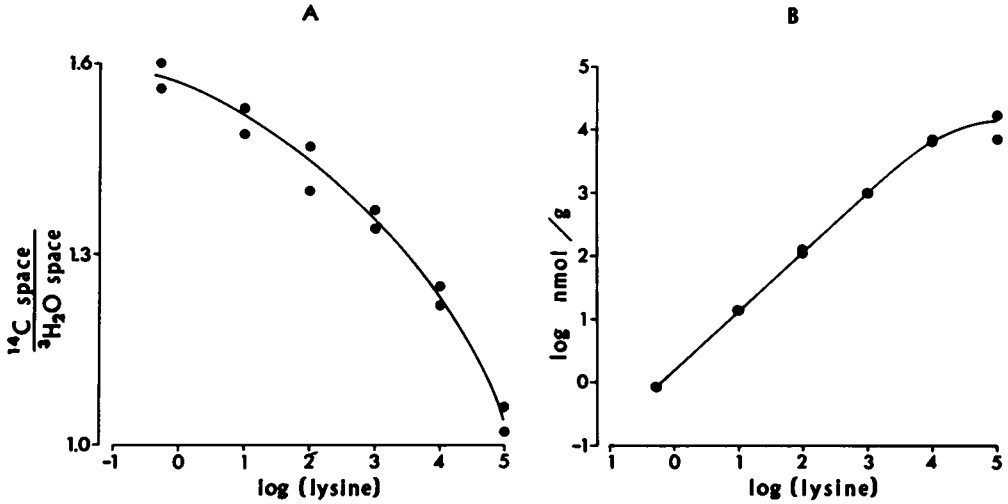

FIGURE 1 Uptake of L-[^{14}C] lysine at varied concentrations of L-lysine. In *A*, the L-[^{14}C] lysine/$^{3}H_2O$ distribution ratio is plotted as a function of the logarithm of the micromolar concentration of L-lysine in the incubation medium. In *B*, the logarithm of the nanomoles of L-lysine per gram of protein taken up in excess of equilibration is plotted against the logarithm of the micromolar concentration of L-lysine in the incubation medium. The amount of L-lysine taken up in excess of equilibration was calculated by subtracting the $^{3}H_2O$ space from the L-[^{14}C]-lysine space, multiplying by the L-lysine concentration in the medium and dividing by the protein content of the mitochondrial sample. The mitochondria (6.4 mg protein/ml) were incubated for 5 min at 20°C in media containing 180 mM sucrose, 17 mM Tris Cl, pH 7.4, trace amounts of $^{3}H_2O$ and L-[^{14}C] lysine, and the concentrations of L-lysine indicated.

TABLE I

EFFECT OF pH ON L-[^{14}C]LYSINE UPTAKE

Mitochondria (6.3 mg protein/ml) were incubated at 20°C in media containing 200 mM sucrose, 16 mM Tris, trace amounts of L-[^{14}C] lysine (approx. 0.55 μM) and $^{3}H_2O$, with or without added unlabeled L-lysine as indicated. The media were titrated with HCl to the pH values shown.

pH	8.0 mM L-lysine added	^{14}C space/^{3}H space	
		45 s	5 min
6.8	—	1.25	1.48
		1.23	1.43
7.4	—	1.53	1.75
		1.49	1.68
8.0	—	2.14	2.25
		2.12	2.17
6.8	+	1.09	1.21
		1.08	1.26
7.4	+	1.25	1.40
		1.24	1.39
8.0	+	1.41	1.47
		1.43	1.50

TABLE II

EFFECT OF ADDED REAGENTS ON L-[^{14}C]LYSINE UPTAKE AND MITOCHONDRIAL K^+

Mitochondria (3.7 mg protein/ml) were incubated at 20°C in media containing 200 mM sucrose, 15 mM Tris Cl, pH 7.4, 100 μM L-lysine, and trace amounts of L-[^{14}C] lysine and ^3H_2O. Additional reagents were present as indicated, at the following concentrations: succinate, 1 mM; KCl, 12 mM; valinomycin, 6.8 ng/mg protein; 2,4-dinitrophenol, 100 μM; and nigericin, 34 ng/mg protein. The values of mitochondrial K^+, determined by atomic absorption, are not corrected for contaminating extra-mitochondrial K^+ which is estimated to be less than 2 μmol/g of protein for samples to which no K^+ was added, 26–31 μmol/g of protein for samples to which KCl was added.

Additions	^{14}C space/^{3}H space		Micromoles K^+/gram protein	
	45 s	5 min	45 s	5 min
None	1.40	1.60	137	136
	1.46	1.79	140	126
Succinate	1.24	1.37	152	148
	1.26	1.36	148	148
KCl	1.18	1.54	192	180
	1.21	1.42	182	184
KCl + succinate	1.11	1.13	182	177
	1.11	1.18	183	180
KCl + succinate	0.97	1.15	262	274
+ valinomycin	1.02	1.07	243	258
Dinitrophenol	1.47	1.80	122	87
	1.60	1.83	117	77
KCl + dinitrophenol	1.35	1.65	156	134
	1.40	1.57	159	141
Nigericin	2.09	1.97	10	10
	2.21	1.92	9	8

transmembrane K^+ flux. Reagents which either stabilize or increase the net intramitochondrial K^+ content (added KCl, succinate, valinomycin) are found to inhibit the uptake of L-lysine. Reagents which induce net efflux of endogenous K^+ (dinitrophenol, nigericin) increase the L-[^{14}C]lysine/^3H_2O distribution ratio.

Measurements of mitochondrial K^+ were carried out by atomic absorption to determine whether outward exchange of endogenous K^+ accompanies L-lysine uptake in the absence of ionophorous reagents. The addition of up to 100 mM L-lysine to the incubation medium was found to be without affect on the mitochondrial K^+ content.

The observed accumulation of L-[^{14}C]lysine cannot be accounted for on the basis of exchange between externally added L-[^{14}C]lysine and endogenous L-lysine. Mitochondria prepared in this laboratory have been found to contain only about 2 μmol of acid-soluble amino acids, including 0.1 μmol of lysine, per gram of protein (King and Diwan, 1972). The possibility that some of the cationic L-lysine may be adsorbing to anionic sites on the mitochondrial membranes cannot be excluded by the data. It has been determined that rat liver mitochondria can bind approximately 35 μmol of alkali metal cations per gram of protein by a respiration-independent process (Gear and Lehninger, 1968).

Yet the data do not support the conclusion that the measured distribution spaces only reflect surface binding. Externally added K^+ inhibits L-lysine uptake. Nigericin, which increases the external K^+ level by inducing K^+ efflux, stimulates L-lysine uptake (for example, in the experiment of Table II nigericin was found to increase external K^+ from 0.32 mM to 1.03 mM). These results are not consistent with a purely external competitive effect of K^+. Rather the changes in L-lysine uptake appear to correlate with the transmembrane K^+ movements. On the other hand, the effects of induced K^+ flux cannot be clearly distinguished from possible effects of the proton (or hydroxyl) fluxes which accompany the K^+ movements under the conditions studied (Pressman et al., 1967). Furthermore, increasing the external pH is known to increase the rate of net efflux of endogenous K^+ (Diwan, 1973). Nevertheless, the observed effects on L-lysine uptake of conditions which affect K^+ flux rates are at least consistent with the conclusion that L-lysine is entering the mitochondria and that its distribution is sensitive to transmembrane movements of other charged solutes.

It has been suggested that under energized conditions permeant cations equilibrate with a membrane potential that is negative in the interior of the mitochondria relative to the surrounding medium (e.g. Rottenberg, 1973). It should be noted that the observed distribution of L-lysine under conditions favoring energized K^+ uptake is not consistent with the view that L-lysine is in equilibrium with a net negative charge in the interior of the mitochondria.

Future studies will attempt to identify the counterion moving during L-lysine uptake in the absence of added ionophorous reagents.

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REFERENCES

DIWAN, J. J. 1973. *Biochem. Biophys. Res. Commun.* **50**:384.
GAMBLE, J. G., and A. L. LEHNINGER. 1973. *J. Biol. Chem.* **248**:610.
GEAR, A. R. L., and A. L. LEHNINGER. 1968. *J. Biol. Chem.* **243**:3953.
JOHNSON, J. H., and B. C. PRESSMAN. 1969. *Arch. Biochem. Biophys.* **132**:139.
KING, M. J., and J. J. DIWAN. 1972. *Arch. Biochem. Biophys.* **152**:670.
KING, M. J., and J. J. DIWAN. 1973. *Arch. Biochem. Biophys.* **159**:166.
PRESSMAN, B. C., E. J. HARRIS, W. S. JAGGER, and J. H. JOHNSON. 1967. *Proc. Natl. Acad. Sci. U.S.A.* **58**: 1949.
ROTTENBERG, H. 1973. *J. Membr. Biol.* **11**:117.