TRANSPORT OF ARGININE BY AN IN VITRO SYSTEM

W. Dorsey Stuart and A. Gib DeBusk

Genetics Group, Department of Biological Science, Florida State University
Tallahassee, Florida 32306

Received April 23, 1973

SUMMARY

Cell surface glycoproteins of Neurospora crassa conidia have been shown to bind amino acids and to be genetically associated with the previously defined amino acid transport systems of that organism. L-arginine does not readily permeate a film of Neurospora conidial lipids. Addition of glycoprotein extracts from Neurospora to the lipid film enhances permeation of arginine at an initial rate 1000 times the rate of permeation through lipid alone. The initial rate of passage exceeds the rate of unhindered passage (no lipid film) through the same cross sectional area by 10 fold.

INTRODUCTION

It has been suggested that cell surface molecules which bind specific transportable substrates could be further implicated in the transport process if such binding molecules restored transport either in a transport deficient in vivo system or in an artificial in vitro lipid membrane system.[1]

We have previously reported[2] the isolation and purification of glycoproteins associated with the amino acid transport systems in conidia of the eucaryote Neurospora crassa. We wish to report that under identical experimental conditions, the initial rate of passage of L-arginine through a crude lipid-glycoprotein film is approximately 10 times greater than unhindered passage through the same cross sectional area and approximately 1000 times greater than passage through a crude lipid film in the absence of glycoprotein extracts.

METHODS AND MATERIALS

Amino acid binding glycoproteins were extracted from 7 day old cultures of wild type strain Tatum SY7A (FGSC No. 622) by methods previously[2] reported. Lipids were extracted by harvesting conidia from comparable cultures directly in 25 ml chloroform. The suspension was shaken vigorously for 10 minutes and filtered through a nitrocellulose filter (Millipore type HA .45) to remove the solid debris. The filtrate containing chloroform soluble material was evaporated to dryness under vacuum at 60°. A crude lipid-glycoprotein mixture was effected by combining equal volumes of the crude lipid and crude glycoprotein extracts. Although the amount of lipid and glycoprotein extracted per gram of conidia varies slightly from culture to culture the

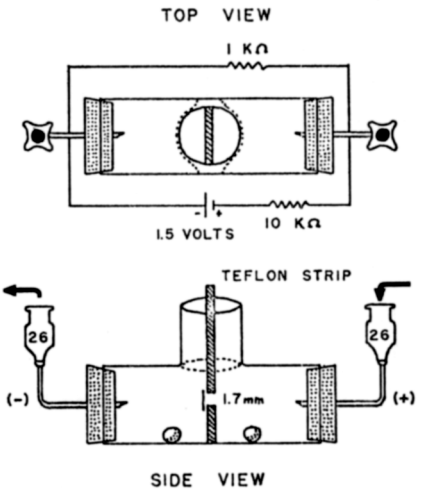

Figure 1. The experimental vessel consists of a glass "T" divided by a teflon strip. The strip is held in place by epoxy cement. The ends of the vessel are closed by rubber stoppers through which are inserted No. 26 needles for introduction and removal of samples. A large glass bead in each sub-chamber effects mixing of the solutions when the entire vessel is shaken 60 times a minute in a direction parallel to the ends of the chamber. The vessel is 6 cm in length. The glass tubing of which it is contructed is 1.4 cm in diameter. The 1.7 mm hole drilled in the teflon strip is covered by a 43 micron stainless steel filter on the side facing the negative chamber. The open cross sectionial area is 1.085 mm^2. The 150 mv potential is applied to the stem of the needles inserted into each sub-chamber.

---

average composition of these mixtures is 90:1, lipid: glycoprotein (w/w). These mixtures were evacuated to dryness at 60°. Dry preparations were stored at 4°. Immediately prior to an experimental run, a few drops of chloroform were added to the dry lipid or lipid-glycoprotein mixture to produce a saturated solution.

The experiments reported here were performed in a glass chamber of original design illustrated in Figure 1. The vessel is dried by filling both sides with acetone, removing the excess liquid and blowing a gentle stream of air into both chambers. The vessel is set on the positive pole end and 0.1 ml of the saturated lipid-chloroform or lipid-glycoprotein-chloroform mixture is applied in micro drops to the 43 u filter screen. The chloroform is allowed to evaporate. Distilled water is slowly added to the positive chamber, care being taken neither to leave an air bubble in the teflon opening nor to displace the lipid film from the filter. The vessel is gently righted while slowly filling the negative chamber with distilled water. It

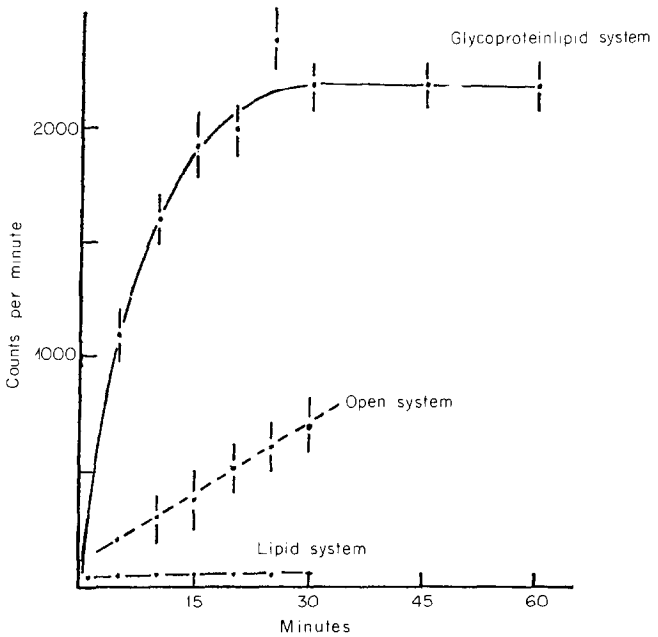

Figure 2. The appearance of L-arginine in the negative chamber through time. The top curve represents appearance of L-arginine after passage through the crude lipid-glycoprotein film. The middle curve represents unhindered passage through a lipid film in the absence of glycoproteins. The L-arginine concentration is measured experimentally as counts per minute per 0.05 ml of the contents of the negative chamber. (1000 counts per minute = 3.3×10^{-6}M L-arginine or a total of 9.9×10^{-9} moles of L-arginine in the negative chamber). All experiments were performed at 25°.

---

has been reported that the membrane of Neurospora has a potential in the range of 150 mv.[3] In an effort to orient the film on the screen, a 150 mv potential is applied throughout the entire experimental run. Concentrated KCl is added to the positive chamber to yield a final concentration of 0.025 M KCl in that chamber.

The experimental run is initiated by adding 0.05 ml of ^{14}C L-arginine (0.5 uCi, specific activity 360) giving a final concentration of 2.4×10^{-4}M ^{14}C L-arginine in the positive chamber. In the competition studies 2.4×10^{-3}M ^{12}C amino acid was added with the ^{14}C amino acid as a mixture.

The vessel is shaken 60 times per minute in a direction 90° opposed to the film surface. Mixing is enhanced by the glass beads in each chamber. The appearance of ^{14}C-L-arginine in the negative chamber through time is monitored by withdrawing 0.05 ml samples from the negative chamber through

the needle inserted in the rubber stopper. At the completion of an experimental run the integrity of the vesicle film is tested by adding a concentrated solution of Blue Dextran 2000 (Pharmecia) to the positive chamber. An intact film will retain the dextran in the positive chamber. A ruptured film will permit passage of the blue sugar into the negative chamber. Experiments in which the lipid film did not remain intact throughout the run were terminated. All experiments were performed at 25°.

RESULTS AND DISCUSSION

The passage of L-arginine through an open screen, a crude lipid-glycoprotein film and through a crude lipid film without glycoproteins is represented in Figure 2. It can be seen that almost no L-arginine is passed through the lipid barrier during the 30 minute experimental period. The slope of the best fit straight line by the method of "least squares" is 0.25 counts per minute per minute (cpm/m). Unhindered passage through the open filter screen occurs in a linear fashion with a slope of 20 cpm/m at this initial amino acid concentration. The slope of L-arginine passage through the lipid glycoprotein mixture as represented by the 30 second and 5 minute point is 220 cpm/m. The rate slows, thereafter, until at 30 minutes the rate is essentially that of passage through the crude lipid film without glycoprotein present. Current experimental efforts are directed towards determining if this decrease in rate is caused by energy depletion, glycoprotein depletion, absence of a regenerative coupling system or yet another, unpostulated phenomenon. The present data are the average of three experimental runs in each case. Observation with a light microscope at 16X reveals the formation of a vesicle extending from the filter screen into the negative chamber upon addition of the KCl. If the KCl is not added to the positive chamber passage of the amino acid through the lipid-glycoprotein film occurs at a rate 6 to 8 times greater than passage through a lipid film but slightly less than the rate of unhindered passage.

Experiments were performed in which 10 times ^{12}C L-arginine or 10 times ^{12}C D-arginine were added to the positive chamber with the ^{14}C isotope. The amount of ^{14}C L-arginine which appears in the negative chamber through time is 0.1 times that which appears when no competing amino acid is present. This is true for both the D and L isomer forms of arginine. We have not yet determined if the reduced passage of labeled L-arginine is caused by competition between the isomers at the binding step or is due to actual passage of the D isomer. Competition could be caused by partial recognition of the epsilon amino group of the D isomer thereby reducing the amount of the L isomer transported. Since the *in vivo* transport systems in *Neurospora*

conidia are stereo specific[4,5] future experimentation will be directed towards understanding the nature of this competition.

In summary, we have demonstrated the passage of ^{14}C L-arginine through a crude lipid-glycoprotein film *in vitro* at an initial rate 10 times that of unhindered passage and 1000 times that of passage through a crude lipid film containing no glycoprotein. After 30 minutes the rate of permeation through the lipid-glycoprotein complex slows to essentially that of the lipid film. We believe this technique will be valuable in establishing both quantitative and qualitative parameters of transport *per se* as distinct from such potentially masking *in vivo* effects as metabolic channeling, free pools and pool regulation.

ACKNOWLEDGEMENTS

This work was supported in part by a National Institute of Health Public Health Service Traineeship in Genetics (Y01 GM01316).

REFERENCES

1. Pardee, A.B., Science 162: 632 (1968).
2. Stuart, W.D. and DeBusk, A.G., Arch. Biochem. Biophy. 144: 512 (1971).
3. Slayman, C.L., J. Gen. Physiol. 49: 69 (1965).
4. Roess, W.B. and DeBusk, A.G., J. Gen. Microbiol. 32: 421 (1968).
5. DeBusk, B.G. and DeBusk, A.G., Biochem. Biophys. Acta 104: 139 (1965).