PHOSPHOLIPID AS A POSSIBLE COMPONENT OF CARRIER SYSTEM IN β-GALACTOSIDE PERMEASE OF ESCHERICHIA COLI

Hiroshi Nikaido

Biochemical Research Laboratory
Massachusetts General Hospital and Harvard Medical School
Boston 14, Mass.

Received October 29, 1962

It has been reported that in Escherichia coli there exist systems which transport amino acids and sugars across the cell membrane at the expense of energy, and that these systems are similar to enzymes in their rigid specificity toward substrates, in their kinetics of action, and in their inducibility by specific inducers (Cohen and Rickenberg, 1956; Rickenberg et al., 1956; Cohen and Monod, 1957). The word 'permease' was therefore proposed for these specific transport systems. It was also proposed that the substrates form transient complexes with hypothetical 'acceptors' or carriers in the membrane, and thus are transported across the membrane. Although many studies have been made on the kinetics and properties of various permease systems, there have been hardly any results indicating the chemical nature of the carriers.

In extensive studies on animal tissues, Hokin and Hokin (1959; 1960a) investigated the relationship between the incorporation of P^{32} into phospholipids and the secretion of various substances --- digestive enzymes, catecholamines, and sodium ion. The strong positive correlation between these two phenomena led them to postulate that phospholipids act as carriers of these substances. For example, it was suggested that

phosphatidic acid forms a salt with sodium ions, and the lipid-soluble salt is transported through the lipid layer of the cell membrane; the transported sodium ions are finally liberated by the conversion of phosphatidic acid into α,β-diglyceride (Hokin and Hokin, 1960b). This idea was supported by several other lines of evidence, and was further extended by many workers. Particularly noteworthy is the work of Tsukada et al.(1960), who reported the increased turnover of phospholipids in brain slices accumulating γ-aminobutyric acid from the external medium.

In view of these results, it appeared interesting to examine whether phospholipids function as 'carriers' in the permease systems of bacteria. The present report deals with studies utilizing β-galactoside permease of E. coli, which transports β-galactosides and β-thiogalactosides.

Various strains of E. coli* were grown overnight with shaking in mineral medium '63' (Cohen and Rickenberg, 1956) with 0.4% sodium succinate as carbon and energy source. The next morning the culture was diluted tenfold with fresh medium, and shaking was continued at 37°C for 3 hours. In some cases, IPTG† (10^{-3}M) was added at the time of dilution in order to induce the synthesis of β-galactoside permease (and also of β-galactosidase). The cells were harvested by centrifugation, and were washed four times with cold deionized water. The final pellet was suspended in a buffer solution (Tris-HCl, pH 7.5, 0.05M; $MgSO_4$, 5×10^{-4}M; NaH_2PO_4, 2.5×10^{-4}M) containing 0.4%

* These were the kind gifts of Drs. S.E. Luria and Barbara E. Wright
† Abbreviations: IPTG, isopropyl-β-D-thiogalactoside; TMG, methyl-β-D-thiogalactoside; TCA, trichloroacetic acid.

sodium succinate, and the optical density of the suspension was adjusted to 1.0 at 650 mμ. The suspension was distributed among several small flasks, which were shaken in a water bath at 37°C. After temperature equilibration, permease substrate (TMG, 10^{-3}M) was added, simultaneously with P^{32} (1.0 μC/ml) in the form of inorganic orthophosphate (control flasks received only P^{32}). 2.0ml aliquots were withdrawn from the incubation mixture and were pipetted into 10% TCA, usually 0, 10, and 20 minutes after the addition of P^{32}. After the addition of several milligrams of non-radioactive carrier bacteria, the samples were washed five times with cold 5% TCA containing 0.025M non-radioactive inorganic phosphate. The pellet was then extracted twice with ethanol-ether (3:1); the first extraction was made at 0°C, and the next at 55°C. The two supernatants were combined, and this fraction was designated 'phospholipids'. The residue after this extraction was dissolved in 0.1 N NH_4OH, and was called 'residual fraction'. Appropriate aliquots were plated and the radioactivity was counted. Typical results are shown in Table 1.

The following observations can be made in the Table*: (1) When the cells actually possess active β-galactoside permease at the time of incubation with P^{32} (this requires that the cells are genetically capable of forming permease --y^+, and also have been preinduced with IPTG), the incorporation of P^{32} into

* The wide variation in the absolute amount of incorporation is probably due to the fact that these strains are not isogenic and are different in the rate of general metabolism. Even with the same strain, the absolute amount of incorporation was affected very much by slight differences in the physiological conditions of the cells, etc.

the 'phospholipid' fraction is definitely stimulated by the presence of TMG. This stimulation is completely reproducible, and so far in 21 determinations, not a single case was encountered with a stimulation less than twofold. The stimulation has a tendency to be greater in the shorter incubations; in the 10 minute incubations, threefold to fourfold stimulation was usually observed.

TABLE I
The incorporation of P^{32} into phospholipid fraction*

Exp. No.	Strain	Genotype†	Pre-induction with IPTG	Incubation with P^{32}	Total P^{32} incorporated into phospholipid		Stimulation by TMG over control
					-TMG	+TMG	
				(min.)	(cpm)	(cpm)	(%)
65	ML30	y^+z^+	+	20	5,450	14,700	170
75	ML30	y^+z^+	+	10	2,050	5,960	191
				20	6,500	14,250	119
66	W4680	y^+z^{del}	+	20	680	2,460	262
66	200R	y^-z^+	+	20	2,140	2,430	13
67	200R	y^-z^+	-	20	1,560	1,780	14
67	W4680	y^+z^{del}	-	20	516	532	3
70	ML30	y^+z^+	-	10	1,260	1,550	23
72§	W4680	y^+z^{del}	+	10	423	2,350	455
				20	1,670	4,500	196
72§	W3133	$y^{del}z^{del}$	+	10	890	1,010	12

*Obtained as described in text. Results were almost the same when the extraction of phospholipids was done according to the method of Hokin and Hokin (1958). More than 90% of the radioactivity thus extracted behaved as typical lipids when chromatographed on silicic acid-impregnated paper by the technique of Marinetti and Stotz (1956). The identification of the phospholipids is in progress.

†Genes y and z control the synthesis of β-galactoside permease and β-galactosidase respectively. y^{del} and z^{del} mean deletions in y and z genes respectively.

§ In this experiment, lactose (10^{-3}M) was used as permease substrate instead of TMG.

(2) When the cells do not possess active permease (i.e., cells having an inactive permease gene --y^-, or not preinduced* at the time of incubation with P^{32}), the presence of TMG stimulated the incorporation into phospholipids only very slightly, if at all.

Furthermore, even with the permease-positive cells, the incorporation into the 'residual fraction' increased only slightly in the presence of TMG (average increase was 8%), which indicates that the stimulation observed in the phospholipids is specific and is not the result of the general enhancement of metabolism brought about by TMG.

The degree of stimulation was measured with varying concentrations of TMG, using preinduced cells of strain ML30. The degree of stimulation, when plotted against TMG concentration, showed a typical saturation curve of Michaelis-Menten type, and the 'K_M' of the system was calculated to be approximately 6×10^{-4}M. This is very close to the known K_M of β-galactoside permease for TMG measured in the same strain, 4.3×10^{-4}M. (Rickenberg et al., 1956).

These facts, together with the fact that TMG is not hydrolyzed at all inside the cell, strongly suggest the intimate relationship between the functioning of the permease and the turnove of phospholipids. TMG, however, is known to be acetylated inside the cell, and it can be argued that the enhanced metabolism of phospholipids might be related to the acetylation reaction. Therefore, similar experiments were carried out using lactose instead of TMG as the substrate for permease, since the enzymatic acetylatic of lactose is very much slower than that of TMG (Zabin et al.,

*With such short incubation time and with non-growing cells, induction of permease is not expected to occur to an appreciable extent during incubation with TMG.

1962). Mutants which do not have even traces of β-galactosidase and hence are unable to hydrolyze lactose were used. The results were essentially the same as those obtained with TMG. (See the last three rows in Table I).

Recently Hokin and Hokin (1962) have modified their hypothesis concerning the mechanism of sodium transport. They assume that sodium is bound to the protein portion of the phosphatidic acid-protein complex, and that the dephosphorylation of phosphatidic acid to produce diglyceride changes the conformation of the protein and thus alters the affinity of the protein for sodium ions; this results in taking up and releasing of sodium ions by the lipoprotein complex, through the cyclic changes between phosphatidic acid and diglyceride. In view of the results presented above and also this hypothesis, we might propose a similar working hypothesis as to the nature of the carrier system in β-galactoside permease. We can postulate that the y gene produces a protein having affinity for permease substrates, that this protein forms a complex with the phospholipid, and that the whole complex functions as carrier through conformational changes in the protein moiety brought about by cyclic chemical changes in the phospholipid moiety. This hypothesis can explain the inducibility, specificity, kinetics, and energy requirement of permease, together with the above-mentioned stimulation of incorporation of P^{32} into phospholipids associated with the function of permease.

ACKNOWLEDGMENTS

This study was supported in part by a grant from National Science Foundation (G-22138) to Dr. H.M. Kalckar. The author is grateful to Drs. S. E. Luria, L.E. Hokin, and H.M. Kalckar for their encouragement.

References

Cohen, G.N., and Monod, J., Bact. Revs., 21, 169 (1957).
Cohen, G.N., and Rickenberg, H.V., Ann. Inst. Pasteur, 91, 693 (1956
Hokin, L.E., and Hokin, M.R., J. Biol. Chem., 233, 805 (1958).
Hokin, L.E., and Hokin, M.R., Gastroenterology, 26, 568 (1959).
Hokin, L.E., and Hokin, M.R., Int. Rev. Neurobiol., 2, 99 (1960a).
Hokin, L.E., and Hokin, M.R., J. gen. Physiol., 44, 61 (1960b).
Hokin, L.E., and Hokin, M.R., Fed. Proc., in press (1962).
Marinetti, G.V., and Stotz, E., Biochim. Biophys. Acta, 21, 168 (195
Rickenberg, H.V., Cohen, G.N., Buttin, G., and Monod, J., Ann. Inst. Pasteur, 91, 829 (1956).
Tsukada, Y., Nagata, Y., and Hirano, S., Nature, 186, 474 (1960).
Zabin, I., Kepes, A., and Monod, J., J. Biol. Chem., 237, 253 (1962)