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Initial Translocation Reaction in the Biosynthesis of Peptidoglycan by Bacterial Membranes

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Bacteria metabolize, grow, and divide in an environment that is very different from their cytoplasmic milieu. In order to protect against this osmotically hostile environment, bacteria possess protective cell walls. Thus, it is of primary importance to understand how constituent polymers are elaborated for incorporation into the wall.

The biosynthesis of these polymers, *e.g.*, peptidoglycan, lipopolysaccharide, and teichoic acid, requires enzymes that are intimately associated with the membrane. Precursors for these polymers are synthesized *via* cytoplasmic pathways and then transferred to the membrane sites that are concerned with polymer formation. This process results in the transfer of precursors from the hydrophilic environment of the cytoplasm to the hydrophobic environment of the membrane.

One of the features of this process concerns the interchange of carriers that are compatible with the particular microenvironments. In the example to be described in this Account, a wall precursor is transferred from uridylic acid as a cytoplasmic carrier to an undecaprenyl phosphate as a membrane carrier. Hence, we have proposed the name for this process, translocation, and that the enzyme catalyzing this process be named a translocase.[2]

Of prime importance to this problem is our understanding of membrane structure. Although information in this area is not sufficient for a well-defined picture of the membrane, the past few years have witnessed a greater understanding of this structure. Many investigators feel that the membrane is a matrix of lipoproteins that function in a particular capacity, whereas others adhere to the traditional concept of a lipid bilayer surrounded by specific proteins. Thus, how enzymes catalyze the translocation process is a fundamental and intriguing question.

The biosynthesis of peptidoglycan occurs in three stages.[3] (1) The synthesis of the nucleotide precursors, UDP-NAc-glucosamine[4] and UDP-MurNAc-pentapeptide, and of the intermediates involved in the cross-linking of peptidoglycan constitutes the cytoplasmic stage. (2) In the second stage NAc-glucosamine and phospho-MurNAc-pentapeptide as well as other intermediates are translocated to the membrane where membrane-associated enzymes catalyze the synthesis of nascent peptidoglycan. (3) In the third stage the nascent peptidoglycan is cross-linked by extracellular enzymes into an external net. The purpose of this Account is to examine the first reaction of the second stage in peptidoglycan synthesis, *i.e.*, the reaction catalyzed by phospho-MurNAc-pentapeptide translocase.

The Reaction. When membrane preparations from *Staphylococcus aureus* Copenhagen are incubated with the peptidoglycan precursor UDP-MurNAc-[^{14}C]pentapeptide (Figure 1) and Mg^{2+}, there is a rapid transfer of radioactivity to an acceptor associated with the membrane.[5,6] Labeling studies reveal that one of the phosphate groups of UDP-MurNAc-pentapeptide is incorporated into UMP[6,7,8] and the other is incorporated into a membrane product.[7] The transfer of 1 mole of phospho-MurNAc-pentapeptide to membranes from UDP-MurNAc-pentapeptide results in the formation of 1 mole of UMP.[9] On the basis of these results, the transfer reaction may be formulated.

UDP-MurNAc-pentapeptide + acceptor ⟶
acceptor–phospho-MurNAc-pentapeptide + UMP (1)

Structure of the Product. The product of the transfer reaction contains a pyrophosphate link between a lipid moiety and MurNAc-pentapeptide.[10] This link-

(1) U. S. Public Health Service Research Career Development Awardee (1-K3-AI-6950).
(2) UDP-MurNAc-L-Ala-D-γ-Glu-L-Lys-D-Ala-D-Ala: undecaprenylphosphate–phospho-MurNAc-pentapeptide transferase.
(3) J. L. Strominger, K. Izaki, M. Matsuhashi, and D. J. Tipper, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **26**, 9 (1967).
(4) Abbreviations used are: MurNAc, *N*-acetylmuramyl; GlcNAc, *N*-acetylglucosamine; UMP, uridine 5′-monophosphate; UDP, uridine diphosphate; FUMP, 5-fluorouridine 5′-phosphate. For reactions 6–16 the abbreviation UMPPMp is used for UDP-MurNAc-pentapeptide. Phospho-MurNAc-pentapeptide (PMp) is covalently linked to UMP *via* a pyrophosphate bridge.
(5) W. G. Struve and F. C. Neuhaus, *Biochem. Biophys. Res. Commun.*, **18**, 6 (1965).
(6) J. S. Anderson, M. Matsuhashi, M. A. Haskin, and J. L. Strominger, *Proc. Nat. Acad. Sci. U. S.*, **53**, 881 (1965).
(7) J. S. Anderson, M. Matsuhashi, M. A. Haskin, and J. L. Strominger, *J. Biol. Chem.*, **242**, 3180 (1967).
(8) J. S. Anderson, P. M. Meadow, M. A. Haskin, and J. L. Strominger, *Arch. Biochem. Biophys.*, **116**, 487 (1966).
(9) W. G. Struve, R. K. Sinha, and F. C. Neuhaus, *Biochemistry*, **5**, 82 (1966).
(10) C. P. Dietrich, M. Matsuhashi, and J. L. Strominger, *Biochem. Biophys. Res. Commun.*, **21**, 619 (1970).

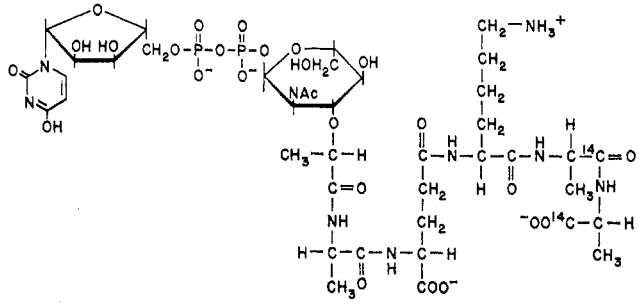

Figure 1. UDP-MurNAc-[^{14}C]pentapeptide. For many of the experiments the terminal D-Ala-D-Ala moiety is labeled with carbon-14. In others the uracil moiety is labeled with tritium.

age is readily hydrolyzed by nucleotide pyrophosphatase.[9] The elucidation of the lipid structure was achieved by Higashi, Strominger, and Sweeley,[11] who found that the acceptor is a C_{55} isoprenoid alcohol phosphate, undecaprenyl phosphate. This lipid acceptor also participates in the biosynthesis of O-antigen of *Salmonella typhimurium*[12,13] and *Salmonella newington*.[14,15] The product of the transfer reaction (eq 1) may be formulated as in Figure 2. Recently, the free C_{55} isoprenoid alcohol (undecaprenol) from *S. aureus* Copenhagen was extensively characterized and found to contain two internal trans double bonds.[16,17] In addition to its role in peptidoglycan and lipopolysaccharide biosynthesis, it has been implicated in the synthesis of mannan[18,19] and it has also been suggested as participating in teichoic acid[20,21] and capsular polysaccharide synthesis.[22] Similar lipid intermediates have also been detected in plant and animal systems.[23,24] In the case of mannan synthesis, the transfer reaction proceeds according to reaction 2.

GDP-mannose + undecaprenyl phosphate $\rightleftharpoons$ (undecaprenyl phosphate)-1-mannose + GDP (2)

Reverse Reaction. A rapid decrease with time in the rate of labeling of membranes was observed, and it was suggested that an inhibitor was being formed during the reaction.[5] Specificity studies with various nucleotides demonstrated that UMP was the most effective inhibitor. For example, if 2.5×10^{-5} *M* UMP was added at zero time, only 11% of the transfer was observed. If UMP was added at 15 min, a rapid loss of label from the membranes resulted. The addition of UMP to a reaction mixture containing lipid product and membranes results in the formation of UDP-Mur-NAc-pentapeptide according to reaction 3.[9] These

acceptor–phospho-MurNAc-pentapeptide + UMP $\longrightarrow$ UDP-MurNAc-pentapeptide + acceptor (3)

results, *i.e.*, the forward and reverse reaction, support a reversible reaction according to eq 4. Both the forward

UDP-MurNAc-pentapeptide + undecaprenyl-P $\rightleftharpoons$ undecaprenyl-PP-Mur-NAc-pentapeptide + UMP (4)

and reverse reaction require the presence of Mg^{2+}.[9] In addition to a requirement for Mg^{2+}, the transfer reaction is stimulated by either K^+, Rb^+, Cs^+, or NH_4^+.[25]

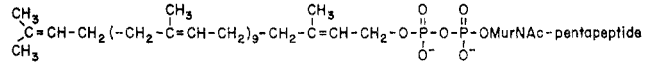

Figure 2. Structure of the lipid product. For details, see ref 11 and 16.

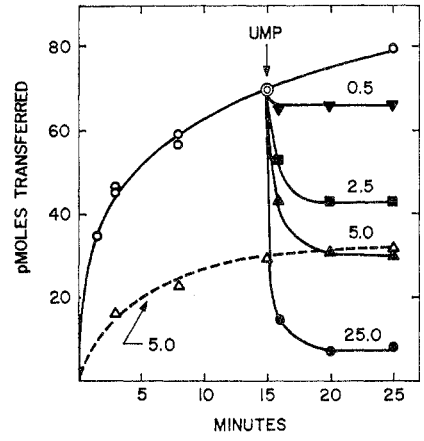

Figure 3. Effect of UMP on the concentration of lipid product. The concentrations of UMP are: ▼-▼, 5.0×10^{-7} *M*; ■-■, 2.5×10^{-6} *M*; ▲-▲, 5×10^{-6} *M*; ●-●, 2.5×10^{-5} *M*. With △-△, 5.0×10^{-6} *M* UMP was added at zero time to the reaction mixtures. For details, see ref 9.

Equilibrium Constant. The addition of increasing concentrations of 5′-UMP resulted in the increased loss of label from acceptor–phospho-MurNAc-pentapeptide (Figure 3). If 5′-UMP is added from zero time, the amount transferred is equivalent to the amount of acceptor–phospho-MurNAc-pentapeptide that remained after adding this concentration of 5′-UMP at 15 min. Thus, equilibrium conditions may be reached by either route. From the results in Figure 3, K_{eq} was calculated to be 0.25 ± 0.04. This corresponds to a $\Delta G^{\circ\prime}$ of +0.98 kcal ($[Mg^{2+}] = 0.01$ *M*,

(11) Y. Higashi, J. L. Strominger, and C. C. Sweeley, *Proc. Nat. Acad. Sci. U. S.*, **57**, 1878 (1967).
(12) M. J. Osborn and R. Y. Tze-Yuen, *J. Biol. Chem.*, **243**, 5145 (1968).
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(14) A. Wright, M. Dankert, P. Fennessey, and P. W. Robbins, *ibid.*, **57**, 1798 (1967).
(15) A. Wright, M. Dankert, and P. W. Robbins, *ibid.*, **54**, 235 (1965).
(16) Y. Higashi, J. L. Strominger, and C. C. Sweeley, *J. Biol. Chem.*, **245**, 3697 (1970).
(17) Protons of methyl group trans to olefinic hydrogens.
(18) M. Scher, W. J. Lennarz, and C. C. Sweeley, *Proc. Nat. Acad. Sci. U. S.*, **59**, 1313 (1968).
(19) M. Scher and W. J. Lennarz, *J. Biol. Chem.*, **244**, 2777 (1969).
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(21) D. Brooks and J. Baddiley, *Biochem. J.*, **115**, 307 (1969).
(22) F. A. Troy and E. C. Heath, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **27**, 345 (1968).
(23) M. Tetas, H. Chao, and J. Molnar, *Arch. Biochem. Biophys.*, **138**, 135 (1970).
(24) N. H. Behrens and L. F. LeLoir, *Proc. Nat. Acad. Sci. U. S.*, **66**, 153 (1970). For additional references see W. J. Lennarz, *Annu. Rev. Biochem.*, 359 (1970).
(25) M. G. Heydanek, Jr., R. Linzer, D. D. Pless, and F. C. Neuhaus, *Biochemistry*, **9**, 3618 (1970).

pH 7.8). The calculation assumes that the endogenous undecaprenyl - diphosphate - MurNAc - pentapeptide equilibrates with the UDP-MurNAc-pentapeptide and is much lower in concentration than the UDP-MurNAc-pentapeptide. Thus, the transphosphorylation formulated in reaction 4 proceeds without loss in free energy. The equilibrium constant for the first reaction in O-antigen biosynthesis has also been established.[12]

$$\text{UDP-galactose} + \text{P-lipid} \rightleftharpoons \text{galactose-PP-lipid} + \text{UMP} \quad (5)$$

As in the case of phospho-MurNAc-pentapeptide translocase, this reaction is readily reversible with an apparent equilibrium constant of approximately 0.5.

Exchange Reaction. The reversible reaction formulated in (4) predicts that [^{3}H]UMP will exchange with the UMP moiety of UDP-MurNAc-pentapeptide (UMPPMp) according to reaction 6. In practice,

$$[^3\text{H}]\text{UMP} + \text{UMPPMp} \rightleftharpoons [^3\text{H}]\text{UMPPMp} + \text{UMP} \quad (6)$$

[^{3}H]UDP-MurNAc-pentapeptide ([^{3}H]UMPPMp) is incubated in the presence of unlabeled UMP with membranes and 10^{-2} M $MgCl_2$.[26] Thus, the exchange proceeds according to reaction 7.

$$\text{UMP} + [^3\text{H}]\text{UMPPMp} \rightleftharpoons \text{UMPPMp} + [^3\text{H}]\text{UMP} \quad (7)$$

In the second stage of the assay, bacterial alkaline phosphatase is added to the mixture and the [^{3}H]UMP is converted to [^{3}H]uridine. The labeled nucleoside is readily separated from the labeled UDP-MurNAc-pentapeptide. From exchange experiments a rate of exchange (E) can be calculated.[9] In experiments with a wide variety of membrane preparations, the rate of exchange (E) always exceeds the rate of transfer (reaction 1). Routinely we find that the ratio of the exchange rate to the transfer rate (E/T) varies from 6 to 24.[26] In order to account for this difference as well as other observations, we propose that an initial transphosphorylation to a nucleophilic center on the enzyme occurs according to the sequence

$$\text{E} + \text{UMPPMp} \rightleftharpoons \text{E}\cdots\text{UMPPMp} \quad (8)$$

$$\text{E}\cdots\text{UMPPMp} \rightleftharpoons \text{UMP}\cdots\text{E-PMp} \quad (9)$$

$$\text{UMP}\cdots\text{E-PMp} \rightleftharpoons \text{E-PMp} + \text{UMP} \quad (10)$$

$$\text{E-PMp} + \text{acceptor} \rightleftharpoons \text{acceptor}\cdots\text{E-PMp} \quad (11)$$

$$\text{acceptor}\cdots\text{E-PMp} \rightleftharpoons \text{E}\cdots\text{acceptor-PMp} \quad (12)$$

$$\text{E}\cdots\text{acceptor-PMp} \rightleftharpoons \text{E} + \text{acceptor-PMp} \quad (13)$$

If the appropriate components are present under saturating conditions, the E/T ratio would indicate that the composite rate constant, k_e, for reactions 8–10 is larger than the composite rate constant, k_t, for reactions 8–13. Clearly, the detection of the proposed enzyme intermediate (E-PMp) will be of primary importance in the analysis of the mechanism of this enzyme.

Kinetics of Product Formation. In the short time intervals (15 min) described in the determination of the equilibrium constant, there is a stoichiometric release of one molecule of UMP for each molecule of phospho-MurNAc-pentapeptide transferred.[9] However, in experiments designed to titrate the lipid acceptor of membrane preparations, it was observed that UMP continued to be formed at a zero-order rate when the lipid product attained a steady-state level.[26] These experiments utilized doubly labeled [^{3}H]UDP-MurNAc-[^{14}C]pentapeptide. Thus, both products were followed simultaneously, as illustrated in Figure 4. In addition to the lipid product and UMP, an additional ^{14}C-labeled compound was detected in the reaction mixture, *i.e.*, MurNAc-[^{14}C]-pentapeptide. Since bacterial alkaline phosphatase was present during the experiment, the initial product would be dephosphorylated. In the absence of the alkaline phosphatase, phospho-MurNAc-pentapeptide (PMp) is formed in addition to the lipid product and UMP. Thus, the results in Figure 4 may be represented by reactions 14 and 15.

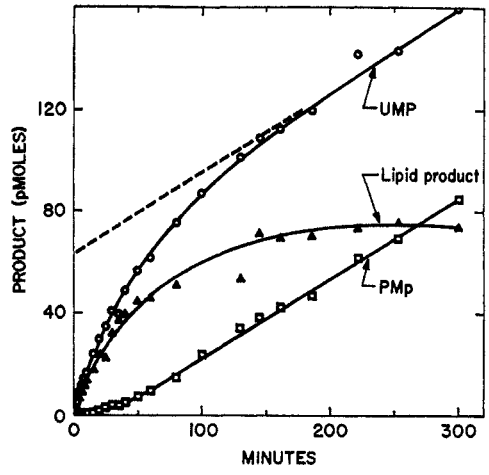

Figure 4. Formation of UMP, lipid product, and phospho-MurNAc-pentapeptide as a function of time. See ref 26 for details.

$$[^3\text{H}]\text{UMPPM}[^{14}\text{C}]\text{p} + \text{acceptor} \longrightarrow \text{acceptorPM}[^{14}\text{C}]\text{p} + [^3\text{H}]\text{UMP} \quad (14)$$

$$[^3\text{H}]\text{UMPPM}[^{14}\text{C}]\text{p} \longrightarrow [^3\text{H}]\text{UMP} + \text{PM}[^{14}\text{C}]\text{p} \quad (15)$$

It is apparent from the kinetics that there is a lag in the formation of phospho-MurNAc-pentapeptide. The lag is consistent with the hydrolysis of either the lipid product or the proposed enzyme intermediate (E-PMp). With the depletion of lipid acceptor, it would appear that hydrolysis of E-phospho-MurNAc-pentapeptide could occur according to reaction 16.[27]

$$\text{H}_2\text{O} + \text{E-PMp} \longrightarrow \text{E} + \text{PMp} \quad (16)$$

Effect of Monovalent Cations. Dialysis of the membrane suspension from *S. aureus* Copenhagen gave a preparation that was dependent on Mg^{2+} and was markedly stimulated by the addition of K^+ when the exchange assay was used (Table I).[25] Although a 20-fold stimulation of the exchange assay could be

(26) M. G. Heydanek, Jr., W. G. Struve, and F. C. Neuhaus, *Biochemistry*, **8**, 1214 (1969).

(27) For a more complete discussion of this suggestion, see ref 26.

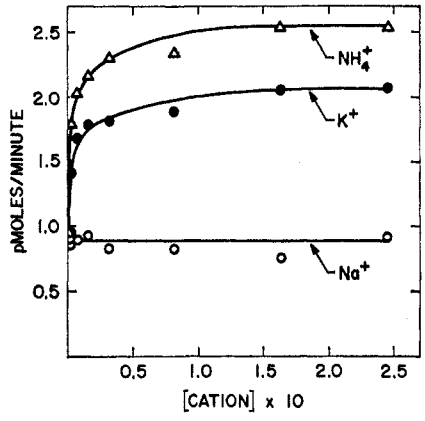

Figure 5. Effect of monovalent ion concentration on the transfer reaction (data from ref 25).

Table I
Metal Ion Activation of Phospho-MurNAc-pentapeptide Translocase

Additions[a]	Act., pmoles/min Exchange	Act., pmoles/min Transfer
None	0	0
Mg^{2+}	2.77	0.94
$Mg^{2+} + Li^+$	5.32	1.06
$Mg^{2+} + Na^+$	2.59	0.91
$Mg^{2+} + K^+$	40.0	2.25
$Mg^{2+} + Rb^+$	40.0	2.55
$Mg^{2+} + Cs^+$	33.7	2.15
$Mg^{2+} + NH_4^+$	53.5	2.60
$Mg^{2+} + K^+ + Li^+$	34.7	2.30
$Mg^{2+} + K^+ + Na^+$	36.0	2.38
$Mg^{2+} + K^+ + Rb^+$	40.5	2.45
$Mg^{2+} + K^+ + Cs^+$	39.7	2.34
$Mg^{2+} + K^+ + NH_4^+$	46.6	2.49

[a] The concentrations of the added monovalent cations were 0.17 M in the presence of 0.04 M $MgCl_2$ (data from ref 25).

demonstrated with K^+, an absolute requirement has not been observed. Routinely, all dialyzed membrane preparations have shown from 5 to 10% of the maximal activity in the exchange assay. In contrast to the 20-fold stimulation observed in the exchange assay, the addition of monovalent cations to the transfer assay resulted in only a 2-fold stimulation of activity. Thus, 5% of the exchange activity is sufficient for a major part (50%) of the transfer activity (Figure 5). With membranes depleted of K^+, the formation of enzyme-phospho-MurNAc-pentapeptide is the rate-determining sequence (reactions 8–10). In the presence of excess K^+ the rate-determining sequence (reactions 11–13) is the transfer of phospho-MurNAc-pentapeptide from the enzyme intermediate to lipid acceptor.[28]

A comparison of the K_m for K^+ ions in the exchange assay (0.01 M) and the K_m for K^+ ions in the transfer assay (2–4 $\times$ 10^{-3} M) provides additional evidence for the above conclusion.[25] Increasing the rate of enzyme-phospho-MurNAc-pentapeptide formation by the addition of 0.01 M K^+ to dialyzed membranes results in the maximal rate of transfer to the lipid

(28) In this model, although the rate-limiting step is suggested to be the transfer of PMp from E-PMp, the rate-limiting step could be inherent in either reaction 11, 12, or 13.

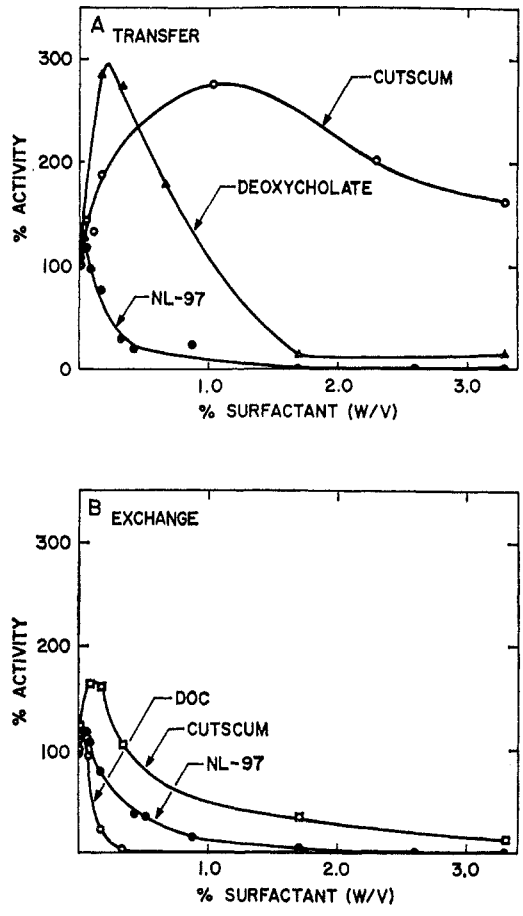

Figure 6. Effect of surfactants on the transfer reaction (A) and exchange reaction (B) (data from ref 26). NL-97 is sodium lauroylsarcosinate.

acceptor and 50% of the exchange rate. Additional potassium ions do not affect the rate of transfer, whereas additional potassium ions increase the exchange rate. Thus, in the concentration range from 0.01 to 0.1 M K^+, it is possible to regulate the rate of enzyme-phospho-MurNAc-pentapeptide formation under conditions in which the transfer rate is constant. Thus, the conclusions derived from a comparison of exchange and transfer rates and from the kinetics of labeling are further substantiated with the effector ion K^+.[25]

Effect of Detergents. In an attempt to analyze the reaction sequence 8–13, additional effectors have been sought that would allow one to control the formation of the proposed enzyme intermediate and undecaprenyl diphosphate-MurNAc-pentapeptide. During the course of our attempts to solubilize the translocase, various surfactants were tested in the exchange and transfer assays.[26] As illustrated in Figure 6A, deoxycholate and cutscum (isooctylphenoxypoly(oxyethylene)ethanol) enhance the transfer reaction whereas sodium lauroylsarcosinate inhibits the transfer reaction. Additional deoxycholate (>1%) markedly inhibits the transfer reaction.

The biphasic stimulation–inhibition of the transfer assay can be correlated with the model described in reactions 8–13. It is proposed that deoxycholate and cutscum inhibit a site(s) in reactions 8–10, as shown

in Figure 6B, by the effective inhibition of the exchange reaction. In contrast, these surfactants appear to enhance the transfer of phospho-MurNAc-pentapeptide to the lipid acceptor (reactions 11–13). On the basis of the exchange to transfer ratios (E/T), we have proposed that the rate constant for the formation of the enzyme intermediate (E-PMp) is 20 times larger than the constant for the rate-limiting reactions involved in the transfer of phospho-MurNAc-pentapeptide from the proposed enzyme intermediate to lipid acceptor. Thus, partial inhibition of reactions 8–10 would not be expected to restrict the transfer to the lipid acceptor. If the surfactant enhances the ability of the enzyme to recognize or bind acceptor in reactions 11–13 and inhibits reactions 8–10, we can visualize the biphasic stimulation–inhibition curves that are observed in the transfer assay.[26]

Effect of Vancomycin. The elucidation of three stages in peptidoglycan synthesis and the enzymes that catalyze the reactions in each stage has provided systems for testing the *in vitro* action of many antibiotics that inhibit cell wall formation. For example, vancomycin is an effective inhibitor of cell wall biosynthesis, and the inhibition is accompanied by the accumulation of UDP-MurNAc-pentapeptide.[29,30] In *in vitro* test systems this antibiotic inhibits the synthesis of peptidoglycan, and it has been proposed that peptidoglycan synthetase, the last reaction in stage two, is one of the primary sites of action.[6–8] The first reaction in this stage, phospho-MurNAc-pentapeptide translocase, is also affected by vancomycin.[5,9,31,32] For example, the addition of low levels (60 μg/ml) of vancomycin to the reaction mixture results in a marked enhancement of undecaprenyl diphosphate-MurNAc-pentapeptide formation, whereas increasing concentrations of vancomycin (>200 μg) inhibit the transfer reaction. In contrast, if the exchange assay is used, a pronounced inhibition is observed at low concentrations (60 μg/ml) of vancomycin.[9,31] The effects of vancomycin mimic the action of cutscum and deoxycholate. In the case of the exchange assay, however, we propose that vancomycin forms an adduct with either UDP-MurNAc-pentapeptide or E-phospho-MurNAc-pentapeptide. Vancomycin effectively binds to the terminal acyl-D-Ala-D-Ala moiety of several test compounds.[33] In the transfer assay, vancomycin could also act as a surfactant and enhance the formation of lipid product. Since the formation of enzyme-phospho-MurNAc-pentapeptide is 6–24 times faster than the transfer reaction, inhibition of enzyme-phospho-MurNAc-pentapeptide formation by this antibiotic would not be expected to restrict the transfer reaction except at high concentrations. Thus, the dichotomy[9] of results previously reported in the two assays may be explained. Similar results have also been observed with ristocetin.

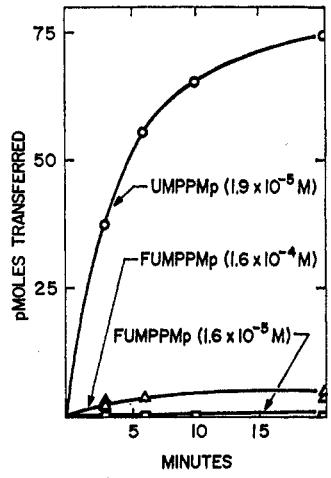

Figure 7. Transfer of phospho-MurNAc-[^{14}C]pentapeptide from UMP and FUMP. See ref 42 for details.

Analogs of UDP-MurNAc-pentapeptide. Although 5-fluorouracil was synthesized as a potential inhibitor of tumor growth,[34] it was observed that this analog inhibits bacterial growth at concentrations significantly lower than those required to inhibit the growth of tumors.[35,36] When *Escherichia coli* K-12 is grown in the presence of 5-fluorouracil, spheroplasts are formed which lyse in a medium of low osmotic strength.[37–39] In addition, it is observed that 5-fluorouracil inhibits the incorporation of α,ϵ-diaminopimelic acid into the cell wall and that large amounts of N-acetylhexosamine esters accumulate. *S. aureus* that is grown in the presence of 5-fluorouracil accumulates the 5-fluorouracil analog of UDP-MurNAc-pentapeptide.[40] In mutants of *S. aureus* that are resistant to 5-fluorouracil, no accumulation of FUDP-MurNAc-pentapeptide is observed.[41]

When UDP-MurNAc-pentapeptide is replaced in the transfer assay (reaction 1) with the fluoro-substituted nucleotide, the rate of transfer is decreased to less than 2% of that observed for the unsubstituted nucleotide (Figure 7).[42] In addition, the fluoro-substituted nucleotide is a competitive inhibitor in the transfer reaction ($K_i = 1.2 \times 10^{-4}\ M$). The inability of the translocase to utilize the fluoro-substituted nucleotide may be the result of either a low affinity or a low catalytic rate constant for the fluoro substrate.[42]

Other uracil analogs have also been tested for their

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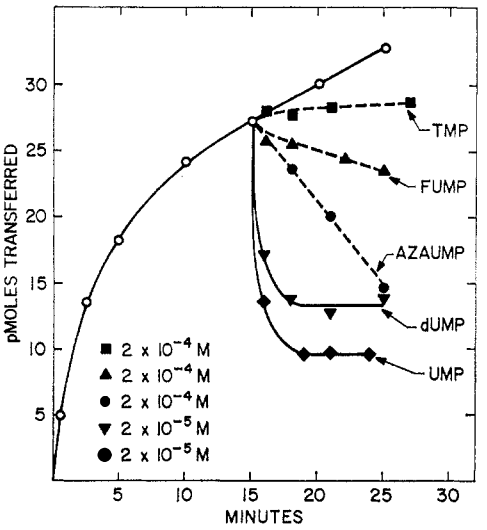

Figure 8. The effect of nucleotide analogs on the loss of label from membranes.[44] The experiment is similar to that described in Figure 3, and the concentration of nucleotide added at 15 min is indicated in the figure. The amount of lipid product was established by the method described in ref 26.

ability to accumulate analogs of UDP-MurNAc-pentapeptide. Of the compounds tested, only 6-azauracil induced the accumulation of *N*-acetylhexosamine esters.[39,43] Analogs of uridylic acid have been tested in the reverse reaction (eq 3) in two ways.[42] In the first experiment, analogs of uridylic acid were incubated with membrane fragments containing undecaprenyl diphosphate-MurNAc-[^{14}C]pentapeptide. Three analogs of uridylic acid are effective in the reverse reaction: 6-azauridylic acid, deoxyuridylic acid, and fluorouridylic acid. In a separate series of experiments (Figure 8),[44] it was observed that the addition of azauridylic acid results in an increased loss of label from lipid product when compared with FUMP. From these results it is inferred that azauridylic acid is a more effective substrate in the reverse reaction than FUMP. The substitution of a fluorine atom for a hydrogen atom on position 5 of uracil lowers the pK_a of the uracil moiety from 9.5 to 7.7.[45] In the case of azauracil, the pK_a is 6.8.[45] Since azauridylic acid is a more effective substrate than FUMP in the reverse reaction, it is not possible to correlate these results with the ionization of the uracil moiety. Other more subtle effects are being sought to explain these results. The high specificity for the uracil moiety suggests that the pyrimidine may play a key role in the reaction. This role may be concerned with either the conformation of the nucleotide or the participation of the pyrimidine in the formation of the enzyme intermediate.

A specificity profile of the pentapeptide moiety of UDP-MurNAc-pentapeptide indicates additional features of the nucleotide that are required for activity with membrane preparations from *M. lysodeikticus*.[7]

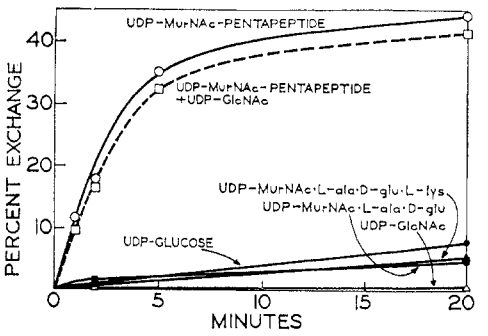

Figure 9. Exchange of [^{14}C]UMP with UDP-MurNAc-pentapeptide and other nucleotides (from ref 7).

As illustrated in Figure 9, UDP-GlcNAc and UDP-MurNAc do not participate in the exchange reaction (reaction 6). Three analogs, UDP-glucose, UDP-MurNAc-L-Ala-D-Glu, and UDP-MurNAc-L-Ala-D-Glu-L-Lys, substitute for UDP-MurNAc-pentapeptide in the exchange assay at 10% or less of the activity observed with the complete nucleotide. On the basis of these results, the terminal D-Ala-D-Ala moiety of UDP-MurNAc-pentapeptide is an essential feature of the substrate. In the case of membrane preparations from *E. coli*, UDP-MurNAc-L-Ala-D-Glu-*meso*-DAP-D-Ala is from 15 to 20% as effective as UDP-MurNAc-L-Ala-D-Glu-*meso*-DAP-D-Ala-D-Ala in the transfer reaction (reaction 1).[46] Three additional modifications of the pentapeptide moiety have been studied.[47] For example, UDP-MurNAc-L-Ala-D-Glu-L-Lys(N^{ϵ}-L-Ala)-D-Ala-D-Ala and UDP-MurNAc-L-Ala-D-Glu-L-Lys-(N^{ϵ}-L-Ser)-D-Ala-D-Ala are utilized as effectively as UDP-MurNAc-pentapeptide for the synthesis of lipid product in membrane preparations from *Lactobacillus viridescens*. The aminoacyl substitution on the ϵ-amino group of lysine is involved in the interpeptide bridge structure of this organism. A third modification of the ϵ-amino group involves the synthesis of UDP-MurNAc-pentapeptide-pentaglycine *via* the reverse reaction (reaction 3)[32]

undecaprenyl diphosphate-MurNAc-pentapeptide-pentaglycine + UMP ⟶ UDP-MurNAc-pentapeptide-pentaglycine + undecaprenyl phosphate (17)

In the transfer reaction (reaction 1) UDP-MurNAc-pentapeptide-pentaglycine is utilized at ~30 and 70% of the amount observed with UDP-MurNAc-pentapeptide in membrane preparations from *S. aureus* and *M. lysodeikticus*, respectively.[32] When UDP-MurNAc-L-Ala-D-Glu-(α-NH_2)-L-Lys-D-Ala-D-Ala is used in the transfer assay (reaction 1), the observed velocity is less than 1% of that found with UDP-MurNAc-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala.[48] In studies on the mode of *O*-carbamyl-D-serine in *S. faecalis*,[49] the analog UDP-MurNAc-L-Ala-D-Glu-L-Lys-D-Ala-*O*-carbamyl-D-Ser is

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as effective as UDP-MurNAc-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala in the exchange reaction (reaction 6). Clearly a systematic modification of the pentapeptide moiety will be necessary in order to define additional features of this molecule that are necessary for activity.

Conclusion

Our knowledge of membrane function and structure is in its infancy. There is a need for basic enzymology of those enzymes that are intimately involved with the function of the membrane. A mechanistic analysis of the translocase would be greatly facilitated if the enzyme could be purified in soluble form. Although solubilization has been achieved with several diverse agents,[50] these preparations have not been successfully fractionated. With information from mechanism studies, we can begin to ask what is the relationship of this enzyme to the structure of the membrane? Does the ordered structure of the membrane have a profound effect on the activity of the enzyme? Alternately, we will ask whether this enzyme catalyzes the translocation process as part of a membrane subunit. Is the enzyme located at the point of new cross-wall formation or is it uniformly distributed throughout the plasma membrane? Is this enzyme one of the key control points? The many questions that are posed indicate that only the surface of this fascinating problem has been probed.

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Some Aspects of the Theory of Liquids

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There has been much progress during the past few years in understanding the properties of liquids, but much of this work is so recent that the theory of liquids is still widely regarded as an unsolved problem.

It is interesting to note that the ideas which form the basis for the modern successful theory of liquids have been well known for almost a century. The work of van der Waals[1] in 1873 implied that the structure of a liquid is primarily determined by the repulsive forces between its molecules, so that a liquid may be regarded as a system of hard spheres with the attractive forces providing a uniform background energy. A century ago the properties of a system of hard spheres were not known. Thus van der Waals made drastic approximations to obtain the hard-sphere equation of state. It is these approximations, rather than weaknesses in his physical ideas, which are responsible for the inadequacies of his equation of state.[2]

Recently, these ideas have been revived. Zwanzig[3] and others[4,5] have shown that the effect of the attractive portion of the intermolecular potential, $u(R)$, can be obtained by means of a perturbation expansion in the strength of the attractive potential using a system of hard spheres as the unperturbed or reference system. Rowlinson[6] has shown that the effect of the repulsive portion of $u(R)$ can be obtained by means of a perturbation expansion in the inverse steepness of the repulsive potential, again using hard spheres as the reference system. Neither the Zwanzig nor the Rowlinson expansion was adequate for the liquid state. McQuarrie and Katz[7] combined these two expansions. However, their scheme had the effect of making the perturbations large and, as a result, the series did not converge at liquid temperatures and densities. An alternative scheme which is fully satisfactory has been developed by Barker and Henderson and will be treated in detail below.

We shall consider only the equilibrium properties of simple liquids in which quantum effects may be ignored and in which the potential energy results from additive contributions of intermolecular potentials which are functions only of the intermolecular separations. The physical ideas used in the theory of liquids may thus be seen without excessive complexity. Such liquids are idealizations and do not exist in nature. As a result, the thermodynamic properties and the radial distribution function (RDF), $g(R)$, which is the probability of finding a pair molecules separated by a distance R, must be obtained by computer simulation studies which are discussed in the next section.

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