Initial Stage in Peptidoglycan Synthesis. Mechanism of Activation of Phospho-*N*-acetylmuramyl-pentapeptide Translocase by Potassium Ions*

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ABSTRACT: The initial reaction in the biosynthesis of peptidoglycan is catalyzed by phospho-*N*-acetylmuramyl-pentapeptide translocase (UDP-MurNAc-L-Ala-D-γ-Glu-L-Lys-D-Ala-D-Ala: C_{55}-isoprenoid alcohol phosphate phospho-MurNAc-pentapeptide transferase). In addition to the transfer reaction, the enzyme catalyzes the exchange of [^{3}H]uridine monophosphate with the uridine monophosphate moiety of UDP-MurNAc-pentapeptide. In addition to an absolute requirement for Mg^{2+}, the translocase is stimulated by either K^+, NH_4^+, Rb^+, or Cs^+. Addition of K^+ (0.17 M) to dialyzed membranes stimulates the exchange reaction 20-fold and the transfer reaction 2-fold. The stimulation by K^+ gives a normal hyperbolic saturation curve with a Michaelis–Menten constant of 0.01 M in the exchange reaction. The value for n (1) established from Hill plots suggests the absence of homotropic effects and that a single binding site for K^+ exists in association with each catalytic center. The binding of K^+ is rapid and reversible and does not cause a significant shift in the pH optimum. On the basis of a kinetic analysis, the primary site of K^+ action is in the formation of the proposed enzyme–phospho-MurNAc-pentapeptide intermediate. With membranes depleted of K^+ ions, the formation of enzyme–phospho-MurNAc-pentapeptide is the rate-determining sequence whereas in the presence of excess K^+ ions the rate-determining sequence is the transfer of phospho-MurNAc-pentapeptide from the enzyme intermediate to lipid acceptor.

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. Soluble preparations of the translocase show identical enhancement by K^+, Rb^+, Cs^+, and NH_4^+.

The biosynthesis of peptidoglycan, the major structural heteropolymer of bacterial cell walls, is catalyzed by a series of enzymes associated with the bacterial membrane (Anderson *et al.*, 1966, 1967; Araki *et al.*, 1966; Chatterjee and Park, 1964; Meadow *et al.*, 1964; Matz and Strominger, 1968). The initial reaction in this series is catalyzed by phospho-MurNAc-pentapeptide translocase (UMP)[1] (Anderson *et al.*, 1965; Struve and Neuhaus, 1965; Struve *et al.*, 1966; Stickgold and Neuhaus, 1967; Heydanek *et al.*, 1969; Heydanek and Neuhaus, 1969) according to reaction 1, where the lipid acceptor is C_{55}-isoprenoid alcohol (Higashi *et al.*, 1967). The reaction is reversible and has a $K_{eq} = 0.25 \pm 0.04$ (Struve *et al.*, 1966). The enzyme also catalyzes the exchange reaction

$$\text{UDP-MurNAc-pentapeptide} + \text{lipid acceptor–phosphate} \xrightleftharpoons{Mg^{2+}} \text{UMP} + \text{lipid acceptor–diphosphate-MurNAc-pentapeptide} \quad (1)$$

$$[^3\text{H}]\text{UMP} + \text{UDP-MurNAc-pentapeptide} \xrightleftharpoons{Mg^{2+}} [^3\text{H}]\text{UDP-MurNAc-pentapeptide} + \text{UMP} \quad (2)$$

In our initial experiments (Struve and Neuhaus, 1965), it was observed that membrane preparations washed in 0.005 M Tris-HCl (pH 7.8) containing 1 M KCl yielded preparations of higher specific activity than those washed with 0.005 M Tris-HCl (pH 7.8). In addition, Park (1965) observed that either KCl or NH_4Cl stimulated the incorporation of radioactivity from UDP-MurNAc-pentapeptide into peptidoglycan. It is the purpose of this paper to define the stimulation by K^+. The activation by this ion is used as a probe of the mechanism of the translocase and as a means to compare the membrane-associated translocase with the soluble translocase.

Experimental Section

Materials. Staphylococcus aureus Copenhagen (kindly provided by Dr. Jack Strominger) was grown to mid-log phase in a New Brunswick 14-l. fermentor with aeration at 37° in a medium containing 0.5% K_2HPO_4, 1% yeast extract, 1% glucose, 1% peptone, and 0.01% antifoam. The preparation of the membrane fragments, solubilized enzyme, nucleotides, and other chemicals have been previously described (Struve *et al.*, 1966; Stickgold and Neuhaus, 1967; Heydanek *et al.*,

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[1] Abbreviations used are: MurNAc, *N*-acetylmuramyl; UMP, uridine 5′-monophosphate; UDP, uridine diphosphate.

TABLE I: Metal Ion Activation of Phospho-MurNAc-pentapeptide Translocase.[a]

Additions	Act. (pmoles/min) Exchange	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 exchange assay and transfer assay B were used with 60 μg of membranes that were dialyzed for 16 hr against 0.02 M Tris-HCl (pH 7.8). The concentrations of the added monovalent cations were 0.17 M in the presence of 0.04 M $MgCl_2$.

1969; Heydanek and Neuhaus, 1969). K^+ was removed from the membrane fragments by dialysis against 1000 volumes of 0.02 M Tris-HCl (pH 7.8) for 16 hr at 4°. Ammonium ions were removed from UDP-MurNAc-pentapeptide and bacterial alkaline phosphatase by gel filtration on a column (0.9 × 20 cm) containing Sephadex G-25 with 0.02 M Tris-HCl (pH 7.8) as the eluent.

Assays. Transfer assay B and the exchange assay have been previously described in detail (Heydanek *et al.*, 1969). In summary, transfer assay B measures the release of [^{3}H]UMP from [^{3}H]UDP-MurNAc-pentapeptide followed by hydrolysis of [^{3}H]UMP to [^{3}H]uridine. Bacterial alkaline phosphatase is present in the assay so that only the forward direction of reaction 1 is observed. Therefore, transfer assay B is a function of the enzyme and lipid acceptor concentrations. The exchange assay involves the exchange of UMP with the [^{3}H]-UMP moiety of [^{3}H]UDP-MurNAc-pentapeptide according to reaction 3. Transfer assay B and the exchange assay con-

$$\text{UMP} + [^3\text{H}]\text{UDP-MurNAc-pentapeptide} \overset{Mg^{2+}}{\rightleftharpoons} \text{UDP-MurNAc-pentapeptide} + [^3\text{H}]\text{UMP} \quad (3)$$

tained 0.04 M $MgCl_2$ and 0.2 M KCl. One modification has been incorporated into these assays. After terminating the reaction with 0.2 ml of cold 0.2 M acetic acid, the mixture was quantitatively applied to a Dowex acetate X-8 (200–400 mesh) column (5 × 20 mm) and washed twice with 0.4 ml of 0.2 M acetic acid. The increase in the amount of acetic acid from 0.4 (Heydanek *et al.*, 1969) to 0.8 ml increased the recovery of [^{3}H]uridine. For convenience the standard exchange was modified for the results presented in Figure 6B. This modification involves the exchange of [^{3}H]UMP with the UMP moiety of

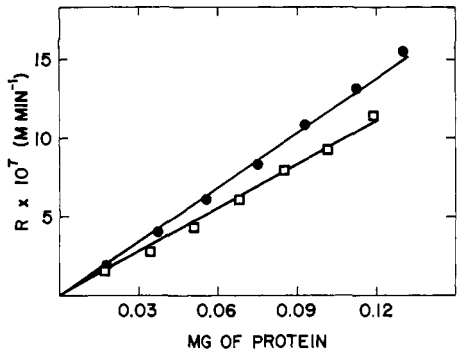

FIGURE 1: Variation of exchange rate with enzyme concentration. The exchange assay was used with dialyzed membranes (●—●) and dialyzed membranes solubilized with sodium lauroyl sarcosinate (□—□) in the presence of 0.20 M KCl.

UDP-MurNAc-pentapeptide according to reaction 2. The assay mixture contained 0.05 M Tris-HCl (pH 7.8), 0.04 M $MgCl_2$, 5.0×10^{-6} M [^{3}H]UMP, UDP-MurNAc-pentapeptide, and KCl as indicated, and 73 μg of membrane preparation in a total volume of 60 μl. The reaction mixture was incubated at 25° for 5 min and the reaction was terminated by boiling at 100° for 2 min. The addition of bacterial alkaline phosphatase and the separation of substrates from product were performed by the methods described by Stickgold and Neuhaus (1967). As illustrated in Figure 1 the rate of exchange, R, presented in moles per liter per minute is proportional to the concentration of membranes. In addition, with the enzyme solubilized with sodium lauroyl sarcosinate the rate of exchange is proportional to the amount of enzyme. Each of the assays contains 0.2 M KCl. In previous work (Struve *et al.*, 1966), the R showed a sigmoidal response in the absence of added K^+.

Analytical Procedures. Measurements of radioactivity were made as previously described (Heydanek *et al.*, 1969). Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard.

Results

Effect of Monovalent Cations. Phospho-MurNAc-pentapeptide translocase has an absolute requirement for Mg^{2+} (Struve *et al.*, 1966). The membrane preparation from *S. aureus* Copenhagen that was used as a source of the enzyme for these experiments was washed four times in 0.005 M Tris-HCl (pH 7.8) containing 1 M KCl. Dialysis of the membrane suspension against 1000 volumes of 0.02 M Tris-HCl (pH 7.8) for 16 hr at 4° 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). As shown in Figure 2, maximal activity in this assay is observed when 0.17 M KCl is added to the assay mixture. The Michaelis–Menten constant for K^+ established from these data is 9.8×10^{-3} M (Figure 2). Although a 20-fold stimulation of the exchange assay could be demonstrated, an absolute requirement for K^+ has not been observed. Routinely, all dialyzed membrane preparations have shown from 5 to 10% of the maximal activity. The specificity of this enhancement in the exchange assay is illustrated in Table I. Ammonium, Rb^+, and Cs^+ also stimulate the exchange reaction. Li^+ enhances the reaction to a

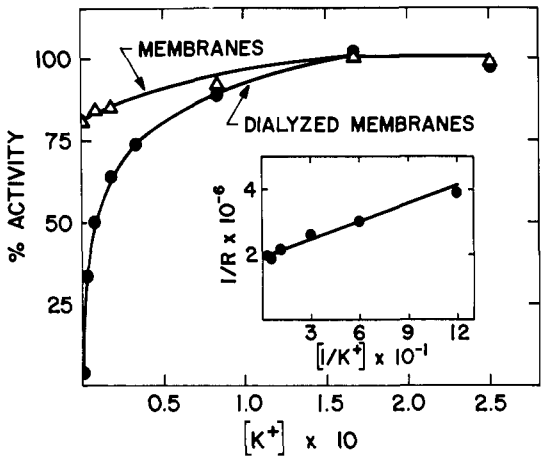

FIGURE 2: Effect of K^+ on the exchange rate. The exchange assay was used with membranes (△—△) prepared according to Struve *et al.* (1966) and dialyzed membranes (●—●).

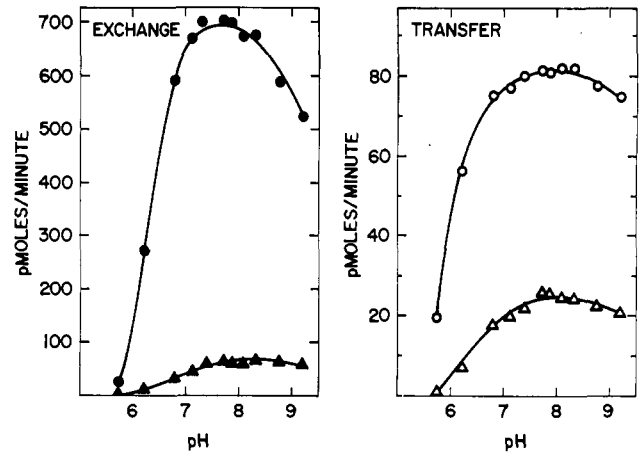

FIGURE 4: Effect of K^+ on the pH optima of the translocase in the exchange assay (A) and transfer assay (B). The exchange and transfer assays were used with 0.021 M Tris-maleate buffer adjusted to the desired pH with NaOH. The activity in the presence (●, ○) and the absence (▲, △) of 0.2 M KCl is presented.

small extent whereas Na^+ has no effect. In every experiment with the exchange assay, NH_4^+ is more effective than either K^+, Rb^+, or Cs^+. The addition of equimolar concentrations of either Rb^+ or Cs^+ to the assay mixture containing K^+ does not affect the activity, whereas, the addition of either Li^+ or Na^+ inhibits to a small extent. On the other hand, the addition of NH_4^+ enhances the activity. The specificity of activation suggests that these effects are not the result of an increase in ionic strength. This was further substantiated by the observation that tetramethylammonium chloride does not activate the enzyme.

As in the case of the exchange assay, the addition of K^+, Rb^+, Cs^+, and NH_4^+ enhances the transfer assay (Table I). However, in contrast to the 20-fold stimulation observed in the exchange assay, the addition of monovalent cations to the transfer assay mixture results in only a 2-fold stimulation of activity. The addition of equimolar concentrations of Li^+, Na^+, Rb^+, Cs^+, or NH_4^+ to the transfer assay containing K^+ does not affect the transfer of phospho-MurNAc-pentapeptide to the lipid acceptor. The effects of increasing concentrations of Na^+, K^+, and NH_4^+ in the transfer assay are illustrated in Figure 3. Na^+ has no effect on the transfer reaction. Ammonium ions are more effective than K^+ ions at each concentration tested. Although the K_m for K^+ is difficult to establish from these data, we have estimated that the K_m for the enhancement in the transfer assay is approximately 2–4 $\times 10^{-3}$ M. This value differs significantly from that established from the exchange assay.

With L-threonine dehydrogenase and yeast aldehyde dehydrogenase, the activation by K^+ is time dependent (Black, 1951; Green, 1964). In order to test the time dependency of activation, two sets of reaction mixtures for the exchange assay were prepared. In the first set, the dialyzed membrane preparation was preincubated for 20 min at 25° in the presence of 0.20 M KCl, and in the second set the dialyzed membrane preparation was preincubated in the absence of KCl. In the second set 0.2 M KCl was added at zero time. No lag in activity

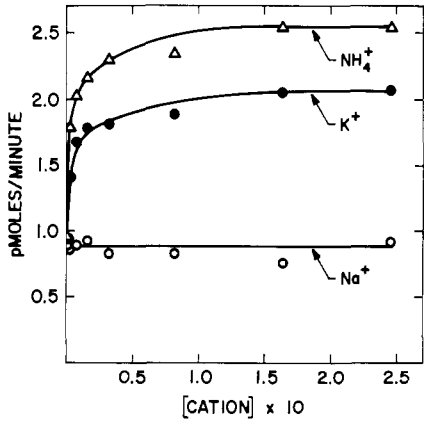

FIGURE 3: Effect of monovalent ion concentration on the transfer reaction. Transfer assay B was used with varying concentrations of K^+ (●—●), NH_4^+ (△—△), Na^+ (○—○), and 60 μg of dialyzed membranes.

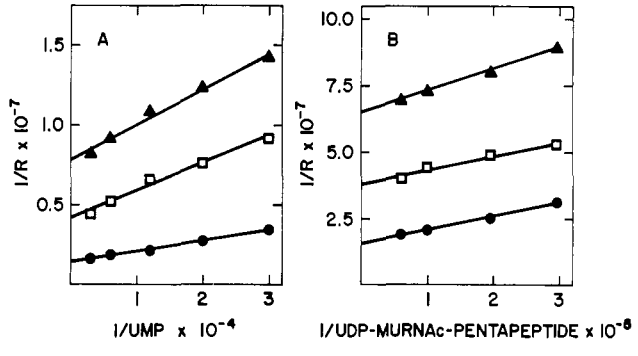

FIGURE 5: Effect of K^+ concentration of K_m and V_{max} for UMP (A) and UDP-MurNAc-pentapeptide (B). The exchange assay was used with 66 μg of membranes in A and 73 μg of membranes in B. For A the concentration of [^{3}H]UDP-MurNAc-pentapeptide was 3.1 $\times 10^{-5}$ M; the concentrations of K^+ were 0.0037 M (▲—▲), 0.017 M (□—□), and 0.20 M (●—●). For B the concentration of [^{3}H]UMP was 5.0 $\times 10^{-6}$ M; the concentrations of K^+ were 0.005 M (▲—▲), 0.01 M (□—□), 0.20 M (●—●). The lines were drawn by using the method of least squares.

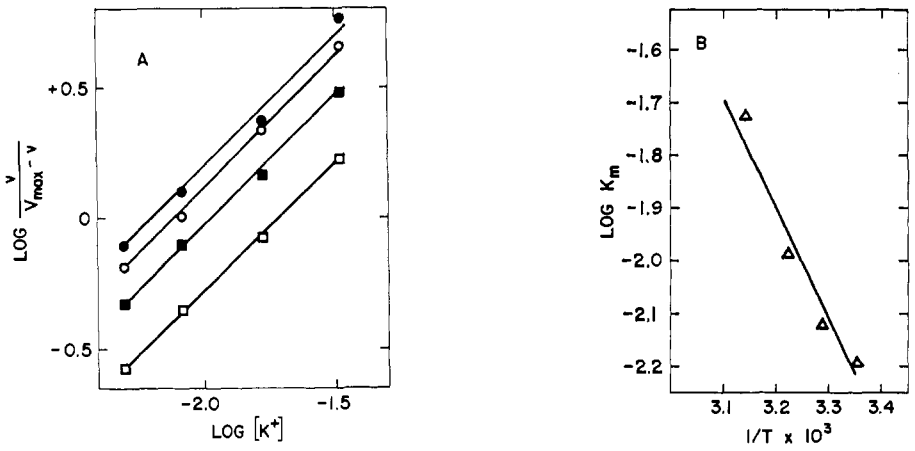

FIGURE 6: Hill plots of exchange reaction at various concentrations of K^+ at different temperatures (A). Temperature dependence of the Michaelis-Menten constant (B). The exchange reaction was used with 60 μg of dialyzed membranes at 25° (●—●), 31° (○—○), 37° (■—■), and 45° (□—□).

was observed in the set that did not contain KCl in the preincubation period. Thus, the activation by KCl is essentially instantaneous and preincubation with KCl is not required in order to observe maximal activity.

pH Optima. The enhancement by K^+ could result from a shift in the pH optima of the translocase. The profiles for the exchange and transfer assays in the presence and absence of K^+ are shown in Figure 4. A small decrease in the pH optimum of the exchange assay (0.5 pH unit) is observed when K^+ is added. This shift does not account for the enhancement in activity.

Effect of K^+ on the Michaelis-Menten Constants of UMP and UDP-MurNAc-pentapeptide. The Michaelis constant, K_m, for UMP (2.7×10^{-5} M) and UDP-MurNAc-pentapeptide (1.8×10^{-6} M) had been established with membranes that were washed in 0.005 M Tris-HCl (pH 7.8) containing 1 M KCl (Struve *et al.*, 1966). Since K^+ has been shown to affect the V_{max} and Michaelis-Menten constants of several enzymes (Neuhaus, 1962; Edwards and Keech, 1968; Tabor and Wyngarden, 1959; Marshall *et al.*, 1961; Giorgio and Plaut, 1967; Bright, 1967; Robinson, 1967, 1969; Nandi *et al.*, 1968), the effects of UMP and UDP-MurNAc-pentapeptide on the exchange assay in the presence of several concentrations of K^+ were examined. In the case of UMP (Figure 5A and Table II), V_{max} increases as the concentration of K^+ is increased. In addition, the values for the K_m of UMP increase with higher concentrations of K^+. The Michaelis-Menten constant and V_{max} for UDP-MurNAc-pentapeptide are also a function of the K^+ concentration. For example, the values for K_m increase from 1.1×10^{-5} to 3.2×10^{-6} M and for V_{max} increase from 0.015 to 0.064 as the concentration of K^+ is increased from 0.005 to 0.20 M (Figure 5B).

Effect of Temperature. The interaction of K^+ with apoenzyme may be expressed by the equilibrium

$$E + nK^+ \rightleftharpoons K_n{}^+E \qquad (4)$$

where E is apoenzyme and $K_n{}^+E$ is the activated holoenzyme with n ions of K^+ associated with the apoenzyme. It is recognized, however, that eq 4 represents only one of the possible mechanisms of K^+ activation (Dixon and Webb, 1964). This reaction can be analyzed with the Hill equation, *i.e.*

$$\log \frac{v}{V_m - v} = \log K_m + n \log [K^+] \qquad (5)$$

where v is the velocity in the presence of activator and V_{max} is the maximum velocity established from a Lineweaver-Burk plot at a defined temperature (Atkinson *et al.*, 1965). K_m is defined as the Michaelis-Menten constant for K^+ and is assumed to be the dissociation constant for eq 4. The slope n is an interaction coefficient that is a function of the number of binding sites and the strength of interaction. In Figure 6A Hill plots are presented for 25, 31, 37, and 45°. At each temperature, the slope (n) is one. The change in enthalpy, $\Delta H°$, calculated from the slope of the plot in Figure 6B, is +9.4 kcal/mole.

Effect of K^+ on the Solubilized Phospho-MurNAc-pentapeptide Translocase. The solubilization of the translocase by

TABLE II: Summary of Values for K_m and V_{max}.[a]

K^+ (moles/l.)	K_m (moles/l.)	V_{max}, R (moles/l. per min) $\times 10^6$
	Expt 1 UMP	
0.0017	2.8×10^{-5}	0.127
0.0037	4.0×10^{-5}	0.238
0.20	4.7×10^{-5}	0.715
	Expt 2 UDP-MurNAc-pentapeptide	
0.005	1.1×10^{-6}	0.015
0.01	1.3×10^{-6}	0.026
0.20	3.2×10^{-6}	0.064

[a] The values were established from the data presented in Figure 5A,B.

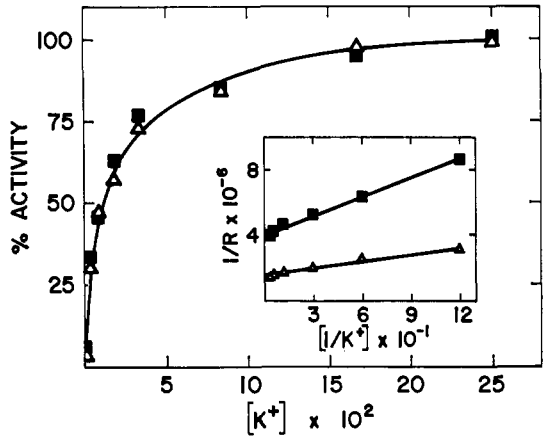

FIGURE 7: Effect of K^+ concentration on soluble translocase. The exchange assay was used with membranes (■—■) solubilized with sodium lauroyl sarcosinate and with membranes (△—△) solubilized with KOH. The soluble preparations of the translocase were prepared according to the methods of Heydanek and Neuhaus (1969). Each preparation was dialyzed for 16 hr against 0.02 M Tris-HCl (pH 7.8).

TABLE III: Metal Ion Activation of Phospho-MurNAc-pentapeptide Translocase Solubilized with Sodium Lauroyl Sarcosinate.[a]

Additions	Act. (pmoles/min)	
	Exchange	Transfer
None	0	0
Mg^{2+}	1.63	0.62
$Mg^{2+} + Li^+$	3.92	0.66
$Mg^{2+} + Na^+$	1.48	0.66
$Mg^{2+} + K^+$	27.2	1.12
$Mg^{2+} + Rb^+$	32.7	1.49
$Mg^{2+} + Cs^+$	23.9	1.02
$Mg^{2+} + NH_4^+$	38.6	1.33

[a] The exchange and transfer assay B were used with 60 μg of solubilized membranes that were dialyzed for 16 hr against 0.02 M Tris-HCl (pH 7.8). The concentration of the added monovalent cations was 0.17 M in the presence of 0.04 M $MgCl_2$.

sodium lauroyl sarcosinate and by KOH has been reported by Heydanek and Neuhaus (1969). In an attempt to characterize the solubilized preparations, the stimulation by KCl has been used as a probe for establishing whether the soluble enzyme is similar to that associated with the membrane. In Figure 7, the effect of KCl on the exchange reaction is shown. From these data the K_m for K^+ was calculated to be 0.01 M in each case. In addition, the specificity of activation by monovalent cations is identical with that observed with the membrane fragments (Table III).

Discussion

Heydanek *et al.* (1969) proposed the following series of reactions (6-11) where E···UMPPMp is the Michaelis complex of enzyme (E) and UDP-MurNAc-pentapeptide, E–PMp is a covalent intermediate between phospho-MurNAc-pentapeptide and enzyme, and UMP···E-PMp is the Michaelis complex of the covalent intermediate and UMP. The exchange

$$E + UMPPMp \rightleftarrows E\cdots UMPPMp \quad (6)$$

$$E\cdots UMPPMp \rightleftarrows UMP\cdots E\text{–}PMp \quad (7)$$

$$UMP\cdots E\text{–}PMp \rightleftarrows E\text{–}PMp + UMP \quad (8)$$

$$E\text{–}PMp + acceptor\text{–}P \rightleftarrows acceptor\text{–}P\cdots E\text{–}PMp \quad (9)$$

$$acceptor\text{–}P\cdots E\text{–}PMp \rightleftarrows E\cdots acceptor\text{–}PPMp \quad (10)$$

$$E\cdots acceptor\text{-}PPMp \rightleftarrows E + acceptor\text{–}PPMp \quad (11)$$

assay involves reactions 6–8, whereas the transfer assay involves reactions 6–11. In the presence of K^+, the ratio (E/T) of the exchange rate to the transfer rate varies from 6 to 24 (Heydanek *et al.*, 1969). If all components are present under saturating conditions, this ratio would indicate that the pseudocomposite rate constant, k_e, for reactions 6–8 is larger than the pseudocomposite rate constant, k_t, for reactions 6–11 (Heydanek *et al.*, 1969). In the absence of K^+, the ratio (E/T) decreases from the above value to 2 to 3.

On an absolute basis, a 20-fold stimulation of the exchange rate and a 2-fold stimulation of the transfer rate is observed when 0.17 M KCl is added to dialyzed membranes. The low-exchange activity (5–10%) of dialyzed membranes is sufficient for 50% of the maximal transfer activity. With membranes depleted of K^+ ions, the formation of enzyme–phospho-MurNAc-pentapeptide is the rate-determining sequence whereas in the presence of excess K^+ ions the rate-determining sequence is the transfer of phospho-MurNAc-pentapeptide from the enzyme intermediate to lipid acceptor (reactions 9–11). 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 ($2\text{–}4 \times 10^{-3}$ M) provides additional evidence for the above conclusion. Increasing the rate of enzyme–phospho-MurNAc-pentapeptide formation by the addition of 0.01 M K^+ to dialyzed membranes (compare Figures 2 and 3) results in the maximal rate of transfer to the lipid acceptor and 50% of the exchange rate. Although additional K^+ ions (>0.01 M, Figure 2) increase the rate of enzyme–phospho-MurNAc-pentapeptide formation, additional K^+ (>0.01 M, Figure 3) ions do not affect the rate of transfer to the lipid acceptor. 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. These results suggest that the primary site of action of K^+ is in reactions 6–8.

It is difficult to establish the precise stage where K^+ functions in reactions 6–8. Table II summarizes the values of K_m and V_{max} obtained from the kinetic analysis presented in Figure 5A, B. V_{max} for the exchange assay increases as the concentration of K^+ is increased. In addition, the Michaelis–Menten constants for both UDP-MurNAc-pentapeptide and UMP become larger as the concentration of K^+ is increased. The fact that K_m increases as the concentration of K^+ ions is

elevated may reflect that the rate-limiting constant(s) of reaction 7 as expressed in V_{max} is an important factor in the value of K_m. Thus, the K_m values for UMP and UDP-MurNAc-pentapeptide may not reflect true dissociation constants.

Our data indicate that one binding site for K^+ exists in association with each catalytic center. The stimulation by K^+ gives normal hyperbolic saturation curves, and the value for n (1) established from Hill plots suggests the absence of homotropic effects. The binding of K^+ to the catalytic center is rapid and reversible and does not cause a shift in the pH optimum.

An objective of this work was to establish criteria for comparing the membrane-associated enzyme with the solubilized preparations of translocase. The activation by monovalent cations provides a method to compare the various soluble preparations of translocase. As illustrated in Figure 7, the solubilized preparations show identical enhancement by K^+ when compared with the preparation of membrane fragments. In addition, the specificity of activation by monovalent cations is identical for the soluble preparation and intact membrane fragments.

References

Anderson, J. S., Matsuhashi, M., Haskin, M. A., and Strominger, J. L. (1965), *Proc. Nat. Acad. Sci. U. S. 53*, 881.

Anderson, J. S., Matsuhashi, M., Haskin, M. A., and Strominger, J. L. (1967), *J. Biol. Chem. 242*, 3180.

Anderson, J. S., Meadow, P. M., Haskin, M. A., and Strominger, J. L. (1966), *Arch. Biochem. Biophys. 116*, 487.

Araki, Y., Shirai, R., Shimada, A., Ishimoto, N., and Ito, E. (1966), *Biochem. Biophys. Res. Commun. 23*, 466.

Atkinson, D. E., Hathaway, J. A., and Smith, E. C. (1965) *J. Biol. Chem. 240*, 2682.

Black, S. (1951), *Arch. Biochem. Biophys. 34*, 86.

Bright, H. J. (1967), *Biochemistry 6*, 1191.

Chatterjee, A. N., and Park, J. T. (1964), *Proc. Nat. Acad. Sci. U. S. 51*, 9.

Dixon, M., and Webb, E. C. (1964), Enzymes, New York, N. Y., Academic, p 429.

Edwards, J. B., and Keech, D. B. (1968), *Biochim. Biophys. Acta 159*, 167.

Giorgio, A. J., and Plaut, G. W. E. (1967), *Biochim. Biophys. Acta 139*, 487.

Green, M. L. (1964), *Biochem. J. 92*, 550.

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

Heydanek, M. G., Jr., Linzer, R., Pless, D. D., and Neuhaus, F. C. (1970), *Bacteriol. Proc.*, 73.

Heydanek, M. G., Jr., and Neuhaus, F. C. (1969), *Biochemistry 8*, 1474.

Higashi, Y., Strominger, J. L., and Sweeley, C. C. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 1878.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

Marshall, M., Metzenberg, R. L., and Cohen, P. P. (1961), *J. Biol. Chem. 236*, 2229.

Matz, L., and Strominger, J. L. (1968), *Bacteriol. Proc.*, 64.

Meadow, P. M., Anderson, J. S., and Strominger, J. L. (1964), *Biochem. Biophys. Res. Commun. 14*, 382.

Nandi, D. L., Baker-Cohen, K. F., and Shemin, D. (1968), *Biochemistry 243*, 1224.

Neuhaus, F. C. (1962), *J. Biol. Chem. 237*, 778.

Park, J. T. (1965), 149th National Meeting of the American Chemical Society, April, Detroit, Mich.

Robinson, J. D. (1967), *Biochemistry 6*, 3250.

Robinson, J. D. (1969), *Biochemistry 8*, 3348.

Stickgold, R. A., and Neuhaus, F. C. (1967), *J. Biol. Chem. 242*, 1331.

Struve, W. G., and Neuhaus, F. C. (1965), *Biochem. Biophys. Res. Commun. 18*, 6.

Struve, W. G., Sinha, R. K., and Neuhaus, F. C. (1966), *Biochemistry 5*, 82.

Tabor, H., and Wyngarden, L. (1959), *J. Biol. Chem. 234*, 1830.