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INITIAL MEMBRANE REACTION IN PEPTIDOGLYCAN SYNTHESIS

PERTURBATION OF LIPID-PHOSPHO-*N*-ACETYLMURAMYL-PENTAPEPTIDE TRANSLOCASE INTERACTIONS BY *n*-BUTANOL

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Summary

Phospho-*N*-acetylmuramyl-pentapeptide translocase, the initial membrane enzyme in the biosynthesis of peptidoglycan, requires a lipid microenvironment for function. *n*-Butanol was reversibly intercalated into membranes to perturb the hydrophobic interactions in this microenvironment in order to define further the role of lipid. In the concentration range for maximal stimulation of enzymic activity (0.12—0.18 M), *n*-butanol causes a 40% decrease in the fluorescence emission of the dansylated product, undecaprenyl diphosphate-(N^{ϵ}-dansyl)pentapeptide. Since no change in emission maximum occurs below 22°C in the presence of 0.12 M *n*-butanol, it is concluded that intercalation of this alkanol causes an increase in fluidity. Above 22°C this concentration of *n*-butanol causes both a decrease in the fluorescence emission and a red shift in the emission maximum. It is concluded that a polarity change as well as fluidity change occurs above 22°C. *n*-Butanol also causes a significant change in the phase transition experienced by the dansylated lipid product. Thus, it is possible with *n*-alkanols, e.g. *n*-butanol, to perturb lipid-translocase interactions resulting in an increase in fluidity in the microenvironment of the enzyme. This change in fluidity correlates with a stimulation of enzymic activity.

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Abbreviations: MurNAc, *N*-acetylmuramyl; dansyl, 5-dimethylaminonaphthalene-1-sulfonyl. Unless stated all abbreviations of residues denote the L-configuration. The omission of the hyphen, i.e., -DAla- for -D-Ala- conforms with the suggestion cited in the IUPAC-IUB, CBN, Recommendations (1966) Biochemistry 5, 2485, or (1966) J. Biol. Chem. 241, 2491. Although not stated, all D-glutamic acid residues are linked through the γ-carboxyl group to the diamino acid.

Introduction

The assembly of peptidoglycan is catalyzed by a system of membrane-associated enzymes that require undecaprenyl phosphate as a lipid carrier. In this system, cytoplasmic precursors are transferred to the membrane where intermediates covalently linked to this prenyl phosphate are assembled and translocated or extruded across the membrane to the sites of cell wall assembly. The initial enzyme in this system, phospho-MurNAc-pentapeptide translocase, requires a lipid microenvironment for function [1—3]. The product of this reaction, undecaprenyl diphosphate-MurNAc-pentapeptide, is immobilized within an anisotropic microenvironment close to the membrane surface and within 4—6 Å of the lipid matrix [4]. The physical state of this matrix has a significant effect on the catalytic activity of the translocase [5]. The precise mechanism by which bilayer lipid affects the catalytic activity of the translocase is not understood.

Intercalation of *n*-alkanols at relatively low concentrations into the membrane perturbs lipid-protein and lipid-lipid interactions, resulting in an increased fluidity of the bilayer [6—10]. This increase results from an enhancement in the mobility of the fatty acyl chains of the lipids. For example, the rotational mobility of intercalated doxyl-labeled stearic acid is increased as *n*-butanol penetrates into the bilayer [11]. This effect is more pronounced in mitochondrial membranes when compared with vesicles prepared from lipids derived from these membranes. This is because the protein-induced constraint of mobility of the fatty acyl chains is removed at low concentrations of alkanol [11]. Thus, the *n*-alkanols not only affect lipid-lipid interactions but they also have a major effect on lipid-protein interactions.

Modification of these hydrophobic interactions by *n*-alkanols provides a useful tool for investigating the role of the lipid matrix in the reaction catalyzed by phospho-MurNAc-pentapeptide translocase. It is the purpose of these experiments to use *n*-butanol to induce changes in the physical state of these lipids in the microenvironment of this enzyme. The specific introduction of a fluorophore into the lipid product, undecaprenyl diphosphate-MurNAc-(N^{ϵ}-dansyl)pentapeptide, gives a method for monitoring microenvironmental changes that can be correlated with changes in enzymic activity. For example, below 20—22°C in the presence of 0.12 M *n*-butanol, we have concluded that an increase in the fluidity of the lipid matrix and not an increase in polarity is responsible for the stimulation of the exchange reaction. Thus, intercalation of *n*-alkanol, e.g. *n*-butanol, allows one to change reversibly the microenvironment of the phospho-MurNAc-pentapeptide translocase.

Materials and Methods

5-Dimethylaminonaphthalene-1-sulfonyl chloride (dansyl chloride) was obtained from Sigma. UDP-MurNAc-Ala-DGlu-Lys-DAla-DAla was prepared from *Staphylococcus aureus* Copenhagen by the method described by Hammes and Neuhaus [12]. UDP-MurNAc-Ala-DGlu-Lys-D[^{14}C]Ala-D[^{14}C]Ala (27 cpm/pmol), UDP-MurNAc-Ala-DGlu-Lys(N^{ϵ}-dansyl)-DAla-DAla and UDP-MurNAc-Ala-DGlu-Lys(N^{ϵ}-dansyl)-D[^{14}C]Ala-D[^{14}C]Ala were prepared by the methods described by Weppner and Neuhaus [13].

Membrane fragments from *S. aureus* Copenhagen were prepared as described by Struve et al. [14]. The membranes were incubated with UMP to generate the highest concentration of undecaprenyl phosphate [12]. Membrane fragments containing undecaprenyl diphosphate-MurNAc-(N^{ϵ}-dansyl)pentapeptide were prepared by a modification of the procedure described previously [13]. A reaction mixture containing 50 μM UDP-MurNAc-Ala-DGlu-Lys(N^{ϵ}-dansyl)-DAla-DAla, 0.61 M KCl, 150 mM Tris-HCl (pH 7.8), 120 mM $MgCl_2$, 53 units of bacterial alkaline phosphatase and membranes (20 mg of protein) was incubated in 2.5 ml for 1 h at 25°C in the dark. The ratio of UDP-MurNAc-(N^{ϵ}-dansyl)pentapeptide to undecaprenyl phosphate (membrane bound) was 3 : 1. The incubation mixture was diluted 15-fold with 20 mM Tris-HCl (pH 7.8) containing 1 M KCl. The membrane fragments were sedimented by centrifugation for 40 min at 230 000 × *g*, washed twice in the same buffer, and resuspended in this buffer. The membranes containing undecaprenyl diphosphate-MurNAc(N^{ϵ}-dansyl)pentapeptide were stored at −196°C in small samples so that each sample was thawed only once before use.

The transfer and exchange assays for phospho-MurNAc-pentapeptide translocase were performed according to procedures described by Hammes and Neuhaus [12]. The transfer assay measured the amount of phospho-MurNAc-[^{14}C]-pentapeptide transferred from UDP-MurNAc-[^{14}C]pentapeptide to undecaprenyl phosphate in membrane fragments. The exchange assay measures the exchange of [^{3}H]UMP with the UMP moiety of UDP-MurNAc-pentapeptide. The dansylated UDP-MurNAc-pentapeptide replaces the normal substrate in both the transfer and exchange reactions as described by Weppner and Neuhaus [13]. In assays of activity where *n*-alkanol was added, the membranes in the buffer were incubated with the *n*-alkanol for 10 min at 25°C before the substrate was added. For low concentrations of *n*-alkanols, methanol was the diluent. The maximal concentration of methanol introduced as diluent was 0.4 M, a concentration that did not affect either of the assays for the translocase.

Fluorescence measurements were made at an angle of 90° in a Farrand Mark I spectrofluorometer. The temperature in the sample compartment was regulated by a Lauda K-2R constant temperature bath (±0.02°C) and monitored with a copper-constantan thermocouple attached to a Fluke digital voltmeter. A bandpass of 5 nm was used on both the excitation and emission beams. The excitation wavelength was 325 nm. An interference filter (Corning 0-52) was placed in the emission beam.

Protein was determined by the method of Lowry et al. [15] using bovine serum albumin as a standard. Radioactivity in aqueous samples was measured in the scintillation fluid described by Patterson and Greene [16].

Results

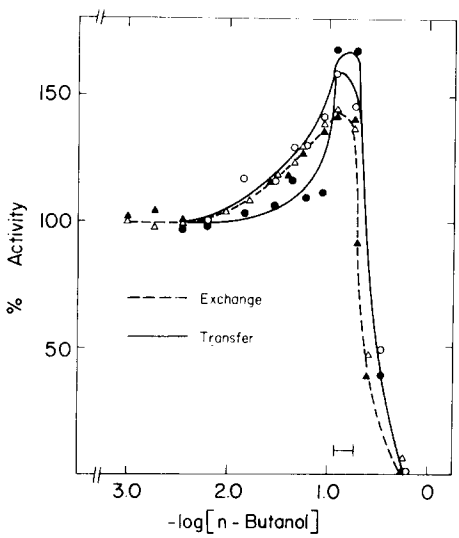
Effect of n-butanol on phospho-MurNAc-pentapeptide translocase

The transfer and exchange activities catalyzed by phospho-MurNAc-pentapeptide translocase with UDP-MurNAc-(N^{ϵ}-dansyl)pentapeptide and UDP-MurNAc-pentapeptide have been compared in the presence of increasing concentrations of *n*-butanol. As illustrated in Fig. 1, maximal stimulation of both activities was observed with 0.12—0.18 M *n*-butanol (|———|). Concentrations

greater than 0.18 M caused a sharp decline in both activities and concentrations greater than 0.56 M completely inhibited the enzyme. The maximal stimulation of the exchange and transfer activities was 40 and 65%, respectively. No differences in response to *n*-butanol in the exchange and transfer reactions were observed between the dansylated nucleotide and the undansylated nucleotide.

This stimulation-inhibition of the translocase by *n*-butanol was also observed by Matsuhashi et al. [17]. It was concluded that this alkanol as well as *n*-octanol affect the interaction of various components of the complex system in the particulate enzyme preparation.

A survey of *n*-alkanols (C_1—C_8) showed that each affected the transfer and exchange activities in a manner similar to that described for *n*-butanol (Fig. 1). The alkanols stimulated the rate between 30 and 100% for the transfer reaction and 20 and 60% for the exchange reaction. As the length of the *n*-alkanol was increased, lower concentrations were needed for the characteristic biphasic response. The relationship between the chain length and the concentration for maximal stimulation of the two activities is illustrated in Fig. 2. The chain lengths of the *n*-alkanols correlated with the negative logarithm of the concentrations. This linear correlation with chain length suggests that the effect of the alkanol is related to its hydrophobicity [11]. *n*-Butanol was chosen for these studies because of the relationship of the concentration range required to



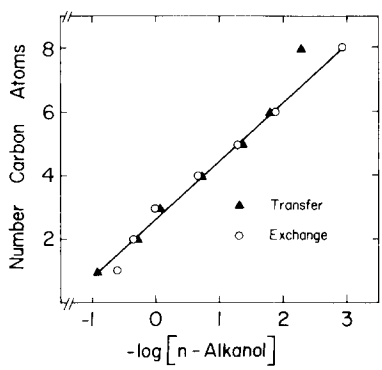


Fig. 1. Effect of *n*-butanol on the transfer and exchange reactions. The exchange assay was used with 39 μg of membrane protein and the transfer assay was used with 118 μg of membrane protein. In the exchange assay the concentrations of UDP-MurNAc-pentapeptide (△) and UDP-MurNAc-(N^{ϵ}-dansyl)pentapeptide (▲) were $3.3 \cdot 10^{-5}$ M. In the transfer assay the concentrations of UDP-MurNAc-[^{14}C]pentapeptide (○) and UDP-MurNAc-(N^{ϵ}-dansyl)[^{14}C]pentapeptide (●) were $2.0 \cdot 10^{-5}$ M. The activities are related to the control (100%). ⊢——⊣, the range of maximal stimulation (0.12—0.18 M).

Fig. 2. Effect of chain length on the concentration of *n*-alkanol for maximal stimulation. Each *n*-alkanol was used in the exchange and transfer assays to establish the concentration of maximal stimulation. The exchange assay was used with 38 μg of membrane protein and the transfer assay was used with 276 μg of membrane protein. The data for *n*-butanol are also shown in Fig. 1.

observe the biphasic response and its solubility, and because of its general use by others as an agent to enhance the fluidity of the membrane [8,10,11].

Effect of n-butanol on the membrane-bound undecaprenyl diphosphate-(N^{ϵ}-dansyl)-pentapeptide

In order to monitor changes in the microenvironment of the translocase induced by *n*-butanol, the dansyl reporter group was specifically incorporated into the lipid product by enzymic action (Fig. 1) [13]. The effect of increasing concentrations of *n*-butanol on the fluorescence emission of this group is illustrated in Fig. 3. The emission decreased 40% in the presence of 0.18 M *n*-butanol. At this concentration, maximal stimulation of enzymic activity was achieved (Fig. 1). At concentrations greater than 0.18 M, both the fluorescence emission and enzymic activity decreased. As the *n*-butanol concentration was increased, the fluorescence emission of the dansyl moiety was red shifted from 495 nm (Fig. 3). At 0.18 M, the concentration for maximal stimulation, the emission maximum was 515 nm. Thus, the addition of 0.18 M *n*-butanol causes both a red shift and a quenching of the fluorescence emission at 22—24° C.

Reversibility of the action of n-butanol

The effects of *n*-butanol that produce an alteration in the microenvironment of the translocase should be reversible in the concentration ranges that are of interest, i.e., 0.12—0.18 M (Fig. 1). Removal of *n*-butanol should give the original enzymic activity as well as the original fluorescence emission of the reporter group.

As illustrated in Table I, the exchange activity is stimulated by 35% in the presence of 0.18 M *n*-butanol. The original activity was restored when the *n*-butanol was removed. In contrast, removal of *n*-butanol from membranes that had

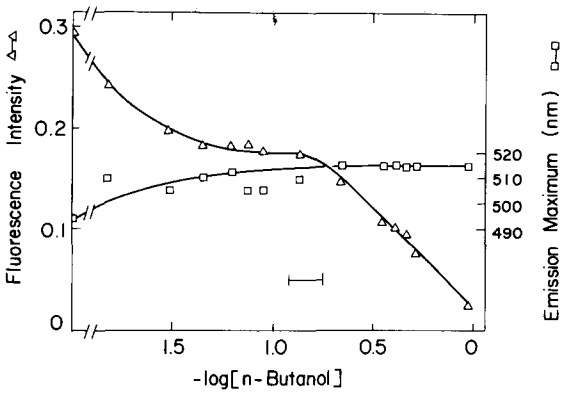


Fig. 3. Effect of *n*-butanol on the fluorescence intensity of membrane-associated undecaprenyl diphosphate-MurNAc-(N^{ϵ}-dansyl)pentapeptide. Membrane fragments (633 μg of protein/ml) containing $3.5 \cdot 10^{-7}$ M undecaprenyl diphosphate-MurNAc-(N^{ϵ}-dansyl)pentapeptide were suspended in 50 mM Tris-HCl (pH 7.8) buffer containing 42 mM $MgCl_2$ and 21 mM KCl. The temperature for these measurements was 22—24° C. The fluorescence emission was monitored from 475 to 525 nm and the relative fluorescence of the peak emission is recorded on the ordinate. The emissions of the dansylated membranes were corrected for the contribution of scattered light. ⊢——⊣, the range of maximal stimulation of the exchange and transfer activties (0.12—0.18 M) (Fig. 1).

TABLE I

REVERSIBILITY OF *n*-BUTANOL ACTION

(a) Exchange assay was performed as described in Materials and Methods. For the control, the membranes (780 μg/ml) were incubated for 20 min at 25°C in 50 mM Tris-HCl (pH 7.8) buffer containing 43 mM $MgCl_2$ and 21 mM KCl. In (1) and (2), the indicated concentrations of *n*-butanol were added to the membrane suspension. Aliquots were assayed for activity, and the remaining membranes in the control, (1) and (2), were isolated by centrifugation from the preincubation mixture which had been diluted with 7 vols. of 20 mM Tris-HCl (pH 7.8) containing 1 M KCl. Aliquots of these membranes were then assayed for exchange activity. (b) The dansylated-membranes (633 μg/ml) were preincubated with the indicated concentrations of *n*-butanol as described in (a). The *n*-butanol was removed as described in (a). This concentration of membrane fragments contains $3.5 \cdot 10^{-7}$ M undecaprenyl diphosphate-MurNAc-(N^{ϵ}-dansyl)-pentapeptide. The emission intensity of membrane fragments (633 μg/ml) lacking the dansylated lipid intermediate was used to correct for the contribution of scattered light.

Condition	Exchange activity (%)			
	+*n*-butanol		*n*-butanol removed	
a. Exchange activity				
1. Membranes + 0.18 M *n*-butanol	135		96	
2. Membranes + 0.56 M *n*-butanol	4		8	
	Fluorescence intensity (*I*)			
	+*n*-butanol		*n*-butanol removed	
	λ_{max} (nm)	*I* (%)	λ_{max} (nm)	*I* (%)
b. Fluorescence intensity				
1. Dansylated membranes	490	100	490	100
2. Dansylated membranes + 0.18 M *n*-butanol	510	62	500	98
3. Dansylated membranes + 0.56 M *n*-butanol	510	25	480	139

been treated with 0.56 M *n*-butanol did not restore the activity. Thus, the effect of 0.18 M *n*-butanol on the exchange reaction is reversible. The fluorescence emission, which was decreased by approximately 40% in the presence of this concentration of *n*-butanol, was restored upon its removal. However, the emission maximum was blue shifted by only 10 nm and not the expected 20 nm. This suggests that the original microenvironment was not precisely attained upon removal of the *n*-butanol, and may reflect that all of the *n*-butanol was not removed. A major change in the microenvironment was observed when the butanol was removed from the membranes treated with 0.56 M alcohol. A 40% increase in fluorescence emission over the untreated membranes as well as a 30 nm blue shift was observed. Thus, the effects of 0.18 M *n*-butanol are essentially reversible and provide one with an alkanol that can be used to perturb reversibly the translocase.

Effect of n-butanol on the exchange activity with UDP-MurNAc-pentapeptide and the fluorescence of undecaprenyl diphosphate-MurNAc-(N^{ϵ}-dansyl)-pentapeptide as a function of temperature

The characteristic temperatures of the phase transition (T_h and T_l), which correlate with the enzymic activity [5], may be changed when the lipid micro-

environment of the translocase is perturbed with butanol. It is the purpose of these experiments to monitor these characteristic temperatures of both the translocase exchange activity and fluorescence of the dansylated lipid intermediate in the presence of 0.12 M *n*-butanol.

As illustrated in Fig. 4, the Arrhenius plot of the fluorescence is characterized by a T_l and T_h of 19°C and 30°C, respectively. These values are in agreement with those established by Weppner and Neuhaus [4]. In the presence of 0.12 M *n*-butanol, the Arrhenius plot is characterized by a single slope change at 22° C. A second feature of these fluorescence measurements is the characteristic emission maximum of the fluorophore. In the absence of butanol, the emission maximum at all temperatures is 495 nm. In contrast, in the presence of 0.12 M *n*-butanol, the emission maximum is 495 nm from 4 to 22° C. Above 22° C it shifts to 510 nm. Thus, in the temperature range 4—22° C, the decrease in fluorescence emission in the presence of this concentration of *n*-butanol is not associated with a red shift whereas above 22° C the decrease in fluorescence is accompanied by a red shift in the emission.

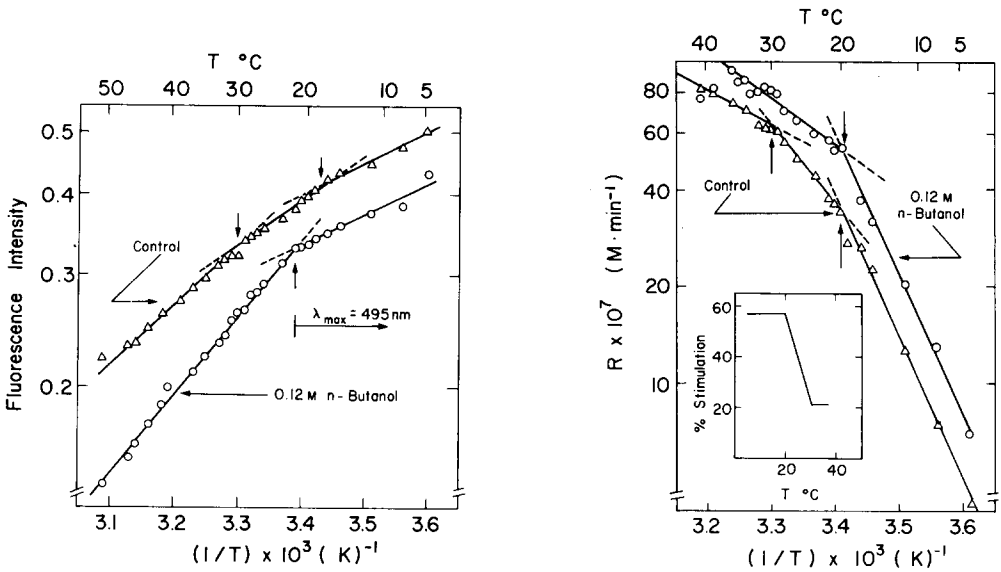


Fig. 4. Effect of *n*-butanol on the temperature dependence of the fluorescence intensity of undecaprenyl diphosphate-MurNAc-(N^{ϵ}-dansyl)pentapeptide. The emission intensity was measured at 495 nm with membrane fragments (633 μg of protein/ml) containing $3.5 \cdot 10^{-7}$ M undecaprenyl diphosphate-MurNAc-(N^{ϵ}-dansyl)pentapeptide in 50 mM Tris-HCl (pH 7.8) buffer containing 42 mM $MgCl_2$ and 21 mM KCl. The emission of the dansylated membranes was corrected for the contribution of scattered light. The emission maximum for the control is 495 nm and the maximum for the dansylated membranes in 0.12 M *n*-butanol is 495 below 22° C and 510 nm above 22° C.

Fig. 5. Effect of *n*-butanol on the temperature dependence of the exchange reaction. The exchange assay was used with 38 μg of membrane protein. The assay mixture contained. 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.8) buffer; 42 mM $MgCl_2$; 21 mM KCl; $3.3 \cdot 10^{-5}$ M UDP-MurNAc-pentapeptide; $3.3 \cdot 10^{-5}$ M [^{3}H]UMP and 0.12 M *n*-butanol in the indicated series. Each reaction mixture was equilibrated for 7 min at a given temperature before the substrates were added to initiate the exchange reaction for 15 min at that temperature. In the presence of this concentration of *n*-butanol, the exchange reaction is inhibited above 36° C. The inset reflects percent stimulation as a function of temperature.

The Arrhenius plot of exchange activity also shows slope changes which reflect the phase transition [5]. In the present work, T_l and T_h are 20 and 30°C, respectively (Fig. 5). These values agree essentially with those previously reported (T_l = 22°C, T_h = 30°C). In the presence of 0.12 M *n*-butanol, the Arrhenius plot is characterized by a single slope change at 20°C.

The interpretation of these Arrhenius plots in the presence of *n*-butanol may be complicated by the effect of temperature on the partition of the alcohol into the membrane. Thus, as the temperature is increased, the actual membrane concentration of the alcohol may increase. In order to remain in the region of activation (0.12—0.18 M) (Fig. 1), these studies were performed in 0.12 M *n*-butanol. It is of interest to compare the relative stimulation by *n*-butanol at each temperature. Below 20°C, the stimulation was 57% whereas above 30°C it was 21% (Fig. 5, inset). Thus, it would appear that the percent stimulation is correlated with the state of the membrane lipid rather than with a temperature dependent partition of the *n*-butanol in the bilayer.

Discussion

The optimal use of *n*-butanol as a perturbant of the microenvironment of a membrane enzyme requires that the induced changes are reversible. Reversible changes in the bilayer imply that the perturbant had not disorganized the supramembranal organization which is required for enzyme function and that it has only induced subtle changes in the fluidity or polarity. In the case of phospho-MurNAc-pentapeptide translocase, the original activity was restored when the *n*-butanol was removed. Moreover, the fluorescence emission of the membrane-associated undecaprenyl diphosphate-MurNAc-(N^{ϵ}-dansyl)pentapeptide was also restored. The emission maximum, however, was blue shifted by only 10 nm and not the expected 20 nm. This difference may reflect that all of the *n*-butanol was not removed. The red shift observed when *n*-butanol is intercalated into membranes can be interpreted as a polarity change. Thus, we conclude that the induced changes caused by low concentrations of *n*-butanol are reversible with the exception that the original polarity of the microenvironment has not been precisely restored.

The microenvironment of the translocase is determined by the interaction of the protein with the bilayer lipid [1,3,5]. In *S. aureus* these lipids are phosphatidylglycerol (37%), diphosphatidylglycerol (24%) and aminoacyl phosphatidylglycerol (40%) [18]. The major fatty acids of these lipids are *iso* and *anteiso* saturated branched methyl C_{15}, C_{17}, and C_{19} fatty acids [18—20]. Of this group C_{15}à and C_{17}à comprise 66% of the fatty acids. Analyses of these membranes indicate that they contain 56% protein, 25% lipid and 14% RNA [20]. In analogy to many membranes [11,21—25], it is reasonable to propose that a substantial percentage of this lipid is immobilized by contact with intrinsic membrane protein. In *Escherichia coli* this percentage is 20—25% [25]. This immobilized lipid is a major feature in defining the microenvironment of the protein. Lenaz et al. [11] proposed that this protein-induced immobility of lipid in mitochondrial membranes is removed by intercalating low concentrations of *n*-butanol. This is accompanied by an increased fluidity of the bilayer lipids.

The biphasic stimulation-inhibition of membrane enzymes by organic solvents has been observed in several cases [8,17,26,27]. For example, membrane ($Na^+ + K^+$)-ATPase, which requires lipid-protein interaction for activity, is stimulated by low concentrations of *n*-hexanol and inhibited by high concentrations of this alkanol. The increased fluidity at low concentrations of alkanol may enable the lipid molecules to interact more effectively with this membrane enzyme [8]. *n*-Butanol at high concentrations destroys the supramembranal organization required for the activity. In the case of phospho-MurNAc-pentapeptide translocase, high concentrations of this alkanol (0.56 M) completely disorganized the system as shown by activity and fluorescence measurements. In the concentration range for maximal stimulation (0.12—0.18 M), the fluorescence emission of membrane-bound undecaprenyl diphosphate-MurNAc-(N^{ϵ}-dansyl)pentapeptide is decreased by 40% and the emission maximum is red shifted to 515 nm. The decrease in fluorescence, which can be correlated with quantum yield, can be associated with a decrease in molecular rigidity resulting from a decrease in microviscosity. A red shift in the emission maximum is usually attributed to an increased polarity of the solvent microenvironment of the fluorophore. An increased polarity may reflect either a change in the solvent or an increased mobility of the solvent dipoles. Generally, however, a shift in emission maximum as well as a change in quantum yield occurs when the polarity of the microenvironment of the fluorophore is altered [28—31]. Thus, before tentative conclusions can be drawn from these spectral characteristics, it is of interest to define experimental conditions under which a change in a single characteristic is detected, i.e., either a change in fluorescence or emission maximum.

Experimental conditions have been found that allow for the *n*-butanol-induced change of the fluorescence yield at constant emission maximum that correlates with enhanced translocase activity. As illustrated in Fig. 4, below 22° C in the presence of 0.12 M *n*-butanol, a decrease in fluorescence without a red shift was correlated with a stimulation (57%) in enzymic activity. Since the emission maximum has not changed under these conditions, we conclude that the polarity of the fluorophore binding site has not been altered below 22° C. This stimulation is interpreted to result from an increase in fluidity of the lipids in the microenvironment of the translocase.

Above 22° C in the presence of 0.12 M *n*-butanol, the emission maximum shifts to 510 nm with a decrease in the fluorescence. The observed stimulation decreases from 57% below 20° C to 21% above 30° C (Fig. 5, inset). A change in the emission maximum can be attributed to a change in the polarity of the fluorophore microenvironment. Mobile solvent dipoles that can deactivate the Franck-Condon excited state will result in a red shift of the emission maximum [28,32]. Thus, we proposed that above 22° C in the presence of 0.12 M *n*-butanol, the polarity of the site also changes. However, the change in polarity is not necessarily correlated with a stimulation in activity since maximal stimulation is observed below 20° C where a red shift does not occur.

The physical state of the lipid bilayer has a significant effect on the catalytic activity of the translocase [5]. This is based on a correlation of slope changes in the Arrhenius plots of the exchange and transfer activities, with the characteristic T_h and T_l of the phase transition of the membrane from *S. aureus*. If *n*-buta-

nol removes the protein-induced constraint of the mobility of the fatty acyl chains as well as perturb lipid-lipid interaction [11], we would predict a significant effect on the characteristic slope changes in the Arrhenius plot for activity. As illustrated in Fig. 5, in the presence of 0.12 M *n*-butanol a single slope change at 20°C is observed in the Arrhenius plot for activity. In the Arrhenius plot for the fluorescence of the dansylated lipid intermediate in the presence of the same concentration of *n*-butanol, a single change is also observed at 22°C. In the absence of *n*-butanol in both the fluorescence and activity measurements, slope changes are observed at 19—20°C and 30°C. Although it is uncertain what physical changes occur at 20—22°C in the presence of 0.12 M *n*-butanol, it is apparent that both the fluorescence of the fluorophore and the activity measurements reflect a similar physical change in the membrane structure.

Since the exchange and transfer activities reflect the same T_h and T_l, Weppner and Neuhaus [5] concluded that the physical state of the lipid matrix has a major effect on the catalytic activity of the translocase, i.e., the intramolecular transfer of phospho-MurNAc-pentapeptide from UMP to undecaprenyl phosphate that is common to both activities. The similar concentration of the alkanol for the biphasic response of both the exchange and transfer reactions suggests that the fluidity change induced by the alkanol affects the same intramolecular transfer process.

The ability of *n*-butanol to perturb the hydrophobic interactions in the microenvironment of the translocase provides a means for defining the role of lipid-protein interactions on the properties of this enzyme. This approach has been particularly successful in the study of membrane ATPase from mitochondria where *n*-butanol reduces lipid-protein interaction resulting in a decrease in the oligomycin sensitivity and the K_m of ATP and an increase in activation energy [9,10]. The increased activation energy is interpreted as the loss of a correct lipid environment of the enzyme. Although we are unable to define the precise role of the lipid in the reaction catalyzed by phospho-MurNAc-pentapeptide translocase, we are certain that this enzyme must function in a lipid matrix and that the lipid provides either the hydrophobic medium for the lipid substrate/product or modulates a specific enzyme conformation required for catalytic activity. Thus, *n*-butanol at low concentrations in a defined temperature range allows one to manipulate reversibly the fluidity in the microenvironment of the translocase which is correlated with the stimulation of enzyme activity.

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