

The Impact of the Outer Membrane and General Porins on Cyanide Diffusion in Gram-Negative Bacteria Vladyslav Yakovliev^{1,*}, Bohdan Lev¹

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Introduction

The outer membrane (OM) of gram-negative bacteria serves as a permeability barrier, protecting the cell against external aggressive environments. The OM consists of an external lipopolysaccharide monolayer, an internal leaflet of a phospholipid monolayer, and β -barrel proteins such as porins. General porins, including OmpF, OmpC, OmpA, and OprF, form water-filled channels in the OM, allowing the passive diffusion of small hydrophilic molecules, such as nutrients and ions. In this study, we investigate the diffusion of various cyanide compounds (free cyanide (CN⁻), thiocyanate (SCN⁻), and metal-cyanide complexes like $[Ag(CN)_2]^-$, $[Fe(CN)_6]^{3-/4-}$, etc.) through general porins embedded in the OMs of *E. coli* (OmpF, OmpC, and OmpA porins) and *Pseudomonas* species (OprF porin). Understanding how these cyanide compounds diffuse is crucial for optimizing cyanide bioremediation and developing efficient biological treatment systems for cyanide-contaminated wastewaters. Other factors impacting the overall OM permeability P include the number n_p and length h of general portions, the fraction of open channels p (especially relevant for portions like OmpA and OprF which have gated states), and the overall surface area $S_{\rm m}$ of the bacteria. For instance, OmpA has only about 2-3% of molecules in an open conformation, and OprF about 5%, significantly affecting their contribution to total permeability [1].



Methods

The permeation of small molecules through the OM can be described as a passive diffusion process. The diffusion rate is determined by Fick's first law of diffusion in terms of the macroscopic permeability coefficient P of the OM:

$$J = PS_{\rm m} \left(C_{\rm out} - C_{\rm in} \right) \tag{1}$$

where J is the diffusion rate of the substance per a single bacterial cell, $S_{\rm m}$ is the surface area of the OM of a single bacterial cell, $C_{\rm out}$ and $C_{\rm in}$ are the concentrations of the substance outside the cell and within the periplasmic space of the cell, respectively.

The diffusion is facilitated through the water-filled channels of general porins. A simple representation assumes a porin as a cylindrical pore with radius R and length h, and the substance is represented by spherical molecules with radius r:

$$J = DS_{\text{eff}} \frac{(C_{\text{out}} - C_{\text{in}})}{h} = D_{\text{eff}} S_{\text{tot}} \frac{(C_{\text{out}} - C_{\text{in}})}{h}$$
(2)

Here, D is the free diffusion coefficient in water, S_{eff} is the effective cross-sectional area of all porins, D_{eff} is the effective diffusion coefficient within a porin, and S_{tot} is the total cross-sectional area of all porins. The free diffusion coefficient D can be estimated using the Stokes-Einstein equation. The relationship between effective and free diffusion coefficients is given by:

$$D_{\rm eff}/D = S_{\rm eff}/S_{\rm tot} = H\left(\xi\right) \tag{3}$$

where $\xi = r/R$ and $H(\xi)$ is the diffusional hindrance factor for a sphere in a cylindrical pore. The widely used Renkin equation represents this factor as:

$$H_{\rm R}(\xi) = (1-\xi)^2 \left(1 - 2.104\xi + 2.09\xi^3 - 0.95\xi^5 \right) \tag{4}$$

However, the accuracy of the Renkin equation is constrained to cases where $\xi \leq 0.4$. For a wider range of $\xi \leq 0.95$, we utilize the Dechadilok-Deen equation:

$$H_{\rm DD}(\xi) = 1 + \frac{9}{8}\xi \ln(\xi) - 1.56034\xi + 0.528155\xi^2 + 1.91521\xi^3$$

Figure 1: Comparison of diffusional hindrance factors. Effect of the solute-to-porin radius on the ratio of the diffusional hindrance factor $H_{\rm R}$ according to the Renkin equation to the diffusional hindrance factor $H_{\rm DD}$ according to the Dechadilok-Deen equation [1].



 $-2.81903\xi^4 + 0.270788\xi^5 + 1.10115\xi^6 - 0.435933\xi^7 \tag{5}$

The comparison in Fig. 1 illustrates the overestimation by the Renkin equation at higher ξ values compared to the Dechadilok-Deen equation.

The modification of the effective diffusion coefficient within the porin due to the presence of the Donnan potential (DP) can be estimated as:

$$D_{\text{eff},\phi} = D_{\text{eff}} W\left(\phi\right) \tag{6}$$

where $W(\phi)$ is the correction factor and ϕ represents the energy of a charged particle in the external potential in units of $k_B T$:

$$\phi = \frac{Ze_c V_D}{k_B T} \tag{7}$$

where $V_D < 0$ is the DP, e_c is the elementary charge, and Z is the charge number of ion. For a linear potential across the porin, $F(x)/k_BT = \phi x/h$, the correction factor $W_{\text{lin}}(\phi)$ is:

$$W_{\rm lin}\left(\phi\right) = \frac{\phi^2}{2\left(e^{\phi} - 1 - \phi\right)}\tag{8}$$

For an intrinsic periodic potential within the pore, e.g., $F(x)/k_BT = 2\phi x/\lambda$ for $0 < x < \lambda/2$ and $2\phi - 2\phi x/\lambda$ for $\lambda/2 < x < \lambda$, the correction factor $W_{\text{per}}(\phi)$ is

$$W_{\rm per}(\phi) = \frac{\phi^2}{(1 - e^{-\phi})(e^{\phi} - 1)} \tag{9}$$

The permeability coefficient is then expressed as:

$$P = \frac{D}{h} \frac{S_{\text{tot}}}{S_{\text{m}}} H\left(\xi\right) W\left(\phi\right) \tag{10}$$

The dependence of the effective diffusion coefficient on ϕ for these potentials is shown in Fig. 2.

Figure 2: Dependence of the effective diffusion coefficient on the energy of a charged particle for linear potential (solid line) and periodic potential (dashed line) [1]

Our calculations reveal substantial differences in permeability coefficients among different cyanide compounds. The effective diffusion coefficient $D_{\rm eff}$ of free cyanide CN⁻ through OmpF porin is 8 to 22 times greater than that of other cyanide compounds, 11 to 35 times greater through OmpC porin, 18 to 78 times greater through OmpA porin, and 3.4 to 5.3 times greater through OprF porin. These differences become even more pronounced when considering the DP effects.

For *Pseudomonas* species, the permeability coefficient for free cyanide exceeds that of other cyanide compounds by factors ranging from 3.4 to 32. In *E. coli*, when considering all three porins (OmpF, OmpC, and OmpA), the permeability coefficient for free cyanide exceeds that of other cyanide compounds by factors of 9 to 155.

The OM of *Pseudomonas* species exhibits greater permeability for larger cyanide compounds compared to *E. coli* OM due to the larger pore size of OprF (2.0 nm diameter) compared to OmpF, OmpC, and OmpA porins (1.0-1.2 nm diameter). However, for free cyanide, the OM of *Pseudomonas* species is less permeable than that of *E. coli* due to the higher percentage of open channels in *E. coli*.

Conclusions

The diffusion of cyanide compounds through the OM of gram-negative bacteria is governed by complex interactions between molecular size, charge, and porin characteristics. The effective diffusion coefficient within porins is significantly reduced compared to free diffusion in water, with this reduction being most pronounced for larger metal-cyanide complexes. The DP across the OM creates additional selectivity based on charge, further influencing the transport of different cyanide species. These insights into bacterial resistance mechanisms have significant implications for optimizing cyanide bioremediation [1,2] as our model enables accurate prediction of cyanide transport rates through bacterial OMs for developing efficient biological treatment systems for cyanide-contaminated wastewaters.

Results and discussion

Our study demonstrates that the effective diffusion coefficient of a cyanide compound within a general porin, D_{eff} , is significantly reduced compared to its free diffusion coefficient in water, D. This reduction is primarily influenced by several factors. The compound-to-porin radius ratio ($\xi = r/R$) is a key determinant, accounted for by the diffusional hindrance factor $H(\xi)$. As shown by the Dechadilok-Deen equation, which is more accurate than the Renkin equation for larger ξ values (see Fig. 1), increased solute size relative to the pore significantly impedes diffusion [1]. The charge of the cyanide ion Z and the DP V_D across the OM collectively influence diffusion through the energy parameter ϕ . The correction factor $W_{\text{lin}}(\phi)$ for a linear potential (approximating the DP) shows that a negative DP -30 mV (typical for bacteria) selectively favors cation permeation and hinders anion permeation, such as cyanide ions [1].

An intrinsic porin potential, which can be periodic or have other forms, further modifies the diffusion via a correction factor like $W_{\text{per}}(\phi)$. This intrinsic potential arises from the fixed charges and geometry within the porin channel itself.

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