Original Article

Nigericin Forms Highly Stable Complexes with Lithium and Cesium[1]

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Nigericin is a monocarboxylic polyether molecule described as a mobile K^+ ionophore unable to transport Li^+ and Cs^+ across natural or artificial membranes. This paper shows that the ion carrier molecule forms complexes of equivalent energy demands with Li^+, Cs^+, Na^+, Rb^+, and K^+. This is in accordance with the similar values of the complex stability constants obtained from nigericin with the five alkali metal cations assayed. On the other hand, nigericin–alkali metal cation binding isotherms show faster rates for Li^+ and Cs^+ than for Na^+, K^+, and Rb^+, in conditions where the carboxylic proton does not dissociate. Furthermore, proton NMR spectra of nigericin–Li^+ and nigericin–Cs^+ complexes show wide broadenings, suggesting strong cation interaction with the ionophore; in contrast, the complexes with Na^+, K^+, and Rb^+ show only clear-cut chemical shifts. These latter results support the view that nigericin forms highly stable complexes with Li^+ and Cs^+ and contribute to the explanation for the inability of this ionophore to transport the former cations in conditions where it catalyzes a fast transport of $K^+ > Rb^+ > Na^+$.

INTRODUCTION

Nigericin is a monocarboxylic polyether molecule (Fig. 1) first described in its ion translocating capability in mitochondrial membranes by Estrada-O. *et al.* (1967a). Two different ion transfer mechanisms have been demonstrated for this ionophore: at concentrations below $1.0 \mu M$, an electrically silent potassium/

proton exchange mechanism was shown by Pressman (1968) and Tosteson *et al.* (1968), this after Mitchell suggested the occurrence of such a model during a Federation Meeting discussion (1968). On the other hand, at concentrations above $1.0 \mu M$, nigericin catalyzes a pH- and concentration-dependent electrogenic potassium transport occurring through mobile dimers (Estrada-O. *et al.*, 1967b; Markin *et al.*, 1975; Toro *et al.*, 1976).

This paper shows that nigericin forms complexes of equivalent energy requirements with lithium, cesium, sodium, rubidium, and potassium, and that the translocator forms highly stable complexes with Li^+ and Cs^+.

These results provide an explanation for the inability of nigericin to transport lithium and cesium in conditions where it catalyzes a fast transport of $K^+ > Rb^+ > Na^+$.
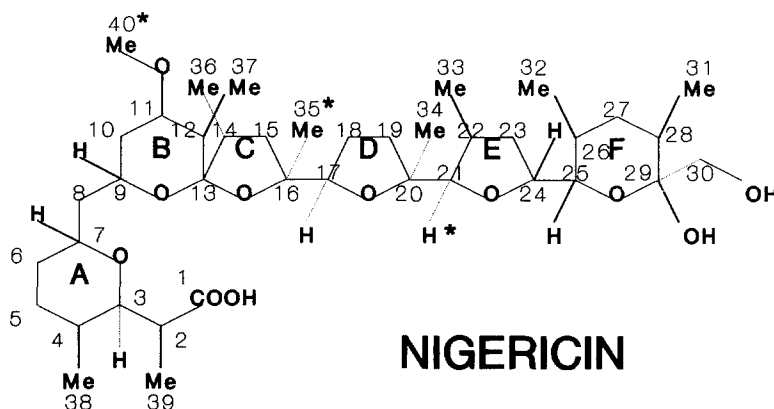
Fig. 1. Chemical formula of nigericin free acid. The numbering sytem follows that in Rodios and Anteunis (1977). Those atoms marked by an asterisk correspond to the signals studied by ^{1}H-NMR as published by Toro et al. (1987).

MATERIALS AND METHODS

Potentiometric Measurements

Stability Constants for Complex Formation

Nigericin free acid was a kind gift from Dr. P. A. Miller, Dr. L. H. Sello, and Dr. J. W. Westley (Hoffman-La Roche, Inc., Nutley, New Jersey).

Stability constants (K_s) for nigericin–alkali metal cation complex formation reactions, in solution, were determined at room temperature by potentiometric techniques. Measurements were made, at equilibrium, having nigericin at 112 ± 6 μM and equivalent concentrations of alkali metal cation chlorides. The experiments were carried out in a single-phase homogenous solution of 1,4-dioxane/H_2O 45% (vol/vol) at pH 7.76, as previously reported for the nigericin–potassium complex (Toro et al., 1987). Ion-selective electrodes (Beckman 39046 for sodium and Beckman 39047 for lithium, potassium, rubidium, and cesium cations) were equilibrated with each alkali metal chloride for a week; their Nernstian response was corroborated before use. All chemicals were analytical grade (Merck).

Binding Isotherms

Binding rates were obtained by potentiometric titration in the same medium used for the determination of K_s. The experiments were carried out with nigericin acid at constant concentration, adding the metal cation and plotting their binding rate as a function of [Bound metal]/[Total metal], following the "isolation" technique described by Smith (1981).

Simultaneous to the titration, pH measurements were carried out with a glass electrode (Beckman 39505).

Proton NMR Spectra

The ^{1}H-NMR spectra were taken in deuterated chloroform ($CDCl_3$) in a Varian EM-390, 90 MHz NMR Spectrometer. Tetramethylsilane (TMS) was used as internal standard.

Nigericin free acid spectrum was obtained directly from the sample provided by Hoffman-La Roche. Nigericin–alkali metal cation complexes were prepared from the free acid and an excess of alkali metal carbonate salts as previously described (Toro et al., 1987). After solvent evaporation, each sample was freeze-dried (Labconco Freeze-Dryer 3) during 3 hours and dissolved in $CDCl_3$. The signal assignments of the spectra were taken from previous reports (Rodios and Anteunis, 1977; Toro et al., 1987).

RESULTS

Feasibility for the Formation of Nigericin–Cation Complexes

Table I shows no statistical difference among K_s values of nigericin bound to lithium, sodium, potassium, rubidium, and cesium cations in homogeneous solution. In fact, the estimation of the corresponding $\Delta G°$ values from the K_s data shows similar feasibility for the formation of nigericin complexes with the five alkali metal cations.

Binding Rates

Nigericin–alkali metal cation binding behavior

Table 1. Log K_s and ΔG° Values of Nigericin–Alkali Metal Cation Complexes[a]

Nigericin complex	log K_s	ΔG° (kcal/mol)
Lithium	4.16 ± 0.34	− 5,681
Sodium	3.91 ± 0.22	− 5,339
Potassium	3.70 ± 0.08	− 5,053
Rubidium	3.70 ± 0.25	− 5,053
Cesium	3.82 ± 0.34	− 5,271

[a]Nigericin-free acid (112 ± 6 μM) was dissolved in a 1,4-dioxane/ H_2O 45% (vol/vol) homogeneous solution (ε 38.48). pH (7.76) was adjusted with triethanolamine and HCl. (ε = dielectric constant.)

was followed by potentiometric titration in homogeneous solution. Figure 2 depicts nigericin–alkali metal cation binding isotherms as a function of total metal at constant nigericin concentration. An apparent first-order reaction is produced with each alkali metal cation tested. However, faster binding rates are obtained for lithium and cesium (Fig. 2a) than for sodium, potassium, and rubidium (Fig. 2b).
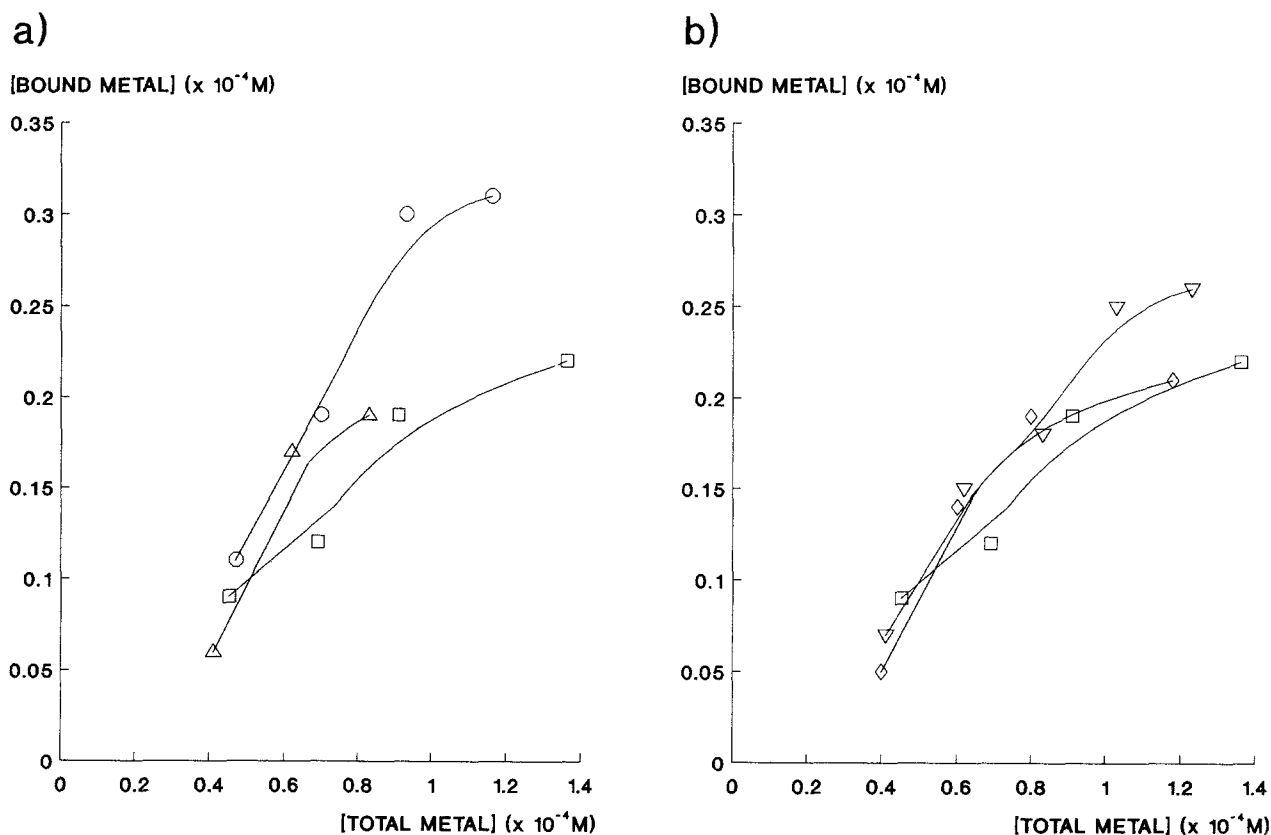
Effect of Alkali Metal Cations on ^{1}H-NMR Signals of Nigericin Free Acid

Table II shows a comparison of the proton shift values (δ), in ppm from TMS internal, of H 21, Me 35, and Me 40 (Fig. 1) for the complexes of nigericin with all the alkali metal cations in $CDCl_3$. It can be seen that wider broadenings of H 21, Me 35, and Me 40 signals, with respect to the free acid, are caused by lithium and cesium; these results suggest an important reduction in the flexibility of the molecule near those loci. On the other hand, only significant $\Delta\delta$ are caused by sodium (H 21 > Me 40 > Me 35), potassium (Me 40 > H 21 > Me 35), and rubidium (Me 40 > Me 35 > H 21) as compared with lithium and cesium.

DISCUSSION

Energy Requirements for Complex Formation

In contrast to previous results on cation binding

a)

b)

Fig. 2. Nigericin–Alkali metal cation binding isotherms in one single-phase homogeneous solution at ε 38.48 and pH 7.76. Initial nigericin concentration was 112 ± 6 μM. Homogeneous solution was prepared as in Table I. (a) Comparison of Li$^+$ (O) and Cs$^+$ ($\triangle$) vs. K$^+$ ($\square$); (b) comparison of Na$^+$ ($\triangledown$) and Rb$^+$ ($\diamond$) vs. K$^+$ ($\square$).

Table II. Proton Shift Values (δ) for Nigericin–Alkali Metal Cation Complexes in $CDCl_3$[a]

	δ signals (ppm)[b]		
Sample	H21	Me35	Me40
Free acid[c]	4.024	1.419	3.353
Nigericin–Li	4.24 (vb)	1.54 (vb)	3.42, 3.44 (b)
Nigericin–Na	4.345	1.591	3.368
Nigericin–K[c]	4.25	1.6	3.62
Nigericin–Rb	4.0	1.46	3.4
Nigericin–Cs	3.9	1.5, 1.48 (b)	3.44, 3.36, 3.33

[a] 90 MHz ^{1}H-NMR spectra. TMS was used as internal.
[b] b: broad; vb: very broad.
[c] Toro et al. (1987).

carried out in bulk-phase partition experiments (Pressman, 1968), where a clear ion selectivity profile for nigericin was observed (K^+ > Rb^+ > Na^+ > Cs^+ > Li^+), this paper shows that the ionophore interacts with almost identical affinity values with the above-mentioned alkali metal cations in homogeneous solutions (Table I).

The finding that no significant differences exist among the K_s values clearly indicates that nigericin may form complexes of equivalent energy demands with the five alkali metal cations tested, irrespective of its translocating capability. Thus, the energy requirements of the complexation reaction seem not to be a limiting step for the transport process and, therefore, they are not responsible for the ionic selectivity observed in multiple phase systems (Taylor et al., 1982), such as bulk phases and lipid membranes.

It is also apparent, from the above results, that there is no correlation between complex stability and cation size (vgr.: ionic, atomic, or hydrated radii) nor electronegativity. Therefore, one may hypothesize that nigericin should be able to complex virtually any metal cation, despite its actual volume.

Binding Intensities Determine the Rate of Ion Transport

The cation-to-polyether binding intensities apparently determine the rate of ion transport. This is derived from the different rates obtained in the ratio existing between bound metal, or formed complex, and total metal concentration shown in Fig. 2, where two general patterns of behavior are observed. First, faster binding rates shown with lithium and cesium (Fig. 2a) suggest greater attraction forces between these cations and the polyether. Second, slower rates

depicted with the cations which are commonly transported, sodium, potasssium, and rubidium, indicate an easier dissociation between these ions and the carrier.

In fact, the observed ^{1}H-NMR broadenings of signals (Table II) and, accordingly, smaller relaxation times, are an indirect reflection of polyether–cation interaction intensity, and suggest that lithium and cesium are strongly tied, not easily dissociated and, therefore, unable to be transported across lipid barriers. Thus, dissociation could be a major limiting step for mobile carrier-mediated transport, as has been recently suggested for potassium and rubidium (Riddell et al., 1988).

The higher affinity and intensity of interaction of lithium and cesium shown for nigericin appear to be related with the higher electron affinity (ea) and reduction potential ($E°$) values of both cations, rather than with their size and electronegativity. This is derived from the fact that $E°$ of Li^+ (-3.02 V) and Cs^+ (-3.02 V) are higher than those of Na^+ (-2.71 V), K^+ (-2.92 V), and Rb^+ (-2.99 V) as indicated by Cotton and Wilkinson (1966), and also by data (Day and Selbin, 1969) showing that the ea of lithium (0.58 eV) is lower than those of rubidium, sodium, and potassium (ea_{Rb^+} 0.6 eV, ea_{Na^+} 0.78 eV and ea_{K^+} 0.92 eV). Consequently, the intrinsic energetic properties of the metal atoms could play a central role in the selectivity of binding and ion transport. These data complement previous evidence indicating that variations on the chemical structure of polyether molecules also determine the transitions of ionic selectivity for transport (Eisenman et al., 1968).

Ionic Interaction Profiles of Nigericin

In conclusion, the correlation of results shown in this paper differs with respect to solvent extraction and transport selectivities previously published for nigericin in several experimental systems (Table III). In addition, they illustrate that the ionophore is capable of interacting with metal cations which are not translocated across lipid phases. Consequently, it is very likely that the profiles of ionic interaction demonstrated in this paper for nigericin could sustain new lines of interpretation to explain the biological activity of carboxylic ionophores (Osborne et al., 1977; Ben-Hayyim and Krause, 1980; Hatefi et al., 1982; Osborne et al., 1982) through the binding with fixed metal ions in enzymes, protein complexes, and surface membrane ligands.

Table III. Nigericin–Alkali Metal Cation Selectivities as Obtained in Different Experimental Systems

Experimental system	Ion selectivity sequence
Mitochondria O_2 consumption[a] Toluene-n-buthanol extraction[b] Transport in mitochondria[cd] BLM at pH $\sim$ 7[d]	K > Rb > Na > Cs > Li
Three-phase transport[e]	K $\sim$ Rb > Na > Cs > Li
BLM at pH 4[f]	K > Rb > Cs > Na > Li
This work:	
K_s	Li $\sim$ Na $\sim$ K $\sim$ Rb $\sim$ Cs
¹H-NMR broadenings	Li $\sim$ Cs > Na $\sim$ K $\sim$ Rb
Cation binding rates	Li $\sim$ Cs > Na $\sim$ K $\sim$ Rb

[a]Graven et al. (1966).
[b]Pressman (1968).
[c]Henderson et al. (1969).
[d]Toro et al. (1976).
[e]Ashton and Steinrauf (1970).
[f]Toro et al. (1987).

ACKNOWLEDGMENTS

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