Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1725–1728

BIOORGANIC &
MEDICINAL CHEMISTRY
LETTERS

A FREE ENERGY CALCULATION CAN BE USED TO PREDICT K^+-BINDING CONSTANTS FOR NEW MACROTETROLIDE ANTIBIOTICS

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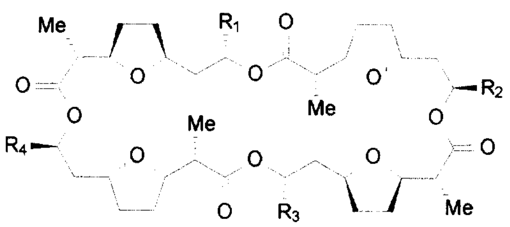
Received 22 April 1998; accepted 2 June 1998

Abstract: A free energy calculation technique was used to predict K^+ binding constants for new macrotetrolides. The technique was validated by successfully predicting affinity constants for known, naturally produced, macrotetrolides. © 1998 Elsevier Science Ltd. All rights reserved.

Introduction

Nonactin (1)[1–9] is the parent compound of a group of ionophore antibiotics, produced by *S. griseus subsp. griseus* ETH A7796, known as the macrotetrolides (Fig. 1). Nonactin has been shown to possess antitumor activity both against mammalian cell lines in vitro and against Crocker Sarcoma 180 in studies in mice.[9] Nonactin was recently shown to be a novel inhibitor of the 170-kDa-P-glycoprotein mediated efflux of 4-O'-tetrahydropyranyl-adriamycin in multidrug-resistant erythroleukemia K562 cells at subtoxic concentrations.[10] The natural macrotetrolide homologues show a wide range of potency. For example, the minimum inhibitory concentration (MIC) of nonactin against *Staphylococcus aureus* and *Mycobacterium bovis* is over an order of magnitude greater than that of dinactin, a difference which is paralleled by the changes in the stability constants of their Na^+ and K^+ complexes.[11]



		R_1	R_2	R_3	R_4
1	Nonactin	Me	Me	Me	Me
2	Monactin	Me	Me	Et	Me
3	Dinactin	Et	Me	Et	Me
4	Trinactin	Et	Et	Et	Me
5	Tetranactin	Et	Et	Et	Et
6	Analog 1	H	H	H	H
7	Analog 2	iPr	iPr	iPr	iPr
8	Analog 3	CH_2OH	CH_2OH	CH_2OH	CH_2OH

Figure 1. The naturally occurring macrotetrolides

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PII: S0960-894X(98)00300-X

Non-natural analogs of the macrotetrolides can be envisaged that may extend the activity trends seen for the natural homologues. Obtaining quantities of such analogs by total synthesis, however, is a difficult undertaking.[12–18] We wish to develop a molecular modeling technique that we can use to predict the K^+ affinity of new macrotetrolides so that they could be evaluated prior to synthesis and our synthetic efforts, therefore, could be directed to the most promising analogs.

In principal, molecular mechanics calculations can be used to give insight into many chemical phenomena, including cation-macrocycle interactions. Dynamics calculations performed on nonactin in methanol have been used to assess the solvation structure of the nonactin-K^+ complex, the absolute ion binding constants, and the mechanism of ion binding to the complex.[19] Additionally, free energy calculations have been used to calculate the relative specificity of certain macrocycles for Na^+ and K^+.[20–22] Previous calculations have perturbed one ion into another within a constant macrocycle structure to determine relative binding constants for the ions.[19] In the case of nonactin, calculations gave simulated values for $\Delta\Delta G_{binding}$ that were in good agreement with experimentally determined constants.[19] In our work we have, instead, kept the nature of the ion constant and have perturbed the structure of the macrocycle to assess relative binding constants of an ion to a series of homologous macrocycle structures.

Results and Discussion

Free energy calculations (FEC) are based on a simple thermodynamic cycle such as depicted in Scheme 1. ΔG_1 and ΔG_3 are, respectively, the free energy changes associated with the binding of a K^+ ion to either of a pair of homologous macrocycles, M_1 and M_2, and represent physical processes. ΔG_2 and ΔG_4 are, respectively, the free energy changes associated with the change of structure of M1 into M2 both with, and without, the complexed ion: these energies can be estimated from molecular dynamics simulations over the course of which a gradual perturbation of the structure is carried out. The utility of the method is expressed in equations 1 and 2 where it can be readily seen that the real $\Delta\Delta G_{binding}$ can be related to the simulated data. The results of our simulations are shown in Table 1. We validated the computational method by using separate perturbations of monactin into nonactin and dinactin, together with an experimentally determined K^+ binding constant for monactin, to predict K^+ binding constants for nonactin and dinactin. The predicted logK values for nonactin

$$\begin{tikzcd}
M_1 + K^+ \arrow[r, "\Delta G_1"] \arrow[d, "\Delta G_4"'] & M_1K^+ \arrow[d, "\Delta G_2"] \\
M_2 + K^+ \arrow[r, "\Delta G_3"'] & M_2K^+
\end{tikzcd}$$

$$\Delta G_1 + \Delta G_2 - \Delta G_3 - \Delta G_4 = 0 \quad \text{(Equation 1)}$$

$$\Delta\Delta G_{binding} = \Delta G_3 - \Delta G_1 = \Delta G_2 - \Delta G_4 \quad \text{(Equation 2)}$$

Scheme 1. Thermodynamic basis of simulation technique

Table 1. Comparison of simulated and real changes in the binding energy of K^+ to a macrocycle when the macrocycle structure is changed.

Simulation	logK predicted for	$\Delta\Delta G_{binding}$ /kcalmol^{-1}	Predicted logK	Experimental logK
monactin **2** → nonactin **1** $R_3 = Et \rightarrow R_3 = Me$	Nonactin (**1**)	- 0.55	3.99	3.88 ± 0.22
monactin **2** → dinactin **3** $R_1 = Me \rightarrow R_1 = Et$	Dinactin (**2**)	0.15	4.51	4.73 ± 0.18
nonactin **1** → **6** $R_{1\text{-}4} = Me \rightarrow R_{1\text{-}4} = H$	**6**	3.60	6.49	-
nonactin **1** → **7** $R_{1\text{-}4} = Me \rightarrow R_{1\text{-}4} = {}^iPr$	**7**	2.82	5.95	-
nonactin **1** → **8** $R_{1\text{-}4} = Me \rightarrow R_{1\text{-}4} = CH_2OH$	**8**	2.44	5.67	-

FOOTNOTE: The nonactin structure was based on X-ray crystal data for the KNCS complex[8] and initial structures for monactin and dinactin derived therefrom. Atomic charges were estimated by semiempirical methods using MOPAC93-R2.[23] Mechanics calculations were done with the AMBER 4.1[24] suite of programs together with the 1994 AMBER force-field parameter set of Cornell et al.[25] Calculations were performed using methanol (all-atom methyl group, with a 'heavy' H atom in the OH group, 750 – 850 molecules in a cube of approximately 40 Å each side) as the solvent as data for the binding of K^+ to nonactin, monactin, and dinactin are available for this system. Coordinates and velocities were taken from an equilibrated dynamics simulation after 40, 45, and 50 ps to act as relatively independent starting points for the FEC simulations which were with and without K^+, using each of the three starting coordinate sets. Each simulation was done using the slow growth algorithm, perturbing the system from its initial state ($\lambda = 1$) to the final state ($\lambda = 0$) over 50 ps of simulation. A timestep of 1 fs was used without application of the SHAKE procedure. logK(monactin) = 4.40 ± 0.27.

and dinactin agree well with the experimental values. Analysis of the coordinate data for each simulation showed a smooth transition from starting compound to end product. No quantitative difference was observed when longer (200 ps) dynamics simulations were run suggesting that the systems are in equilibrium at all times. Notably, any change in the substitution pattern of nonactin, either removing or adding alkyl groups, improves the K^+ binding affinity. Nonactin, however, is the most prevalently produced macrotetrolide in *S. griseus*. We suggest that this is a reflection more of the biosynthesis precursor availability than the necessity to the organism of making the most potent antibiotic. Electrostatic interactions are the most significant factors in promoting K^+ binding in the macrotetrolides. Each analog had average K^+-carbonyl oxygen distance of 2.7 to 2.9 Å. All the analogs, except for **6**, showed the same eight-coordinate, approximately cubic, binding scheme to K^+, with the carbonyl and furan oxygen atoms acting as donors. In the case of **6**, however, two methanol molecules coordinate in place of the two oxygen atoms of one macrotetrolide monomer subunit, with the latter being folded away from the central cation. We propose that increasing the alkyl substitution on the macrotetrolide leads to a more rigid structure and, therefore, leads to a lower entropy penalty for ion binding. In the case of **6**, the backbone is highly flexible so that the improved ion affinity relative to the parent macrotetrolide, nonactin, is achieved by adopting an alternate ion coordination.

In conclusion, we have validated a technique to predict K^+ binding affinities for macrotetrolides. For all the known macrotetrolides, this K^+ affinity is highly correlated with activity. We have, therefore, obtained three initial candidates for our synthetic efforts.

Acknowlegement: This work was supported by the College of Pharmacy, The Ohio State University, and by grant number CA77347 from the National Cancer Institute. We also gratefully acknowledge the AFPE for a 'Gateway' scholarship, awarded to JWL.

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