
Ab Initio Simulation of Chemical Shift Effects from Metal Ion Binding in Bacitracin A

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ABSTRACT: *Ab initio* calculations of a chemical shift at the HF/6-31G(d,p)/B3LYP/6-31G(d) level on a model system representing the interaction between Zn⁺⁺ and the thiazoline ring of Bacitracin A is used for studying the binding mode between the metal ion and the ring system. The simulations show that a reinterpretation of the shift effects seen in the NMR spectra of the zinc–Bacitracin A complex may be needed, because the spectra probably indicate that the Zn⁺⁺ ion is coordinated by the nitrogen atom of the thiazoline ring rather than the previously assumed sulfur atom.
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Introduction

Bacitracin A is a dodecapeptide produced by *Bacillus subtilis* and *B. licheniformis*, and it belongs to a class of closely related compounds¹ including Bacitracin F_A, B₁, B₂, and B₃. It has a macrocyclic ring formed by a covalent bond between the C-terminal carboxyl group of Asn 12 and the side chain of Lys 6 as shown in Figure 1. In the N-terminal part of the molecule there is a thiazoline ring formed by a cyclization of the side chain

of Cys 2. Four of the residues in Bacitracin A have *D* chirality. The bacitracins are made by a synthetase complex rather than through the standard ribosomal pathway, and the complete sequence of this synthetase complex was recently determined.²

Bacitracin A shows antibiotic activity against Gram-positive and a few Gram-negative species of bacteria; a divalent metal ion, most likely zinc, seems to be necessary for this activity.³ The exact mechanism of the antibiotic activity has not been proven, but it is generally assumed that bacitracin inhibits cell wall synthesis of the bacteria by binding the pyrophosphate form of a C₅₅ isoprenyl carrier lipid.^{4,5} Dephosphorylation of this lipid is necessary in order to recycle it into the transport process, and binding by bacitracin probably prevents this dephosphorylation. It has been shown that

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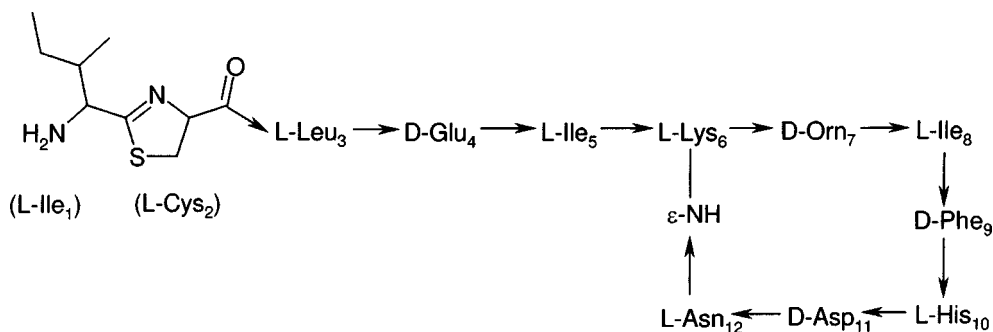


FIGURE 1. The structure of Bacitracin A. The arrows indicate peptide type bonds between the different residues.

equimolar amounts of Bacitracin A and Zn^{++} will give a very strong and selective binding of lipid pyrophosphates.⁶

The structural information on bacitracins is limited, in particular with respect to the zinc–bacitracin complex. The structure of zinc-free Bacitracin A has been determined by NMR⁷ and by X-ray crystallography.^{8,9} For its crystal structure Bacitracin A was studied in complex with subtilisin, which is a protease. There have been several unsuccessful attempts to determine the structure of zinc–bacitracin complexes; for example, an attempt to cocrystallize zinc–Bacitracin A and subtilisin resulted in a zinc-free complex and free zinc ions in the solvent.⁹ It is likely that the structure of zinc–bacitracin is different from the structure of zinc-free bacitracin (see ref. 10 for a discussion), and the structure determinations of zinc-free bacitracin have also shown that Bacitracin A is a very flexible molecule. Therefore, the existing 3-dimensional (3-D) bacitracin structures are of limited value for understanding the properties of zinc–bacitracin.

The groups involved in zinc binding were identified as the side chains of His 10 and Glu 4, the thiazoline ring, and possibly the N-terminal NH_2 group (see, e.g., ref. 10 for a discussion). However, the exact binding mode is still unclear, in particular for thiazoline. The ring system has two potential binding sites, nitrogen (N) and sulfur (S). Electron paramagnetic resonance (EPR) experiments ruled out S as the coordination site for divalent ions,¹¹ and this conclusion seems to be supported by recent extended X-ray absorption fine structure (EXAFS) experiments on the zinc–Bacitracin A complex.¹⁰ However, the EPR experiments were performed with Cu^{++} as the divalent metal ion and previous NMR experiments found no evidence that the thiazoline ring is involved in coordination of Cu^{++} ions.¹² Therefore, these experiments may be less informative with respect to zinc binding. NMR experiments

showed that zinc binding gives a downfield shift and large broadening effects on the H_β protons in the thiazoline ring and a minor upfield shift on H_α .¹³ The broadening effect on H_β was interpreted as a direct consequence of metal ion binding caused by an exchange between the complexed and free form. This interpretation is supported by the fact that the line broadening is observed for all groups assumed to be directly involved in zinc coordination. For a quasislow or quasifast exchange process¹⁴ there will be a significant broadening of all signals affected by the exchange process. If the exchange is quasislow there will be only minor shift effects. In the NMR experiments of Mosberg et al.¹³ a 50% saturation with Zn^{++} was used. Judging from the published NMR spectra, only a fraction of the bacitracin molecules seem to be in a zinc-coordinating form. The relatively small and broad NMR signals from the bacitracin–zinc complex may therefore not be observable in the published NMR spectra. The small shift effects, in particular on H_α , may also be interpreted as a more indirect effect caused by a change in the conformation upon complexation. Such a conformational change will in this case most likely be slow on the NMR time scale, and it will then be observed as a change in chemical shift. Because the broadening of the signal from H_β is the effect that seems to be most directly related to the complexation, this was interpreted as an indication that the metal ion is coordinated by sulfur,¹³ because this coordination site is close to H_β .

The NMR results may be the most relevant data for understanding the active binding mode of divalent ions, because they are based on the zinc complex in solution. However, the conclusion that zinc is coordinated by S is not strongly supported by other experimental data. A library search of the Cambridge structural data base¹⁵ for transition metals coordinated by a thiazoline ring returned 19 entries where the metal ion was bound to the thi-

azoline nitrogen, but no entries where the metal ion was bound to the corresponding sulfur atom.¹⁰ This observation is confirmed by EXAFS data on the zinc–bacitracin complex, which showed that it is unlikely that the zinc ion is coordinated by a sulfur atom.¹⁰ Therefore, although the NMR results are for conditions that are more comparable to the active state of Bacitracin A, the published interpretation of these data leads to a somewhat atypical result.

This means that we seem to have a discrepancy between different experimental methods. EXAFS and maybe EPR identifies nitrogen as the most likely coordination site; NMR identifies sulfur. To try to solve this apparent contradiction it was decided to do *ab initio* simulation of chemical shift values on suitable model systems representing the thiazoline ring of Bacitracin A both in the free form and with Zn^{++} coordinated to N or S.

Experimental

The *ab initio* computations were done using Cadpac¹⁶ (version 6.3) and Dalton¹⁷ (version 1.0) on an SGI Power Challenge L running IRIX 6.2. Cadpac uses the localized orbital/local origin (LORG) method¹⁸ for computation of shift values whereas Dalton uses London atomic orbitals¹⁹ (LAOs), also known as gauge invariant atomic orbitals (GIAOs). Interaction energies were corrected for basis set superposition error (BSSE) by the counterpoise approach.²⁰ Basis sets were taken directly from the Cadpac and Dalton distributions; the 6-31G type basis for zinc²¹ was taken from the Extensible Computational Chemistry Environment Basis Set Database.²² An alternative TZ type basis set for zinc²³ was tested in some initial computations, but it has so far not been used for the computations reported in this article.

The decision was made to do the majority of the computations using structures optimized with a 6-31G(d) basis^{24–26} using B3LYP,^{27,28} followed by shift computations with Hartree–Fock (HF) and a 6-31G(d,p) basis (HF/6-31G(d,p)//B3LYP/6-31G(d)). This decision was based on results from calculations of chemical shifts using a well-defined set of model compounds selected to be representative of typical peptide properties (unpublished data), as well as on similar results reported by other research groups.²⁹ However, because of software limitations in Cadpac not all computations could be done at a full 6-31G(d,p) level. Therefore, a locally dense basis^{30,31} was used for the chemical shift calculations with polarization added to H_α , $\text{H}_{\beta 1}$, and $\text{H}_{\beta 2}$. The validity of this approach was verified using a full

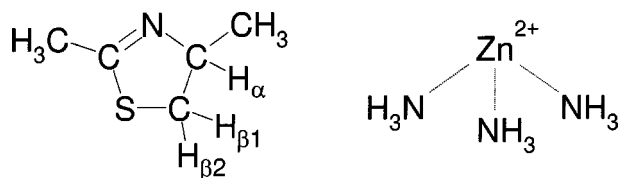


FIGURE 2. The model system used for computations. The zinc ion is coordinated to either nitrogen (N) or sulfur (S).

6-31G(d,p) basis on the thiazoline model (*vide infra*). To explore basis set effects a locally dense basis with 6-311++G(2d,2p) on C_α , H_α , C_β , $\text{H}_{\beta 1}$, and $\text{H}_{\beta 2}$ was also tested. In order to test for correlation effects, shift calculations using density functional theory (DFT) as implemented in Cadpac were also included using BLYP^{28,32} and BP91.^{32,33}

Insight II³⁴ was used for model building and visualization of results. The properties of the model systems were also analyzed with natural bond orbital (NBO³⁵) analysis programs (version 4.0). Input to NBO was generated from the Cadpac dump file using a local software tool. Molecular structures were plotted with Pluto from the Cambridge Structural Database System.¹⁵

Description of Model System

Bacitracin A is a large molecule with 100 heavy atoms. This makes the molecule too large for many computations at an *ab initio* level. In addition to this the 3-D structure of the molecule, in particular in complex with Zn^{++} , is not known. It is therefore necessary to define a model where the features that are relevant for this study are represented in a realistic and unambiguous way. The chosen model system is shown in Figure 2. It basically consists of the thiazoline ring and the Zn^{++} ion. Side chains of the thiazoline ring are represented by methyl groups, and the other coordination sites involved in zinc binding are represented by NH_3 groups.

It is likely that the amide group next to the C_α of the thiazoline ring may have some effect on the shift values of the ring atoms. This shift effect will depend upon the orientation of the amide plane (see, e.g., ref. 36), and without experimental information on the 3-D structure any change in this orientation is very difficult to predict. However, the effect of a change in the amide plane is probably on a different time scale compared to the effect from zinc coordination, which was already discussed. Because the direct (nonbroadening) shift effects in the NMR spectra are small, the shift effects from changes in

the amide plane are also most likely small. This indicates that the error introduced by ignoring the amide group is small. In addition to the thiazoline ring, it is known that His 10, Glu 4, and possibly the N-terminal NH₂ group are involved in coordination of the zinc ion as already discussed. This additional coordination is represented by NH₃ groups in the model system. It turned out to be necessary to add this coordination of Zn⁺⁺ in order to reduce the effective electron affinity of the ion. Without such extra coordination the strong electron withdrawal of the Zn⁺⁺ ion leads to ring opening of the thiazoline ring when coordination to S is modeled.

Results and Discussion

The computations were performed as described on the thiazoline model alone and with Zn⁺⁺ coordination to either S or N.

The final energy-minimized conformations of thiazoline and its zinc complexes are shown in Figure 3. The complex with zinc coordination to nitrogen is close to planar; the angle between the ring plane (defined by N, C, and C_α) and the N–Zn axis is 3.14°. However, the ring plane is slightly twisted compared to the free ring system. This is probably caused by the interaction between the zinc ion and the side chains of the ring system and leads to a small change in conformation for the ring protons. For the alternative complex with zinc coordinated to sulfur the complex is nonplanar with an angle of 56.82° between the ring plane (defined by S, C, and C_β) and the S–Zn axis. In this conformation the zinc ion is trans to the methyl group (side chain) at the C_α position. In order to cover both possible conformations for zinc coordination to sulfur a model with the zinc ion oriented cis to the methyl group was optimized as well; in this conformation the angle to the ring plane is –54.74°. In both cases the conformation of the ring system is almost identical to the conformation of the free form, and this probably reflects a less crowded situation around sulfur compared to nitrogen. The results for the BSSE corrected energy calculations on these conformations are shown in Table I, and the results for the chemical shift calculations are shown in Table II.

The BSSE corrected energies show that we get the lowest energy when zinc is coordinated by nitrogen. The energy values are very similar to estimated bond dissociation energies for Au⁺–NH₃ (71 ± 7 kcal/mol) and Au⁺–H₂S (55 ± 6 kcal/mol).³⁷ This similarity in energies is probably fortuitous, although the Mulliken charge density on the zinc

TABLE I. BSSE Corrected Energies for Complexes at B3LYP/6-31G(d,p)//B3LYP/6-31G(d) Level Using High Density Grid.

Ligand	Geometry	ΔE (hartrees)	ΔE (kcal/mol)
N	—	–0.113705	–71.35
S	cis	–0.086036	–53.99
S	trans	–0.086419	–54.23

atom in the model complex is close to 1.0, which may make it more similar to other single-charge ions. However, this similarity does indicate that the relative order and magnitude of the energies is reasonable; a similar trend was also observed for the interaction of Mn⁺ with N(CH₃)₃ vs S(CH₃)₂,³⁸ as well as for Cu⁺ with C₅H₅N vs S(CH₃)₂.³⁹

The locally dense basis set approach used for the computations listed in Table II was verified by doing a full 6-31G(d,p) calculation on the free thiazoline ring model. For H_α, H_{β1}, and H_{β2} the calculated shift values changed by 0.02–0.03 units compared to the calculation using a locally dense basis. This shows that using a locally dense basis is an acceptable simplification compared to using a fully balanced basis for this molecule.

The results from the chemical shift calculations show that coordination of the Zn⁺⁺ ion to the nitrogen atom of the thiazoline ring gives the largest relative change in chemical shift for the two H_β protons compared to Zn⁺⁺ coordination to sulfur. Also, coordination to nitrogen gives a very small effect on H_α, in particular compared to the effect when the coordination is to sulfur. This trend is consistent for all computations performed in this study including the DFT calculations. Significant effects are also seen in the nitrogen shift where there is a clear correlation between the coordination site and the direction of the shift. A significantly smaller shift change is observed for nitrogen when the DFT approach is used. This effect from including correlation is not surprising, because such interactions are probably of particular importance in the interaction with zinc. However, the direction of the shift effect is unchanged, indicating that nitrogen may be a useful NMR probe for investigating zinc binding in bacitracin. The DFT data also show that the effect of correlation on the proton shifts is relatively small, which may mean that correlation effects are of less importance for estimating the proton shifts in this system.

These computations were done on a simplified model system and should therefore be interpreted

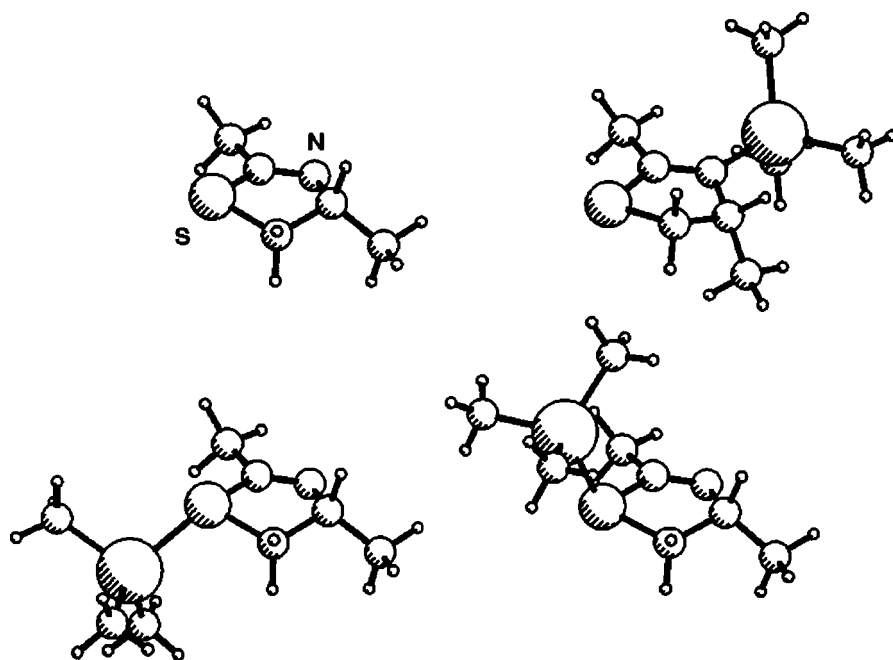


FIGURE 3. The final energy-minimized conformations of the thiazoline model system (upper left) and zinc complexes of the system with Zn^{++} coordinated to N (upper right) and to S in the cis conformation (lower left) and in the trans conformation (lower right).

TABLE II. Changes in Chemical Shift Values for Complexes Compared to Uncomplexed Form of Thiazoline Ring Model.

Ligand	Methods		Basis ^a	N	H _α	H _{β1}	H _{β2}
N	LORG	HF	6-31G(d,p)	115.8159	0.0094	-0.6790	-0.9823
	GIAO	HF	6-31G(d,p)	115.4628	0.0662	-1.1118	-1.0497
	GIAO	HF	6-311++G(2d,2p)	111.9360 ^b	0.0684	-0.8794	-0.9960
	LORG	BLYP	6-31G(d,p)	92.1408	-0.0240	-0.4372	-1.0322
	LORG	BP91	6-31G(d,p)	93.4812	-0.0081	-0.4078	-1.0011
S (cis)	LORG	HF	6-31G(d,p)	-20.0717	-0.8759	-0.7995	0.0682
	GIAO	HF	6-31G(d,p)	-26.6556	-1.0365	-0.8990	0.1786
	GIAO	HF	6-311++G(2d,2p)	-27.2360 ^b	-0.8916	-0.7148	0.2076
	LORG	BLYP	6-31G(d,p)	-3.3813	-0.9623	-0.7581	0.0346
	LORG	BP91	6-31G(d,p)	-3.9332	-0.9419	-0.7498	0.0157
S (trans)	LORG	HF	6-31G(d,p)	-20.4232	-0.4775	-0.1275	-0.6680
	GIAO	HF	6-31G(d,p)	-26.7125	-0.4496	-0.0969	-0.8469
	GIAO	HF	6-311++G(2d,2p)	-26.2302 ^b	-0.3757	-0.0383	-0.6679
	LORG	BLYP	6-31G(d,p)	-7.5530	-0.6107	-0.1611	-0.5433
	LORG	BP91	6-31G(d,p)	-7.9548	-0.6007	-0.1664	-0.5457

^a A locally dense basis was used as described in the text.

^b The values for nitrogen may be less accurate, because the locally dense basis was not used for all atoms directly bonded to nitrogen.

with some care. However, when seen together with other experimental data on bacitracin, the results do indicate that it may be incorrect to expect that the largest effects in the NMR spectra will be ob-

served for the atoms that are closest to the binding site. Therefore, the interpretation of the NMR data on the zinc-Bacitracin A complex¹³ may have to be reevaluated. From the current computations it

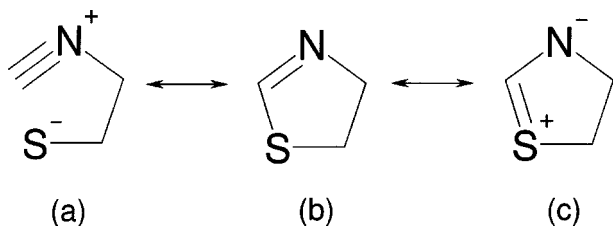


FIGURE 4. The delocalization in the thiazoline ring. The charge separated form (c) will be stabilized by a positive charge close to the nitrogen.

seems much more likely that Zn^{++} coordinates to the nitrogen atom of the thiazoline ring rather than sulfur. However, it is important to realize that this alternative interpretation actually removes the discrepancy between the NMR data and the EXAFS data (and maybe the EPR data), because these data now can be interpreted as representing the same binding mode.

This distance effect in relative shift values is most likely caused by an interaction between the sulfur and the nitrogen atoms of the thiazoline ring by delocalization of a lone pair through a shift of the ring double bond.⁴⁰ A strong electron attracting force at the nitrogen will draw electrons from the nitrogen. This will in principle be compensated for by delocalization of a lone pair on sulfur and a reorganization of the double bond as shown in Figure 4 (although in most cases it is likely that only a partial shift of electrons will take place). This delocalization will cause the largest change in electron distribution to occur at the sulfur atom rather than at the nitrogen.

In order to confirm this hypothesis NBO calculations were performed on the model system with and without zinc complexation. Changes in the Wiberg bond index⁴¹ correspond to the expected changes in bond properties. For the C=N double bond the bond index changes from 1.85 in free form to 1.59 when zinc coordinates to nitrogen whereas it changes to 1.99 when zinc coordinates to sulfur. For the C-S single bond the bond index is 1.03 in the free form, 1.20 for zinc coordinated to nitrogen, and 0.85 for zinc coordinated to sulfur. This shows that a positive charge close to nitrogen will favor the (c) form in Figure 4 with reduced double bond character in the C=N bond and increased double bond character in the C-S bond. A positive charge close to sulfur will have the opposite effect and can in extreme cases lead to ring opening.

This trend is confirmed by a computation using the natural resonance theory⁴²⁻⁴⁴ (NRT) option in the NBO program. The analysis shows that for both the free molecule, as well as when zinc is coordi-

nated to sulfur, the (c) form in Figure 4 contributes ~5% to the various resonance forms. When zinc is coordinated to nitrogen this increases to 11%. The (a) form in Figure 4 seems to be of minor importance; it contributes ~2% in all cases. It is therefore clear that the resonance form with increased negative charge on the nitrogen is important for stabilizing the complex when the nitrogen atom is used for coordinating the zinc ion, and it is likely that this also will affect the chemical shift values for the ring protons.

It is possible to argue that we may be observing a conformational effect rather than an electronic effect. As seen from Figure 3, there is a small change in conformation when the zinc ion coordinates to nitrogen. However, this change affects both H_α and H_β whereas we see effects mainly on H_β . Also, for the two models where we have coordination to sulfur the optimized models show very small structural changes on the ring system whereas we see significant changes in the chemical shift values. This makes it less likely that this is a structural effect and supports the hypothesis that we see effects from changes in the electron distribution in the ring system.

Conclusion

When comparing EPR data,¹¹ EXAFS data,¹⁰ and NMR data¹³ for the interaction of Bacitracin A with a divalent metal ion, there seems to be a contradiction. NMR shows the largest shift effects to be on H_β , which is close to S, whereas EPR and EXAFS data make S less likely as a binding site. This study shows that this contradiction is not real. Coordination of a metal ion to N gives a large shift effect at H_β , probably through a delocalization of electrons. Therefore, the NMR data and the EXAFS data both support N as the coordination site for Zn^{++} in the thiazoline ring. The relative change in estimated chemical shift at the nitrogen atom for these two alternatives (see Table II) indicates that ¹⁵N-NMR may be a suitable experimental method for verifying this result.

Apart from being a useful clarification with respect to how Bacitracin A binds metal ions, this result also has some important implications for the interpretation of other NMR data. In molecules where there is a possibility for relocation of electrons the main shift effect from interaction with charged groups may not always occur at the coordination site, but rather at some alternative position along the relocation pathway of the electrons.

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