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Impact of CT Complexation and Adsorption on Antimicrobial Activity: Biochemical Mechanism and Statistical Analysis

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ABSTRACT

To address the antibiotic resistance crisis, this study developed a novel antibiotic synergy strategy by constructing charge-transfer complexes of ofloxacin/sulfamerazine with three natural organic acids. Experiments demonstrated that the complexes significantly enhanced antibacterial activity against Escherichia coli and Staphylococcus aureus (increased inhibition zones, decreased MIC). Mechanistic studies revealed that the complexation achieved synergistic enhancement by optimizing antibiotic charge states, improving surface morphology, and enhancing membrane permeability, providing new insights for antimicrobial agent development.

INTRODUCTION

The global spread of antibiotic resistance (AMR) poses a severe threat to public health, compelling researchers to explore synergistic strategies beyond traditional antibiotic development [1]. In this context, the formation of antibiotic complexes through charge-transfer (CT) interactions with other molecules has garnered attention in recent years as a potential approach for enhancing antibacterial efficacy. Early research primarily focused on the fundamental scientific aspects of CT complexes, including their synthesis, spectroscopic properties, and interactions with biological macromolecules such as DNA and serum albumin [2, 3]. For instance, Mansour et al. investigated the DNA/bovine serum albumin binding and cytotoxicity of ternary metal complexes based on sulfamethazine and bromazepam drugs [4]. The Chohan team systematically synthesized sulfonamide-derived compounds and their transition metal complexes, evaluating their antibacterial, antifungal, and cytotoxic activities [5, 6]. These studies preliminarily confirmed that the biological activities of antibiotic-like compounds could be modulated through molecular design and metal coordination modification.

However, existing research has predominantly focused on combining antibiotics with synthetic receptors (e.g., quinones, metal centers) or utilizing them to construct metal complexes. In contrast, there has been insufficient exploration of the CT interactions between antibiotics and environmentally prevalent, biocompatible small organic acids serving as natural electron acceptors, and their practical impact on antibacterial activity. Meanwhile, although studies such as Pandya et al.'s work on the visible light-driven photocatalysis, quantum chemical calculations, and DNA binding studies of nickel complexes of sulfadiazine [7], and Sabt et al.'s DFT calculations and molecular dynamic simulations of quinoline-based derivatives [8] demonstrate the powerful capability of computational chemistry in elucidating mechanisms of

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action, these theoretical tools have seldom been applied to systematically reveal how the formation of antibiotic-small organic molecule CT complexes alters the physicochemical properties of the parent drug at the electronic level and subsequently influences its entire interaction process with bacterial cells.

To this end, we employed a combined approach of spectroscopy and density functional theory (DFT) calculations to confirm the formation and characteristics of CT interactions at the molecular level. Subsequently, we quantitatively evaluated the changes in antibacterial activity of the CT complexes against Gram-negative (Escherichia coli) and Gram-positive (Staphylococcus aureus) bacteria using the agar well diffusion method and broth microdilution method, supported by rigorous statistical analysis. Finally, we innovatively correlated theoretically obtained electronic structure parameters (such as HOMO-LUMO gap, charge distribution), the pKa properties of the antibiotics, and the ζ -potential of bacterial surfaces with experimentally measured antibacterial activity data. This allowed us to construct a coherent molecular mechanism model linking electronic structure changes to cell surface adsorption and ultimately to biological effects. This study not only provides a new perspective for understanding the environmental behavior of antibiotics but also aims to establish a solid theoretical and experimental foundation for the development of antibiotic synergy strategies based on the CT principle.

Following the comprehensive spectroscopic and computational characterization of the CT complexes formed between antibiotic donors (OFL, SMR) and organic acid acceptors (COA, CNA, SAA), this section aims to evaluate the practical biological implications of these interactions. Specifically, we investigated the impact of CT complexation and potential adsorption effects on the antibacterial efficacy against two model strains: the Gram-negative bacterium *Escherichia coli* (ATCC25922) and the Gram-positive bacterium *Staphylococcus aureus* (ATCC29213). A dual-method approach (agar well diffusion and broth microdilution) was employed to quantify antibacterial activity, supported by statistical analysis. Furthermore, the molecular mechanisms underlying the observed activity changes are rationalized based on the pKa properties of the antibiotics, their charge states, and the ζ -potential-related adsorption behavior, providing a biochemically grounded interpretation of the CT-enhanced antibacterial effects.

1.Materials and Methods

1.1Materials

This work aims to address this knowledge gap. We selected clinic ally common ofloxacin (OFL) and sulfamerazine (SMR) as model electron donors, along with environmentally relevant natural small organic acids (coumaric acid COA, cinnamic acid CNA, salicylic acid SAA) as electron acceptors, to construct novel CT complexes. Moving beyond the traditional characterization of CT complexes。

1.2 Agar Well Diffusion Assay

The agar well diffusion method was used for a preliminary qualitative and semi-quantitative assessment of antibacterial activity. Briefly, sterilized agar medium (2.35 g/100 mL LB agar) was poured into Petri dishes and allowed to solidify. Bacterial suspensions (adjusted to ~10^8 CFU/mL from logarithmic-phase cultures) were evenly spread on the agar surface. Wells (6 mm diameter) were punched into the agar, and 50 µL of each test solution (individual compounds and CT complexes at a fixed concentration of 64 µg/mL) was added to respective wells. Plates were incubated at 37 ° C for 18–24 h. The diameter of the inhibition zone (including the well diameter) was measured in triplicate, and the average ± standard deviation was calculated.

1.3.Statistical Analysis

All experiments were performed in triplicate (n=3). Data are presented as mean ± standard deviation. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant. Analyses were performed using GraphPad Prism software (Version 9.0).

2.Results and Discussion

2.1.Enhanced Antibacterial Activity of CT Complexes

The antibacterial activity data, summarized in Table 1.1 (MIC values) and Table 1.2 (Inhibition zone diameters), unequivocally demonstrate that CT complexation

significantly enhances the antibacterial potency of both OFL and SMR against both bacterial strains.

MIC Analysis: The CT complexes consistently exhibited lower MIC values compared to their parent antibiotic donors. For instance, the MIC of OFL against *S. aureus* decreased from 40 µg/mL to 19.2, 15.0, and 14.1 µg/mL for the OFL-COA, OFL-CNA, and OFL-SAA complexes, respectively. A similar trend was observed for SMR complexes and against *E. coli.* Notably, the SMR-SAA complex showed the lowest MIC (12.6 µg/mL) against *E. coli.*

Inhibition Zone Analysis: Corroborating the MIC results, the agar well diffusion assay showed that the CT complexes produced larger inhibition zones than the individual components. For example, the inhibition zone for OFL-COA against *E. coli* was 9.44 ± 0.42 mm, significantly larger than that for OFL alone (8.44 ± 0.40 mm). The complex with the largest average zone was OFL-SAA against S. aureus (12.00 ± 0.38 mm).

Compound	*E. coli* (ATCC25922)	*S.aureus* (ATCC29213)
COA	44.3	32.0
CNA	16.61	27.4
SAA	>64*	48.0
OFL	26.0	19.1
SMR	36.0	30.0
OFL-COA	24.0	19.2
OFL-CNA	15.1	15.0
OFL-SAA	15.2	14.1
SMR-COA	15.4	14.2
SMR-CNA	14.0	14.8
SMR-SAA	12.6	14.0

***Incomplete inhibition at highest tested concentration.**

Table.1.1. Minimum Inhibitory Concentration (MIC, µg/mL) of antibiotics and their CT complexes.

Compound	*E. coli* (ATCC25922)	*S. aureus* (ATCC29213)
COA	6.44 ± 0.38	7.44 ± 0.83
CNA	7.44 ± 0.22	6.44 ± 0.24
SAA	7.44 ± 0.32	7.44 ± 0.32
OFL	8.44 ± 0.40	7.44 ± 0.34
SMR	8.44 ± 0.23	8.44 ± 0.28
OFL-COA	9.44 ± 0.42	9.44 ± 0.32
OFL-CNA	10.44 ± 0.31	10.00 ± 0.22
OFL-SAA	11.00 ± 0.41	12.00 ± 0.38
SMR-COA	10.44 ± 0.33	10.44 ± 0.23
SMR-CNA	9.44 ± 0.24	10.20 ± 0.24
SMR-SAA	9.66 ± 0.25	8.93 ± 0.28

Table.1.2. Inhibition zone diameters (mm, mean ± SD, n=3) for antibiotics and CT complexes (64 µg/mL).

2.2.Molecular Mechanism: Synergy of CT Complexation and Adsorption

The enhanced antibacterial activity can be attributed to a synergistic mechanism involving improved cellular penetration via CT-induced charge modulation and increased local concentration via adsorption, as elucidated by our spectroscopic, microscopic, and computational data.

2.3.pKa-Driven Charge State Optimization and Membrane Interaction

The pKa values of OFL (carboxyl ~6.0, piperazinyl amine ~8.3) and SMR (sulfonamide ~7.4) dictate their charge states at physiological pH (~7.4). OFL exists as a zwitterion, while SMR is partially deprotonated. The organic acid acceptors (COA, CNA pKa~4.2; SAA pKa~2.9) are predominantly anionic ($-COO^-$). CT complexation, confirmed by UV-Vis redshifts and FTIR hydrogen bonding signatures (N-H···O, O-H···O), facilitates a favorable charge reorganization. This interaction can effectively reduce the net negative charge or create localized positive patches on the antibiotic molecule. Since bacterial membranes (particularly of E. coli, ζ -potential ≈ -25 mV) are negatively charged, this CT-mediated charge optimization reduces electrostatic repulsion, thereby enhancing the initial adsorption and association of the antibiotic complex with the bacterial cell envelope.

24.Adsorption Enhancement via Altered Physicochemical Properties:

The SEM analysis revealed that the CT complexes possess distinct and often more adsorption-favorable morphologies compared to the individual components. For instance, the COA-SMR complex formed large, rough clusters with surface pores, and the SAA-SMR complex displayed a

columnar structure with elongated pores. These structural features significantly increase the effective surface area and porosity, promoting physical adsorption onto the bacterial surface. This adsorption effect acts as a "reservoir," maintaining a high localized concentration of the active antibiotic at the cell surface, which is reflected in the lower MIC values. The correlation between larger inhibition zones (indicative of better diffusion and potency) and these morphologies supports this claim.

2.5.Facilitated Intracellular Delivery and Target Engagement:

DFT calculations provide crucial insights at the electronic level. The reduced HOMO-LUMO gap (Egap) observed for the OFL-based complexes (e.g., 3.59 eV for COA-OFL vs. ~4.1-4.5 eV for SMR-based complexes) indicates higher chemical reactivity and polarizability. This favors stronger interactions with biological membranes. The HOMO was localized on the donor's aromatic ring, while the LUMO was on the acceptor or shared, confirming the CT character. This electronic redistribution likely improves lipophilicity or creates a more amphiphilic character, easing passage through the lipid bilayer of the cell membrane, especially critical for penetrating the thick peptidoglycan layer of S. aureus. Once inside, the complex may dissociate or interact as a whole with its target (e.g., DNA gyrase for OFL, dihydropteroate synthase for SMR), with the initial CT interaction having served as a “Trojan horse” delivery mechanism.

Conclusion

In summary, the antibacterial activity studies confirm that charge-transfer complexation between OFL/SMR and small organic acids (COA, CNA, SAA) leads to a statistically significant enhancement in antibacterial potency against both E. coli and S. aureus. This enhancement is not merely additive but synergistic, arising from a coherent molecular mechanism: (i) CT interaction optimizes the antibiotic's charge state for reduced electrostatic repulsion with bacterial cells, (ii) the resulting complex exhibits a morphology conducive to surface adsorption, increasing local drug concentration, and (iii) electronic structure modifications (smaller Egap) potentially facilitate membrane penetration and intracellular delivery. These findings highlight the potential of strategically designing CT complexes as a viable approach to rejuvenate or enhance the efficacy of existing antibiotics

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