# Chiral Recognition Is Observed in the Deprotonation Reaction of Cytochrome *c* by (2*R*)- and (2*S*)-2-Butylamine

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The proton transfer reactions of (2*R*)- and (2*S*)-2-butylamine with three charge states of cytochrome *c* (9+, 8+, and 7+) show a systematically strong preference for the *R* enantiomer over the *S*, illustrating an appreciable chiral effect in these deprotonation reactions. The rate constants of reactions involving the R enantiomer are about 10 times greater than those involving S. The multiply charged ions were produced by electrospray ionization (ESI)[1−3] and the proton transfer reactions were monitored with Fourier transform mass spectrometry (FTMS)[2−5] using methods described in greater detail elsewhere.[6−8] For comparison, *n*-propylamine and *tert*-butylamine were reacted with the same three charge states of cytochrome *c*.[9,10] *n*-Propylamine reacted with similar rate constants to (2*S*)-2-butylamine, while *tert*-butylamine was the least reactive of the four amines studied despite its having the greatest gas-phase basicity.

Chiral specific reactions are important and when used to probe multiply protonated proteins may provide information regarding the gas-phase protein surface as well as the environment at the sites of protonation. The use of mass spectrometry to differentiate chirality has received increasing attention.[11−33] However, there are few examples of chiral recognition in a bimolecular gas-phase reaction. Chu *et al*. have observed chiral selectivity in the complexation of a host molecule containing two stereocenters with a chiral guest.[34] Nikolaev *et al*. have shown chiral effects in the unimolecular dissociation and ligand exchange of proton bound dimers of dimethyl tartrates.[35,36] The chiral selectivity presented in this report is the first involving proton transfer, and it involves two fairly small organic enantiomers.

Although the *R* isomer is generally more reactive than the *S*, the two exhibit similar trends in reactivity with respect to the three charge states. Rate constants for the proton transfer reactions are listed in Table 1.[37] The rate constants provided are often precise to within 10%, although the absolute values may be off by a larger amount due to the calibration of the ion gauge.[38] The deviations (one standard unit) of the rate constants, measured three times over different days, are also provided. The rate constants for the 9+ state are $1.5 \times 10^{-11}$ and $2.5 \times 10^{-12}$ (all units in $cm^3/(molecule \cdot s)$) for *R* and *S*, respectively. Similar rate enhancements of *R* over *S* are observed for the other two charge states. We have looked for chiral selectivity in the reactions of 2-butylamine with small protonated peptides (di- and tripeptides of alanine and valine) and have not been successful.[39] We attributed this to the relatively simple interaction and the absence of higher order structures of the protonated species. The reactions presented here have low efficiencies (0.1−0.001%), indicative of a combination of endergonic reactions and large steric interactions in the transition states. The decay of the 9+ state as a function of time is best represented by a single rate constant, while both 8+ and 7+ are best represented each by two rate constants. Of these two rate constants, the larger values are nearly 10 times greater than the smaller ones. The relative amount of fast and slow reacting species are the same for the *R* and the *S* isomers. For the 8+ state the ratio of fast to slow reacting components is nearly 50:50, while for the 7+ state the ratio is about 25:75. The *S* isomer exhibits decreasing reactivity with decreasing charge state, as expected from purely Coulombic considerations. In this regard, both *n*-propylamine and *tert*-butylamine behave in the same manner. With the *R* isomer, the rate constant of the 9+ and the fast rates of the 8+ and the 7+ are approximately equal, suggesting a possible reactive site for the three charge states.

The observation of a single reacting species for the 9+ and two reacting species for 8+ and 7+ charge states is consistent

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**Table 1.** Rate Constant of Deprotonation Reactions Involving Respective Amines and the 9+, 8+, and 7+ Charge States of Cytochrome *c* Produced by Electrospray Ionization[a]

| charge state | (2*R*)-2-butylamine GB[d] = 211.7 | percent contribution[e] | (2*S*)-2-butylamine GB[d] = 211.7 | percent contribution[e] | *n*-propylamine GB[d] = 210.1 | *tert*-butylamine GB[d] = 213.0 |
|---|---|---|---|---|---|---|
| $9^+-8^{+\,b}$ | $1.5 \times 10^{-11}$ (±0.3) | | $2.5 \times 10^{-12}$ (±0.2) | | $2.2 \times 10^{-12}$ | $6.1 \times 10^{-13}$ |
| $8^+-7^{+\,c}$ | $2.3 \times 10^{-12}$ (±0.5) | | $4.6 \times 10^{-13}$ (±1.1) | | $2.9 \times 10^{-13}$ | $3.8 \times 10^{-14}$ |
| $8^+-7^{+\,b}$-FAST | $1.0 \times 10^{-11}$ (±0.3) | 45 | $1.9 \times 10^{-12}$ (±0.4) | 46 | $3.1 \times 10^{-13}$ | $3.7 \times 10^{-13}$ |
| $8^+-7^{+\,b}$-SLOW | $1.4 \times 10^{-12}$ (±0.1) | 55 | $3.7 \times 10^{-13}$ (±1.0) | 54 | | |
| $7^+-6^{+\,c}$ | $2.3 \times 10^{-13}$ (±0.1) | | $8.4 \times 10^{-14}$ (±3.6) | | $7.2 \times 10^{-14}$ | |
| $7^+-6^{+\,b}$-FAST | $1.1 \times 10^{-11}$ (±0.1) | 21 | $1.4 \times 10^{-12}$ (±0.3) | 30 | $1.4 \times 10^{-13}$ | $5.1 \times 10^{-14}$ |
| $7^+-6^{+\,b}$-SLOW | $1.3 \times 10^{-13}$ (±1.1) | 79 | $1.4 \times 10^{-13}$ (±1.9) | 70 | | |

[a] Units for rate constants are $\mathrm{cm}^3$/(molecule·s). Rate constants involving the two isomers of 2-butylamine were determined from three separate experiments performed on different days. [b] Higher charge state is isolated directly from ESI source with reaction proceeding toward the low charge state. [c] Further reaction of the product ion in *b* to a lower charge state. [d] Values obtained from ref 44. [e] Contribution of each rate constant to overall rate constants when two or more are observed.

with published studies of gas-phase cytochrome *c* that suggest different conformers for some charge states. At least three conformers for the 7+ charge state of cytochrome *c* and two for the 8+ state have been proposed by Clemmer *et al.* to explain observations in ion mobility experiments.[40] They further suggest that the relative abundances depend strongly on the ionization conditions. Suckau *et al.* propose two reacting species for 8+ and one for 7+ on the basis of gas-phase H−D exchange.[41,42] Evidence of multiple reacting species has also been presented by Cassady *et al.* for ubiquitin.[43] The different reacting species are not readily observed in the reactions of the achiral bases *n*-propylamine and *tert*-butylamine.

In deprotonation reactions that are rapid, the removal of a second proton is sometimes observed. The rate constants for these reactions were determined and compared to the proton-transfer reactions in which the reactant is initially isolated. The rate constant of the 8+ produced by deprotonation of 9+, reacting with (2*R*)-2-butylamine ($2.3 \times 10^{-12}$) is similar to that of the slowly reacting 8+ isolated directly from the source ($1.4 \times 10^{-12}$). Similarly, the rate constant of 7+, produced by deprotonation of 8+, reacting with (2*R*)-2-butylamine ($2.3 \times 10^{-13}$) is closer to that of the slowly reacting species of 7+ isolated from the ionization source ($1.3 \times 10^{-13}$). This is consistent with the notion of a common highly reactive site for the three charge states. Charges states 7+ and 8+ produced by deprotonation would be missing this site, while some fraction of the corresponding isolated states would contain the reactive sites, as observed. Similar behavior is observed with the *S* isomer.

For comparison, *n*-propylamine, which is 1.6 kcal/mol less basic than (2*R*)- and (2*S*)-2-butylamines,[44] was reacted with the same three charge states of cytochrome *c*. This compound is less hindered than the 2-butylamines, while its lower basicity should render it less reactive in the absence of "steric effects". It further allows an estimate, in energy terms, of the effect associated with chiral differentiation. With the 9+ charge state, *n*-propylamine is as reactive as the *S* isomer, suggesting a rise in the reaction barrier for the *S* isomer equivalent to a 1.6 kcal/mol increase in the endothermicity of the reaction due to its steric effect. The *R* isomer of 2-butylamine, on the other hand, is about seven times as reactive as propylamine, roughly what would be expected from its higher gas-phase basicity. The foregoing suggests that the reactive protonated site discriminates against the 2-butylamine of the unfavorable configuration by the equivalent of 1.6 kcal/mol.[45]

The most basic of the four amines is also the least reactive, undoubtedly because of its steric bulk. The rate constant associated with the reactions of *tert*-butylamine is an order of magnitude less than that of *n*-propylamine for 9+ and 7+ but is nearly the same for 8+. The reactivity of both amines follows the order of 9+ > 8+ > 7+.

Deprotonation reactions of multiply charged gas-phase proteins by neutral amine bases likely involve intermediates with highly specific structures in which steric effects can play a strong influential role. In this regard, chiral probes of gas-phase structure may provide an important and sensitive probe for the conformations of gas-phase proteins. Intermediates of defined structures are also consistent with observations in hydrogen−deuterium exchange involving gas-phase protonated peptides.[46−48] This notion is further supported by the presence of adducted species in the reaction of cytochrome *c* with three of the amines, the two isomers of 2-butylamine and *n*-propylamine, while no adducted species were observed with the more basic but less reactive *tert*-butylamine. Rate constants for adduct formation were estimated to be at least 2−3 orders of magnitude faster than those for proton transfer. The rapid formation of the adducted species precluded calculation of reliable rates.

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