Peptide salt bridge stability: From gas phase via microhydration to bulk water

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1. Motivation

- Salt bridges are prominent non-covalent interactions in proteins.
- Typically occur between titratable amino acid side chains: basic, positively charged groups Lysine, Arginine; acidic, negatively charged Aspartate, Glutamate.
- Competition between attraction of opposite charges and tendency of water to solvate them separately.
- Discussion about relative importance of salt bridges for protein stability.
- Appear on the surface of protein, but also buried inside.
- Upon lowering effective dielectric constant: weak salt bridge => strong salt bridge => neutralization.

2. Model system

- Terminated lysine-glutamate dipeptide.
- Glu is more flexible than Asp.
- Lys unlike has only one geometric way to form a salt bridge.
- Capped (neutral) termini are chosen to reduce the complexity of possible proton transfer patterns and maximize number of peptide bonds.

3. Methods

Ab initio molecular dynamics
- CP2K program package.
- BLYP functional with Grimme correction.
- TZVP/MP2 MOLOPT basis set.
- GTH pseudopotential.
- T = 300 K.
- CSVR thermostat.
- Short simulations: 10–20 ps.
- Mainly for 5 H2O attached, but systems containing up to 64 H2O tested.
- Interplay between neutral and zwitterion.

Classical molecular dynamics
- Gromacs program package.
- OPLS force field, TIP4P water.
- Direct or Umbrella sampling.
- Clusters containing 20–100 H2O molecules restrained to avoid evaporation, and bulk simulations.
- Salt bridge opening.

4. ZOO of structural motifs

Typical for 0.2 H2O. Zwitterionic initial conditions with n≥2 usually end up in this configuration.

Contact neutral:
- Already 1 H2O can stabilize it, if additional stabilization from backbone is provided.
- Typical for n≥3.
- Solvent shared zwitterion:
- Formation from solvent shared neutral by proton transfer through water bridge observed in case of 3 H2O.
- Formation of short-lived solvent shared zwitterions from contact ones observed for n≥4.

5. Proton transfer

- Direct between functional groups. Heavier atoms distance: rN2O < 0.265 nm.
- More frequent through water bridge.
- NHB exceeds two ~50 fs before neutral => zwitterion conversion. For opposite conversion NHB decreases two ~40 fs afterwards.

6. Number of hydrogen bonds

- NHB ~ 25, r < 0.18 nm.
- Zwitterionic NHB > 3.
- Dynamical equilibrium: 2 < NHB = 3.
- Neutral NHB < 2.

7. Salt bridge opening from direct classical MD

- Protein stabilization by a salt bridge.
- Initial conditions with n≥2 usually end up in this configuration.
- Solvent shared neutral:
- Stable for 1–2 H2O for length of our simulation due to weaker attraction of neutral polar groups.
- Formation from solvent shared neutral by proton transfer through water bridge observed in case of 3 H2O.
- Formation of short-lived solvent shared zwitterions from contact ones observed for n≥4.

8. Free energy profile of salt bridge opening

- The free energy difference between contact and solvent shared NH+HCOO– ion pairs, as well as the barrier between them decrease monotonically with the NH+HCOO– due to screening the charges.
- The dependence of free energy profile on NHB for dipeptide is more complex due to surface effect and configurational entropy.

9. Lifetimes

- Logarithm of probability of staying in particular state was fitted by straight line.
- The lifetime of contact decreases with NHB and levels off at ~120 ps.
- The lifetime of solvent shared is ~10 times shorter.
- The lifetime solvent separated ion pair increases with NHB.

10. Conclusions

- Bare dipeptide prefers to be in neutral state.
- Already a single water molecule can trigger zwitterion formation.
- Proton transfer can proceed directly or through a water bridge.
- Zwitterion needs to be stabilized by three additional hydrogen bonds.
- The onset of long-lived solvent shared ion pairs is observed for ~30 water molecules, of solvent separated ion pairs for ~40 water molecules.
- We have shown formation and then subsequent weakening of a salt bridge upon increasing the number of hydrating water molecules.
- Neither too little nor too much hydration is beneficial for a salt bridge, which tends to be most stable upon moderately dehydrated conditions, as in concave pockets at protein surfaces.

Further reading


Acknowledgements

- Evolving molecular interactions in proteins.
- New insight into wet proteins.
- Improved understanding of salt bridge.

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