

Selective Binding of Lisinopril in Angiotensin-Converting Enzyme Domains

Poster Team: Lexie Carpenter, Gail Kingsbury, and Patrick Tierney

Jmol Team: Jennifer Dippel, Whitney Hendrickson, and Sarah Hunt

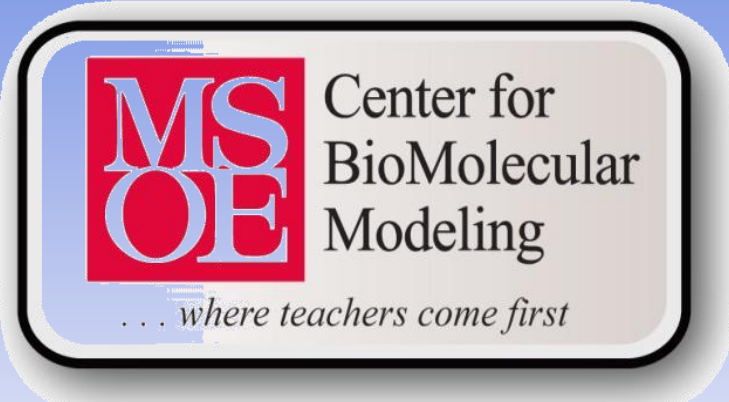
Faculty Advisor and Mentor: Daniel S. Sem, Ph.D.

Concordia University School of Pharmacy, 12800 North Lake Shore Drive, Mequon, WI 53097

Professional Mentors:

Eleanor Shterenfeld - Aurora Medical Center (Grafton, WI)
Dustin P. Ehster, PharmD- VA Medical Center (Milwaukee, WI)
Dominique Tremmel – QuadMed Pharmacy (Sussex, WI)
Steven Kennedy, PharmD – VA Medical Center (Milwaukee, WI)

Meaghan McMurray, PharmD - Aurora Pharmacy (Mequon, WI)
Valerie Huibregtse - CVS (Fox Point, WI)
Ashley G. Rineck, PharmD – Aurora St. Luke's (Milwaukee, WI)
Anne Hammel, PharmD – Aurora Sinai Medical Center (Milwaukee, WI)



ABSTRACT

Angiotensin-converting enzyme (ACE) is a membrane-anchored carboxypeptidase enzyme that cleaves both angiotensin I and bradykinin. Cleavage of angiotensin I yields angiotensin II, and ultimately results in potent vasoconstriction and high blood pressure. Bradykinin acts as a vasodilator, and cleavage of bradykinin blocks this vasodilation, and also leads to a buildup of bradykinin in the tissues. The ACE inhibitor lisinopril binds irreversibly to the active site of ACE, and prevents cleavage of both angiotensin I and bradykinin. Lisinopril successfully reduces blood pressure by blocking the conversion of angiotensin I into angiotensin II. However, the buildup of bradykinin that occurs leads to the development of a dry cough in about 10% of users.

CASE STUDY

Patient presents to the pharmacy with a dry cough keeping him up at night.
Medication History: Lisinopril for high blood pressure
OTC potassium supplements

After reviewing the medication history, the pharmacist explains that dry cough is a side effect of lisinopril, resulting from the structure of lisinopril binding to the protein target. The pharmacist also explains that potassium should be avoided because of concern with the common side effect of high potassium levels in the body associated with taking lisinopril.

HISTORICAL PERSPECTIVE OF DRUG DEVELOPMENT

- ACE plays a dual role in stimulating vasoconstriction in the body by:
 - Catalyzing the cleavage of angiotensin I to angiotensin II, a potent vasoconstrictor
 - Cleaving bradykinin, a vasodilator, into inactive small peptides
- Blocking ACE would serve to reduce vasoconstriction and thereby reduce hypertension
- Exact structure of ACE was unknown but researchers knew it was similar to that of carboxypeptidase A, which binds substrate by:
 - Binding negative carboxylate terminus with a positive Arg145
 - A hydrophobic pocket that stabilizes an aromatic or non-polar residue on the C-terminus
 - A zinc moiety that stabilizes transition state¹
- Using the structure of carboxypeptidase as a guide, researchers developed the first pharmacophore to target ACE, succinyl-L-proline.

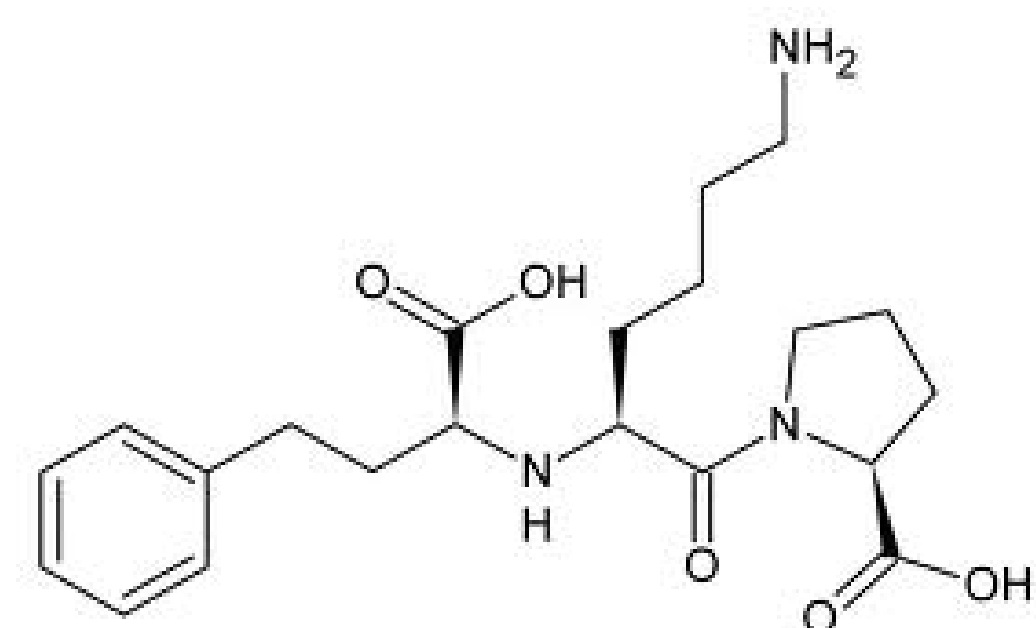


Figure 1. Lisinopril structure.

Succinyl-L-proline served as a basis for further development of ACE inhibitors, leading eventually to lisinopril. Lisinopril varies from other ACE inhibitors with a substituted N-carboxymethyl group and phenylethyl group at R₄ and lysine replacing alanine.²

MOLECULAR STORY

- There are 2 isoforms of ACE: tACE and sACE.
- The larger, somatic isoform, sACE, is a polypeptide chain 1,277 amino acids long.³⁻⁵
- sACE is found on the surface of vascular endothelial, epithelial, and neuroepithelial cells, with concentrations in pulmonary and renal endothelial.³⁻⁵
- The larger sACE is comprised of two domains, the N-terminus and the C-terminus; within each domain is a zinc binding site.³⁻⁵
- The active site contains several water molecules important for substrate binding
- Within the active site, His-Glu-x-x-His hold zinc in place

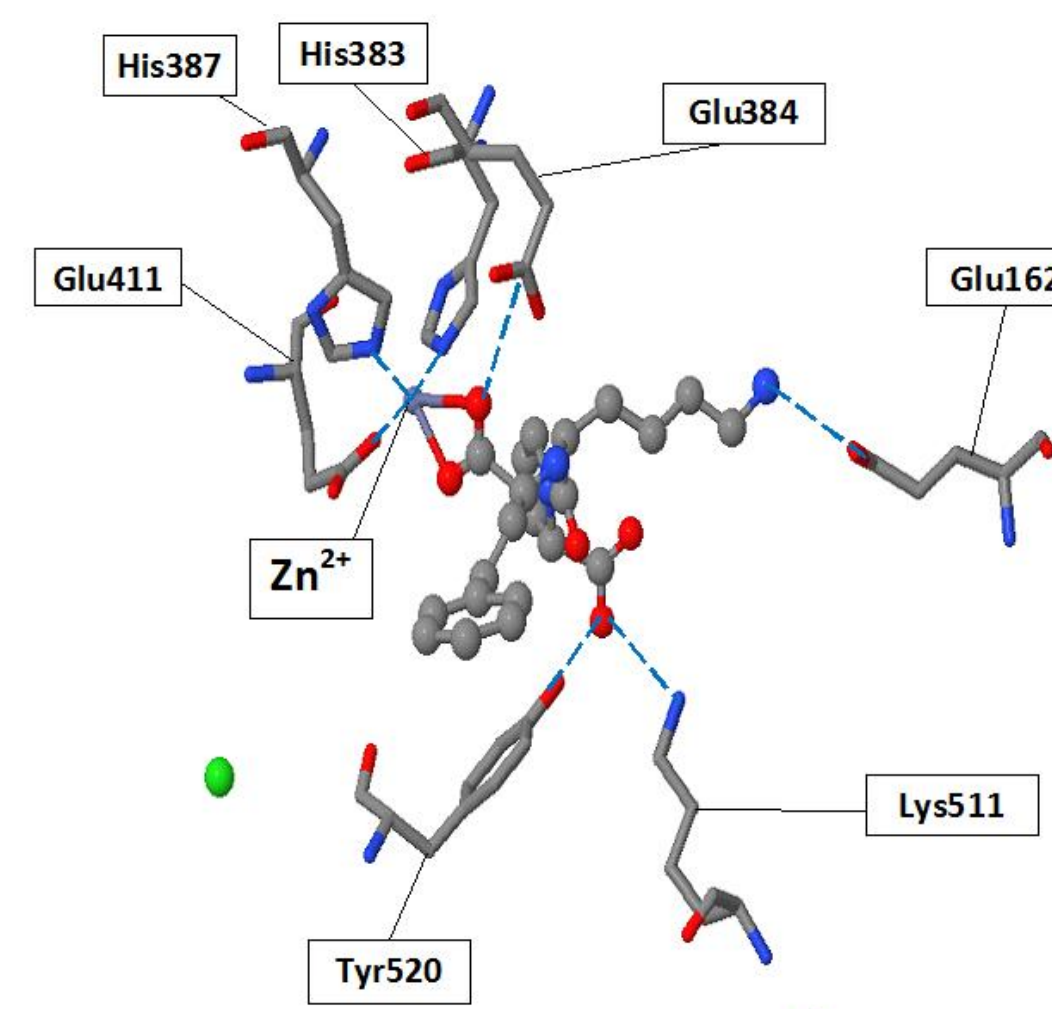


Figure 3. Lisinopril and its key binding points with sACE.

- The carboxyl group, located between the phenylpropyl group and the lysine, binds to the active site of the zinc molecule. (Fig 3.)
- The phenyl ring of lisinopril interacts with the S1 subsite.²
- The lysine of interacts with the S1' subsite.²
- The proline interacts with the S2' subsite.²
- Lisinopril forms hydrogen bonds and ionic interactions with negatively charged amino acid residues. (Asp415, Glu162, Asp377, Glu384, Glu143, Glu376) (Fig 4.)
- Lisinopril also interacts with positively charged amino acid residues through hydrogen bonds, ionic interactions, or entropically favored interactions (Lys511, His513, His353, His383).¹⁰ (Fig 4.)

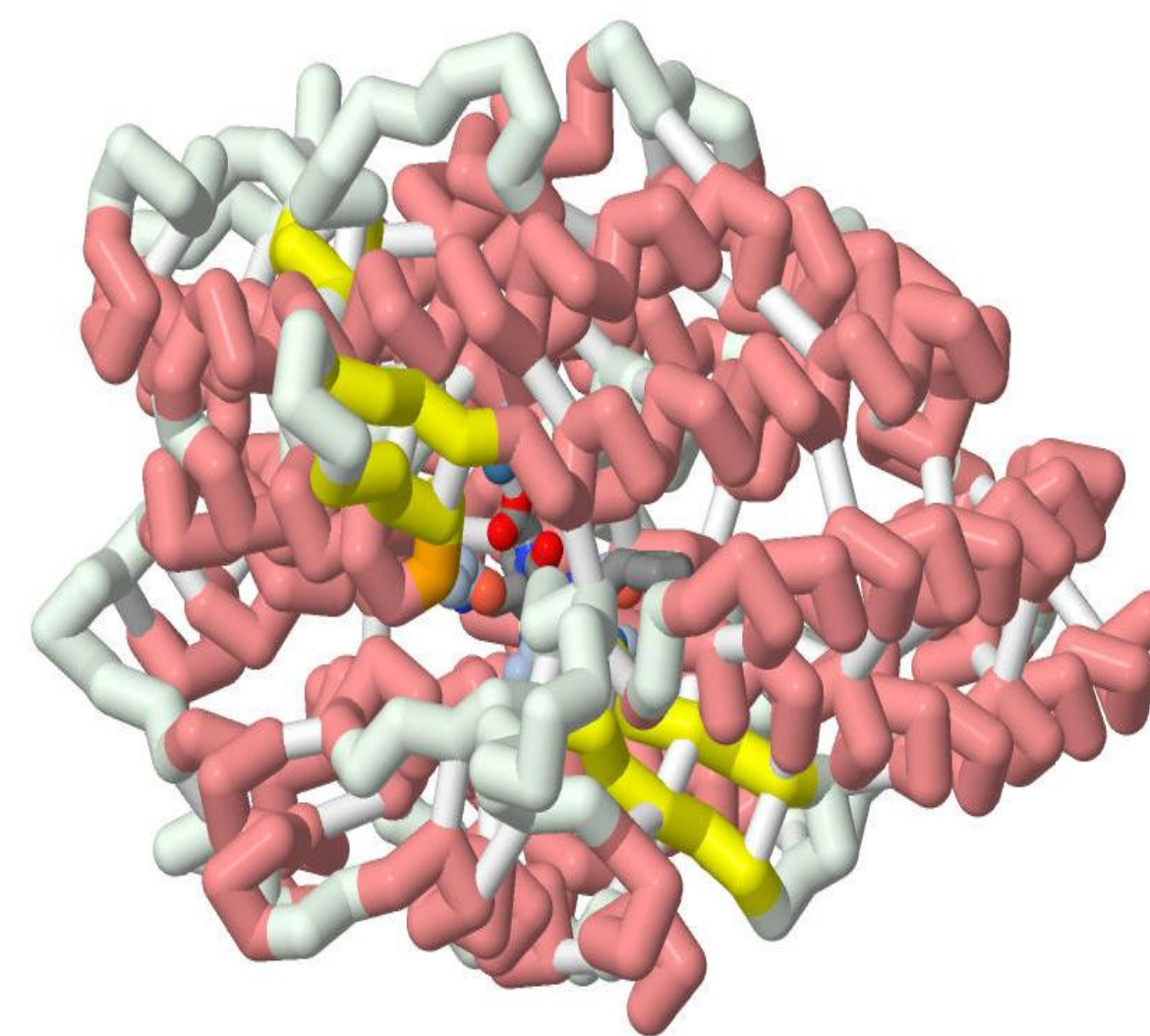


Figure 2. The structure of sACE is mostly α -helical and ellipsoid in shape, and includes two chloride ions with the catalytic zinc ion buried deep in the active-site.⁶

- His383, His387, Glu411 bind via metal covalent bonds to Zn^{2+} of lisinopril.⁷
- Glu384 binds via hydrogen bonding to an oxygen of the carboxylate group located between the phenylpropyl group and the lysine of lisinopril.⁷
- Glu162 binds via hydrogen bonding to the lysine chain of lisinopril.⁷
- Lys511 and Tyr520 binds via hydrogen bonding to the C-terminal proline carboxyl group of lisinopril.⁷
- The zinc ion plays an important catalytic role in ACE. This ion is deep within the active site of ACE. Zinc catalyzes the hydrolysis of the peptide bonds containing hydrophobic amino acids.⁶

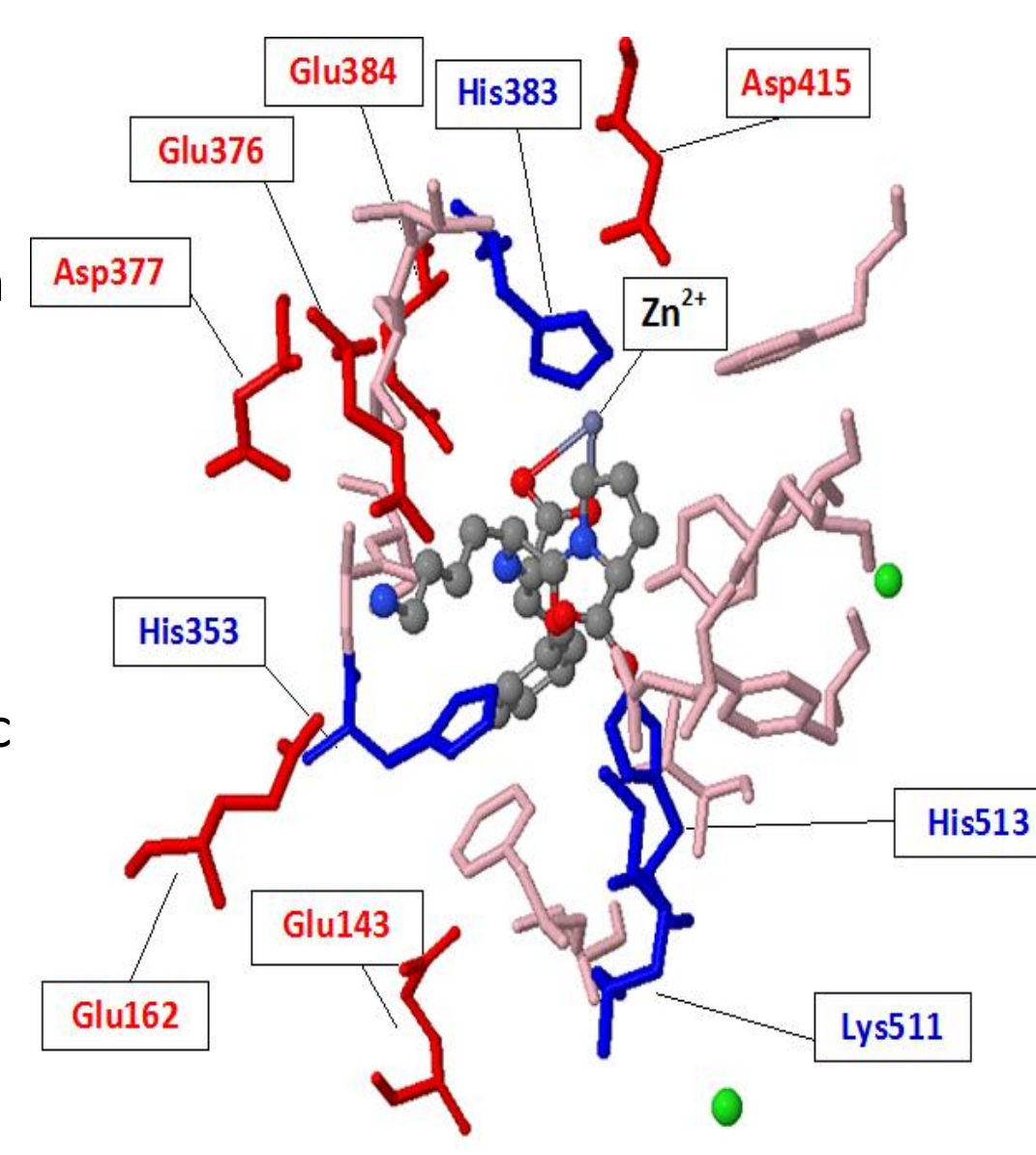
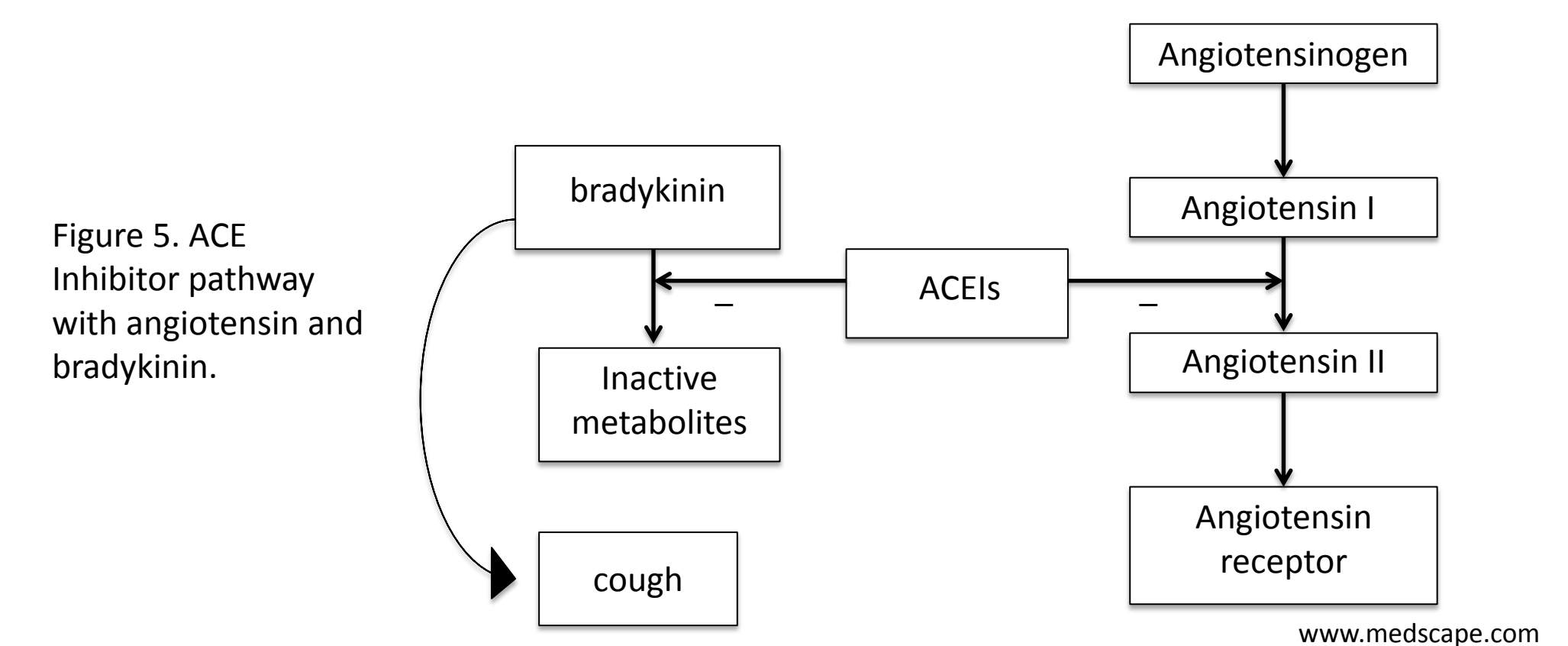


Figure 4. Lisinopril bound to sACE showing significant intermolecular forces.

SIDE EFFECTS



Bradykinin

- Known to have antihypertensive and cardioprotective effects.
- Bradykinin is normally cleaved by ACE, however when this enzyme is inhibited, bradykinin is built up (Fig. 5).⁸⁻⁹
- Build-up of bradykinin causes dry cough in up to 10% of patients.⁸
- Bradykinin may stimulate proinflammatory responses in the lung.⁸⁻⁹
- May also increase lung prostaglandin levels.⁸

Potassium Levels

- ACE inhibits production of aldosterone.
- Aldosterone works in the cortical collecting ducts to increase Na^+ and Cl^- ; inhibition of aldosterone causes K^+ retention in the body (hyperkalemia) due to Na/K ATPase.^{6,10}
- Hyperkalemia present in about 28% of those taking an ACE Inhibitor.^{6,10}

FUTURE WORK

- Explore molecular interactions between bradykinin and the receptors on which it acts to produce dry cough
- Identify genetic markers that determine susceptibility to dry cough
- Reformulate lisinopril to prevent buildup of bradykinin

SUMMARY

- Lisinopril uses many moieties that bind to ACE, some of which include hydrogen bonds and Van der Waals interactions. Zinc also plays a key role in lisinopril binding.
- Build-up of bradykinin can cause increased inflammatory factors and presentation of a dry cough.
- Inhibition of ACE can also cause decreased aldosterone which can lead to hyperkalemia.

REFERENCES

- (1) Erdos, E. (2006). Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/16770001>
- (2) Williams, D.A. (2008). *Foye's Principles Of Medicinal Chemistry*. Lippincott Williams & Wilkens.
- (3) Baudin, B. (2005). Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/15781381>
- (4) Chansel, D. (1987). Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/3038613>
- (5) Fernandez, J. (2003). Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12901857>
- (6) Watermeyer, J.M. (2010). Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/20233165>
- (7) Brew, K. (2003). Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12915047>
- (8) Mukae, S. (2000). Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10904024>
- (9) Mukae, S. (2002). Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12522467>
- (10) Sadjadi, S.A. (2009). Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/19707264>