Notes for capsaicin PowerPoint slides

Jerry A. Bell

Simmons College, Professor of Chemistry (emeritus)

American Chemical Society, Senior Scientist (retired)

Wisconsin Initiative for Science Literacy, Faculty Associate

j\_bell@acs.org

jerryalanbell@gmail.com

Slide 1

This short PowerPoint presentation originated as part of an honors high school chemistry classroom discussion of the chemistry and mode of action of the “hot” component(s) of a spicy salsa. The salsa is the central element of a “chips and salsa” day at the culmination of an extended cumulative assessment near the end of the course.

The opening slide introduces a major “hot” compound, capsaicin, that is found in spicy vegetables, especially chili peppers. A skeletal structure and space-filling model are shown and students asked to identify polar and nonpolar (hydrophilic and lipophilic—water-loving and fat-loving, respectively) areas of the ambiphilic molecule to orient their thinking to the properties of molecular structures. In this case, the collection of electronegative atoms (oxygen and nitrogen) at one end of the molecule and the hydrocarbon tail at the other end.

Asked how they describe the sensation they feel when they eat a very spicy food like a chili pepper, students almost all respond and agree that it feels “hot.” We point out that this is because capsaicin activates the same nerve receptors as are activated by a hot object, so our bodies get the sensation of “hotness,” even though no actual high temperature is involved.

In 1912, in studies to quantify the degree of “hotness” of chili peppers and other spicy foodstuffs, Wilbur Scoville (a pharmacist working for a drug company) devised a somewhat subjective heat unit scale that is named for him. The procedure is to extract the “hot” component from the dried pepper with alcohol and then dilute the extract with sweetened water and have volunteers taste it to see if they get the “hot” sensation. Then the sample is further diluted by mixing part of it into another volume of sweetened water and again asking the volunteers if they get the “hot” sensation from the more dilute sample. The dilutions continue until the volunteers no longer get the “hot” sensation and this final dilution factor is the Scoville heat unit for that foodstuff.

Slide 2

Consider what the Scoville heat unit value for capsaicin, 15,000,000 units, means. A sample of pure capsaicin has to be diluted by a factor of 15,000,000 in order to no longer to produce a “hot” sensation when tasted. This would be equivalent to mixing about one third of a cup of capsaicin into the water in an Olympic-size swimming pool. Examining the Scoville heat unit values for other molecules closely related to capsaicin can provide information about what structural properties of the molecule are important for the heat effect.

How does the structure of dihydrocapsaicin differ from capsaicin? The carbon-carbon double bond in the hydrocarbon tail has been replaced by a single bond, which means that there are two more hydrogen atoms in the new structure, explaining the prefix “dihydro.” The change in structure has no effect on the “hotness” of the molecule, which indicates that the presence of the double bond is not important for the heat effect.

How does the structure of nordihydrocapsaicin differ from capsaicin? The carbon-carbon double bond in the tail is missing and the tail is one carbon shorter than in capsaicin. The change in structure reduces the “hotness” of the molecule to about 60% of the heat effect for capsaicin. Since the loss of the double bond in dihydrocapsaicin has no effect on the “hotness,” it’s very likely that it is irrelevant here as well. The reduction in “hotness” must be a result of shortening the tail.

How does the structure of homodihydrocapsaicin differ from capsaicin? The carbon-carbon double bond in the tail is missing and the tail is one carbon longer than in capsaicin. The change in structure reduces the “hotness” of the molecule to about the same nordihydrocapsaicin, 60% of the heat effect for capsaicin. Again, the loss of the double bond is very likely irrelevant, so the reduction in “hotness” must be a result of lengthening the tail.

How does the structure of homocapsaicin differ from capsaicin? The tail is one carbon longer than in capsaicin with the carbon-carbon double bond in the same place at the end of the tail as in capsaicin. The change in structure reduces the “hotness” of the molecule to about the same as nordihydrocapsaicin and homodihydrocapsaicin, 60% of the heat effect for capsaicin. The presence of the double bond in homocapsaicin compared to homodihydrocapsaicin has no effect on the “hotness” of the molecule, reinforcing the conclusion that the double bond has no relevance to the heat effect.

The conclusion from these data is that the length of the hydrocarbon tail is critical for the “hotness” of capsaicin

Slide 3

This table illustrates the great range of “hotness” of various peppers and capsaicinoid compounds and is available on many internet web sites that discuss chili peppers, salsa, and other products that incorporate peppers in their recipes. Class members can find their favorite or a familiar pepper to compare with others.

Slide 4

The figure represents the structure of the protein with which capsaicin interacts in nerve cells, TRPV1 (the transient receptor potential cation channel subfamily V member 1 – sometimes called the capsaicin receptor). Channel-forming proteins like this are very abundant in cell membranes and help control trafficking of various molecules into and out of the cells. The cell membrane is represented here by the double layer of ambiphilic molecules on the right and left of the figure. These membrane-forming molecules have a polar (hydrophilic) head shown as a blue circle and two long nonpolar (lipophilic) hydrocarbon tails shown in yellow.

The TRPV1 protein is actually a tetramer of four identical proteins shown in dark blue, aqua, green, and cyan that stack together to form a channel for ions to pass through. TRPV1 is present in the cell membranes of nerve cells that send pain messages to the brain, but not in nerves that are responsible for sending messages to muscles. When capsaicin interacts with TRPV1, the channel is opened and ions, particularly calcium ion, Ca2+, can pass through the membrane causing the nerve cell to “fire” and send a sensation of painful “hotness” to the brain. Note the way the protein chains are modeled as ribbons (alpha helices mostly spanning the membrane) and threads of less well-organized loops of the chain. This is one of the usual ways to model protein structures that does not show the individual amino acids that compose the chain, but shows enough detail to make the structure apparent.

In this representation of the protein, four ambiphilic molecules that affect the channel-forming function of TRPV1 are shown as space-filling structures attached to the protein. The one that is easiest to see is on the right-hand side of the protein and seems to be attached between the green and cyan protein monomers. The polar head toward the intracellular side of the protein is mostly red/orange (hydroxy and phosphate groups). The nonpolar hydrocarbon tail in white/light grey extends up in a groove between the two protein monomers. (The other three ambiphilic molecules are somewhat hidden by the protein structure.) These are not capsaicin molecules, but it is likely that capsaicin interacts in a very similar way at other sites on the TRPV1. To fit well in the protein structure, it makes sense that the hydrocarbon tail has to be the right length, which explains why the length of the tail is so important to its “hotness,” as we found in the comparisons among the capsaicinoids in slide 2.

The model shown here is from Wikipedia Commons and based on a model in “Dissection of the components for PIP2 activation and thermosensation in TRP channels,” Sebastian Brauchi, Gerardo Orta, Carolina Mascayano, Marcelo Salazar, Natalia Raddatz, Hector Urbina, Eduardo Rosenmann, Fernando Gonzalez-Nilo, and Ramon Latorre, PNAS, 2007, 104(24), 10246-10251. An experimental structure obtained by electron microscopy, “Structure of TRPV1 channel revealed by electron cryomicroscopy,” Vera Y. Moiseenkova-Bell, Lia A. Stanciu, Irina I. Serysheva, Ben J. Tobe, and Theodore G. Wensel, *PNAS*, **2008**, *105*(21), 7451-7455, complements this computer model and shows the shape of the molecule. (PIP2 is phosphatidylinositol-4,5-bisphosphate, a relative of the ambiphilic molecules that make up cell membranes.)

Slide 5

The computer-generated movie on this slide shows how the relative motions of the monomers in a tetrameric protein channel similar to TRPV1 close and open the channel. The movie is generated as a view looking out through the channel from the inside of the cell. The action of capsaicin on TRPV1 is to “lock” the structure in the open position so cations can pass through the membrane. The movie is from “Mechanism of Voltage Gating in Potassium Channels,” Morten Ø. Jensen, Vishwanath Jogini, David W. Borhani, Abba E. Leffler, Ron O. Dror, David E. Shaw, *Science*, **2012**, *336*(6078), 220-233. This is Movie S1 in Supplementary Materials.

In order to run the movie, the file “voltage-gated K+ channel.mov”, should be in the same folder as the PowerPoint presentation. In presentation mode, clicking any place within the light lilac-colored area starts the movie. Clicking again within the colored area stops the movie, which can be started again by clicking within the colored area. Thus, it is possible to step through the movie stopping at interesting points, for example at a “time” between 130 and 140 us (upper left corner of the figure), just as the channel closes. The movie can be replayed when it has finished by clicking again in the colored area. Clicking outside the colored area at any time advances the program to the next slide.

Slide 6

Lidocaine is commonly used as a local anesthetic for dental procedures. Almost everyone in a high school class has had this experience and questioning them about it always leads to the conclusion that the numbness that lasts for some time after the procedure is an odd sensation that is generally unwelcome. The reason for this is that lidocaine is a relatively nonpolar molecule that can diffuse across cell membranes without need for a special carrier or channel. Once inside a nerve cell, lidocaine blocks a sodium channel protein from the inside and prevents the cell from firing. Hence, you feel no pain. But nerves that should send messages to muscles are also shut down, so you also get the numb feeling.

A possible solution to this issue is represented in this figure. Consider how the structure of QX-314 differs from lidocaine. QX-314 is positively charged (a quaternary amine) and cannot readily pass through cell membranes because of the nonpolar lipophilic interior of these bilayer structures. Once inside a nerve cell, however, QX-314 acts just like lidocaine to block sodium channels and shut down the nerve. Capsaicin interacts only with TRPV1 on pain-sensing nerves and opens this cation channel so there is a pathway for QX-314 to enter pain nerves, but not others. Thus a combination of capsaicin and QX-314 would deaden pain, but have no effect on muscles (no numbness). This combination has been tested in mice and rats and found to block their feeling of pain, but have no effect on movement. (Through late 2013, there does not appear to have been further research toward making this anesthetic combination suitable for use in humans.)

The figure in this slide is from “New Anesthetic Avoids Common Side Effects,” [Sophie Rovner](http://pubs.acs.org/cen/staff/biosw.html), *Chemical & Engineering News*, October 8, 2007, 8, which is a report on this research, “Inhibition of nociceptors by TRPV1-mediated entry of impermeant sodium channel blockers,” Alexander M. Binshtok, Bruce. B. Bean, and Clifford J. Woolf, *Nature*. **2007**, *449*(7162), 607-10. The figure in *C&EN* is based on one in this commentary on the research, “Neuroscince: A local route to pain relief,” Edwin W. McCleskey, *Nature*, **2007**, *449*, 545-546.

Note the way that the membrane proteins are modeled in this figure. These are often called “cartoons” that capture the essence of the function of the protein, but show essentially none of its detailed structure. When you see protein models like these, keep in mind that they represent complex molecules with structures like the model shown in slide 4, which is also simplified to focus on some details, but not the atoms that make up the actual structure.