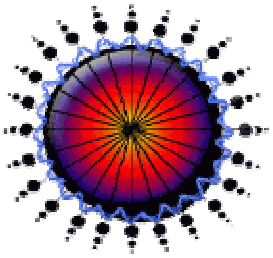

The Characterization of Amino Acids and the Purification of Proteins

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Abstract

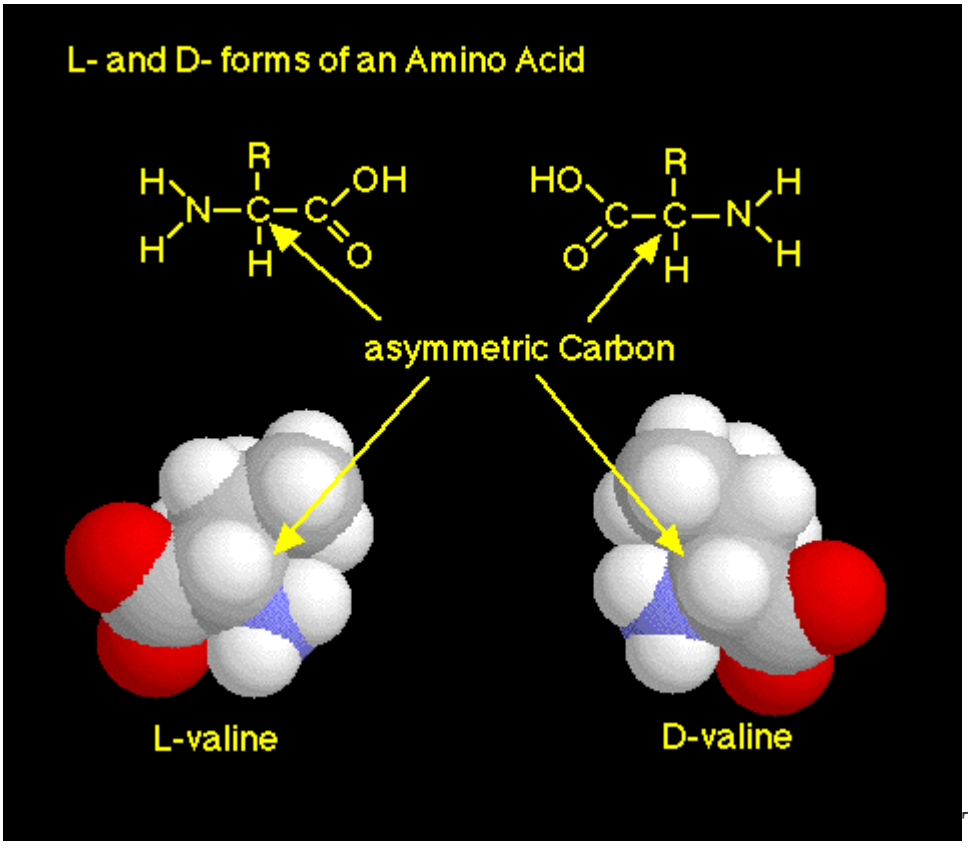
Amino acids are the building blocks of proteins. And it is a compound that contains both the amino and carboxyl groups. Various foods produce amino acids that the human bodies need. Some foods that produce certain proteins are called complete protein and incomplete protein. Proteins are many compounds that make up living organisms. Serine, Tryptophan, and selenatryptophan are the amino acids that are the main focus.

Introduction

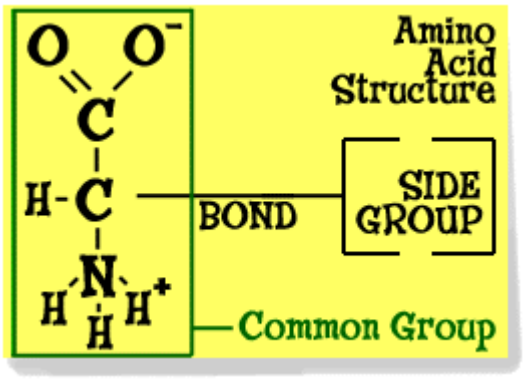
Amino Acids

Amino acids are the basic portion of the molecule, and are capable of reacting with both inorganic and organic acids to form salts, amides, and so on. They are class of organic compounds that contain both the amino and carboxyl groups. Of these acids, 20 serve as the building blocks of proteins. Known as the standard, or alpha, amino acids, they comprise serine and tryptophan, which are 2 of the 20 that are constructed according to a general formula. Some plants and microorganisms can make all the proteins. Humans and high animals cannot make all of the 20 amino acids their bodies need to build tissue. Humans must get at least 9 amino acids from their food. Foods, such as, eggs, meat, milk products, and some vegetables, provide amino acids. Also many amino acids link together to form new proteins and the body can make several different types of proteins. But different sequences determine the proteins function, however, most proteins are complex and contain about 20 amino acids. They may form either parallel chains or global structures. When a cell makes protein, the

carboxyl group of one amino acid is linked to the amino group of another to form a peptide bond. The carboxyl group of the second amino acid is also linked to the amino group of a third, and so on, until a long chain is produced. This chain molecule, which may contain from 50 to a number of hundred amino acid subunits, is called a polypeptide. A protein may be formed of a single polypeptide chain, or it may consist of many chains held together by weak molecular bonds. Each protein is formed according to a precise set of instructions contained within the nucleic acid, which is the genetic material of the cell. The R groups of the amino acid subunits determine the final shape of the protein and its chemical properties; an extraordinary variety of proteins can be produced from the same 20 subunits.



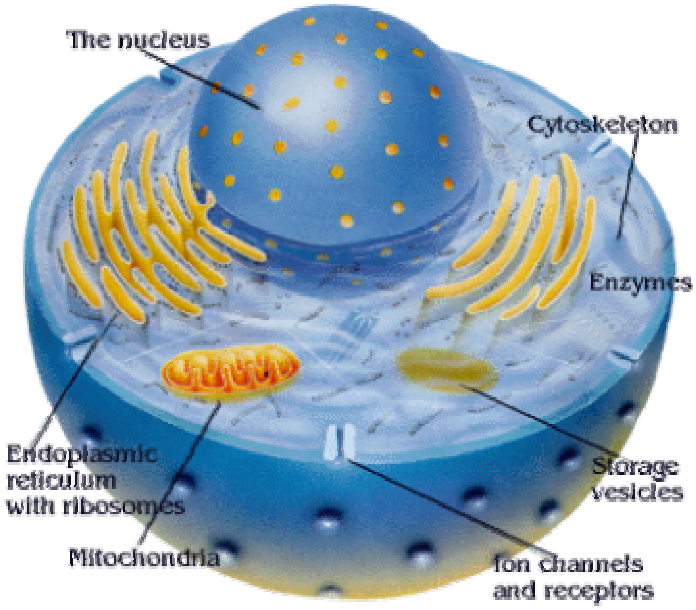
The standard amino acids serve as raw materials for the manufacture of many other cellular products, including hormones and pigments. In addition, several of these amino acids are key intermediates in cellular metabolism.



Proteins

Proteins are a number of organic compounds that make up living organisms and are essential to their functioning. Whether found in humans or in single-celled bacteria, proteins are composed of units of about 20 different amino acids. Proteins are in the 3 main food groups that provide energy to the body and they exist in every cell. Cheese, eggs, fish, meat, and milk contain high protein and are called complete proteins. Others such as, cereal grains, nuts, vegetables are called incomplete proteins. The most basic level of protein structure, called the primary structure, is the linear sequence of amino acids. Different sequences of the acids along a chain, affect the structure of a protein molecule in different ways. Forces such as hydrogen bonds attract positive and negative charges, and hydrophobic ("water-fearing") and hydrophilic ("water-loving") linkages cause a protein molecule to coil or fold into a secondary structure, examples of which are the so-called alpha helix and the beta-pleated sheet. When forces cause the molecule to become even more compact, as in globular proteins, a tertiary protein structure is formed. When a protein is made up of more than one polypeptide chain, as in hemoglobin and some enzymes, it is said to have a quaternary structure. Polypeptide chains are sequenced and coiled in a way that the hydrophobic amino acids usually face inward, giving the molecule stability, and the hydrophilic amino acids face outward, where they are able

to interact with other compounds and mainly other proteins. Globular proteins can join with a specific compound such as a vitamin derivative and form a coenzyme or join with a specific protein and form an assembly of proteins needed for cell chemistry or structure.



Purpose:

To make selenatryptophan more commercially available to the community

Protocols:

Run a TLC (Thin Layer Chromatography)
Purify Proteins

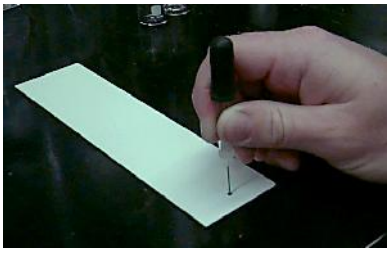
1. Wash mode
2. P-50 Preparation
3. Loading CFE (Cell Free Extract)
4. Stripping Chelating Sepharose
5. After Finishing Your Chromatography

Materials:

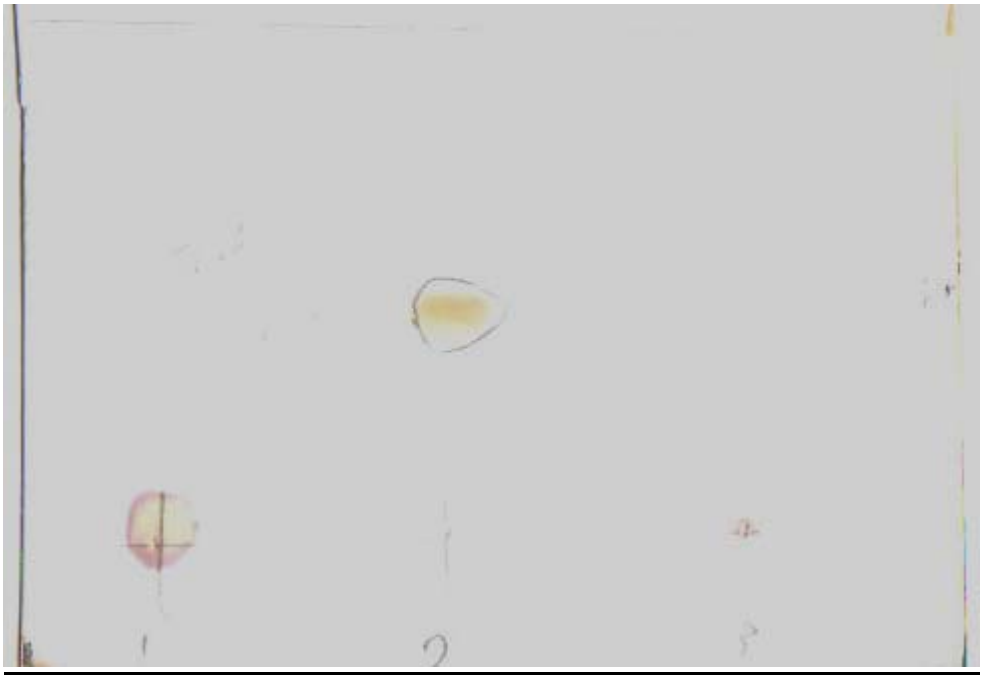
1. TLC Plate
2. TLC Solution
3. Gradifrac
4. Recorder
5. P-50 Pump
6. P-1 Pump

Running a TLC

Dissolve 10mg (0.01g) into 2mL of dH**2**O
Vortex until in solution
Spot sample on a TLC plate
Set in TLC solution for about 10-15mins.
Look at it under a light
Circle samples movement
Set it in a jar of iodine or spray it with ninhydrant
Then dry it with a plow dryer



Results



What this is showing us is that we are positive for 1.Serine, 2.Trytophan, and 3.Selenatrytophan. In addition, because of the ninhydrant making the samples more transparent we are able to see where the samples are located.

Purifying Proteins

Wash mode

Turn on recorder, detector, gradifrac, and p-50 pump. In wash mode with p-1 pump pass water or 10% ethanol through p-1 pump out port 4 to waste. Change valve to inject mode and pass 2-3ml of water or 10% ethanol through the column. Then switch to buffer A for 2 mins.

P-50 preparation

Drop a and b line of p-50 pump into buffer a and b, then change valve to wash mode. After that run method 0 on gradifrac.

Loading CFE (cell free extract)

Change valve to inject mode, then put the p-1 line in buffer a and pump about 2mls of the buffer. Next you go into manual on gradifrac and set it at 0% b, 2ml/min, 8ml fractions, and put line 6 of the valve in buffer a. Later you want to pump buffer a through p-1to equilibrate the column, afterward you press pause on the gradifrac. Next you put p-1 line in CFE and press continue on the gradifrac, then load the CFE. After that switch the p-1 line to buffer a and continue with the buffer through the pump for 10-30mins until the recorder reapproaches the base line. Next press end on gradifac and turn p-1 pump off. Then change the valve to load mode and continue running the method.

Stripping chelating sepharose

Push end on gradifrac and set the valve to inject mode. Pump p-1 line in stripping buffer and pump until the column is white. Then run 10% ethanol through the column for 10mins.

After finishing your chromatography

Push end on gradifrac and set the valve to inject mode. Next you put p-1 line in 10% ethanol for 10mins. Then change valve to wash mode and drop

p-5o lines a and b into 10% ethanol and run method 9 on gradifrac for about 20mins.

Discussion

What we have that is going into the reaction is Se-Indole analog substrates that was synthesized at Los Alamos National Lab, enzymes that were over expressed in bacteria and then purified using medium pressure liquid chromatography, and commercially available buffers to control the pH level. What comes out is selenatrytophan. But a little before that the centripreps were used to remove the enzymes. Then we do a product analysis by TLC. Afterwards we hope to get a better yield in order to make selenatryptophan more commercially available for the community, which makes that our main purpose.
But this is an on going project and it will probably some time before we produce an increasingly better yield.

Lecture notes:

Socio-cultural Anthropology

The study of human society and culture. Ethnology is the comparison of people or the study of ethnicities. I learned that Pasaje is a city located is Ecuador. Also that school was from 8a.m. to 12p.m. then they went back at 3p.m. to 5p.m. If one of the kids wanted to go outside a relative had to know the last name of the other child they were playing with. It helps identify the social background. In addition boys had more freedom then girls.

For example the boys can stay out later than the girls even if it were a school night. Another thing I learned was that the boys, at an early age, begin to help the family by getting a job and working.

Bronx River Oyster Project 2006

This project is to restore the Bronx River to a clean body of water. Also it is to restore and replenish disappearing species by reintroducing oysters. It was also explained that oyster marines are mollusk having two rough irregular shells. The materials that were used were shells, oysters, and buoys. And what they did was fill a net with oyster shells and reintroduce them to the Bronx River. Also filter feeders are a created habitat space for other oysters. Other critters that they found were sea squirts, tube worm, blue claw crab, bryozoans, barnacles, oyster spat, slipper shell, sea anemones, and Gobi fish. Their conclusion was that they noticed that the population of the fish was increasing, the Bronx River is stabilizing, and beavers are starting to return to the river. In addition to that their future plans are to continue monitoring the oyster reef every month for more updates.

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