

3.3.1: Cultural Connections- Anthropology and Protein Stoichiometry

We have examined the idea of amino acid *complementarity*, where different foods in the diet supply the correct balance of amino acids to construct Human protein, in another section. Human proteins require stoichiometric amounts of about ten "essential" amino acids, so if only one is missing, the protein cannot be synthesized, and protein malnourishment results. This illustrates the concept of a "**limiting reactant**", which is a reactant present in less than the stoichiometric amount relative to other reactants.

A diverse group of surviving cultures worldwide have adopted diets, different as they may be, that supply the correct amino acid balance^[1]. Anthropologists believe that fortuitous adoption of these diets provided survival value for the cultures. In areas where food is scarce, cultures that do not adopt diets with correct amino acid balance may not survive. Here are some examples of diets that supply approximately the correct stoichiometric amounts of amino acids:

Tostadas

In Latin America, diets combining corn tortillas or other corn products with beans, like the tostada shown here, are common.



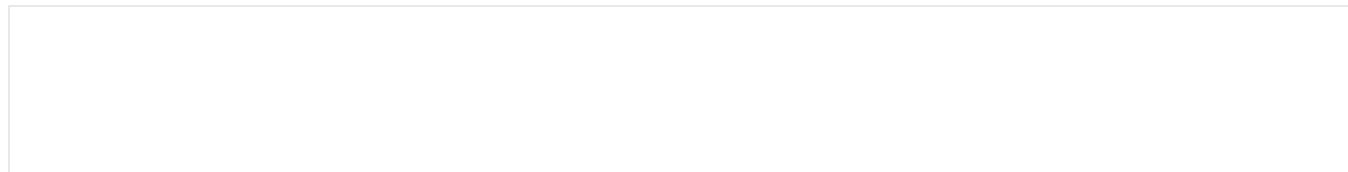
Tostada with corn tortilla, bean topping, and vegetables^[2]

Beans eaten alone provide limiting amounts of sulfur containing amino acids like methionine and cysteine, so these amino acids limit the amount of Human protein that can be synthesized. Beans have large amounts of the amino acids lysine and tryptophan, so these are "**excess reactants**" when used to synthesize Human protein, and will be degraded to urea and wasted. If wheat, rice, or corn are eaten alone, they typically provide amounts of lysine and tryptophan which limit the amount of Human protein that can be synthesized. But if beans are eaten with grains or corn, the **excess reactants** of the beans *complement* the limiting reactants of the grain (and vice versa). Human protein can then be synthesized efficiently, and very small amounts of amino acids are simply excreted as urea.

The origins of this diet are ancient. The Tehuacan Archaeological-Botanical Project (1961–1964) investigated the beginnings of agriculture in the New World and the concomitant rise of autochthonous (indigenous) civilization^[3]. The project is described in six volumes, the first of which explores subsistence and the environment^[4]. The domestication of maize after 8000 B.C.E. and beans (3000–4000 B.C.E.) in the Tehuacan Valley (the "Valley of Mexico") of south central Mexico, gave inhabitants a nutritional advantage in the face of progressively deteriorating resources. Maize cob size increased rapidly under domestication from only a centimeter or two to current size, while *Phaseolus vulgaris* (kidney and pinto beans) similar to modern ones probably existed in preagricultural times. "Wherever these two food crops met, an immediate adaptive combination favored by human selection was formed."^[5]

Falafel and Pita

In the Middle East, a meal may consist of falafel and hummus made from chickpeas eaten with pita bread made from wheat.



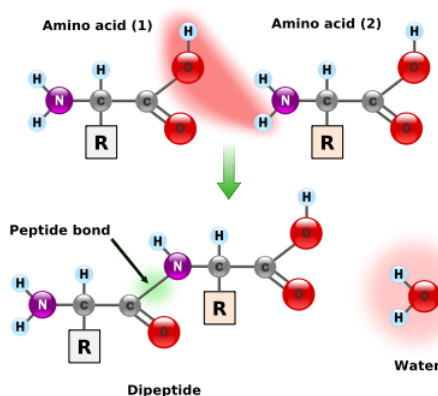
A plate of Indian food, likely a vegetarian meal, featuring white rice, yellow lentil soup (dal), and various vegetable curries. A small bowl of chutney is also visible.

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Amino Acid	Requirement (g/kg)	Amount in corn (g/kg)	Amount in lentils (g/kg)
Lysine (LYS)	7.0	2.4	6.3
Tryptophan (TRP)	1.5	0.7	0.81
Methionine (MET) and Cysteine (CYS)	6.0	3.8	0.77 + 1.18
Histidine (HIS)	3.0	2.0	2.54
Valine (VAL)	6.0	4.6	4.48
Isoleucine (ISO)	5.0	3.9	3.90
Threonine (THR)	5.0	3.9	3.23
Arginine (ARG)	6.0	5.0	6.97
Phenylalanine (PHN) and Tyrosine (TYR)	8.0	9.6	4.45 + 2.41
Leucine (LEU)	7.5	11.0	6.54

How Amino Acids Form Proteins

Amino acids are joined by a very simple "condensation" reaction where two molecules join by eliminating water, as shown in the figure below. The portion of the molecule labeled "R" stands for a variable part of the molecule that distinguishes different amino acids; the rest of the molecule is common to all amino acids.



Formation of a peptide bond between two amino acid shown in terms of ball and stick diagrams. All amino acids have the common $\text{N H}_2 \text{C H C O O H}$ structure. The R group connected to the middle C is unique to each amino acid. The product is a chain of both amino acids with a peptide bond between the C from C O from one amino acid and N from N H of another amino acid.

Formation of a peptide bond ^[13]

This process can continue with different amino acids adding to either end of the protein chain. The H-N of an amino acid adds to the C-OH end of the protein, eliminating water (H-OH) and forming a C-N bond where the water was eliminated (or the C-OH end of an amino acid adds to the N-H end of the protein). This process continues until the protein has hundreds of different amino acids.

Example 1: Stoichiometry of Corn Malnutrition

a. Correct, or Stoichiometric Amino Acid Ratios:

From data in the Table II above, calculate the optimal molar ratio of lysine (LYS, $\text{C}_6\text{H}_{14}\text{N}_2\text{O}_2$, Molar mass 146.19 g/mol) to Arginine (ARG, $\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2$, Molar mass 174.2 g/mol) in the diet.

b. Limiting Amino Acid:

From data in the Table above, calculate which, LYS or ARG, is the **limiting reactant** in a corn diet?

c. How much of the **excess amino acid** will be wasted in 1 kg of corn diet?

Solution

a. The balanced equation will have coefficients that we need to determine:

$x \text{ LYS} + y \text{ ARG} + \text{other amino acids} \rightarrow \text{Protein} + \text{water}$

According to the atomic theory, x mol LYS is required for each y mole of ARG to make useful protein. If the diet is optimal when 7.0 g of LYS is eaten with every 6.0 g of ARG (this ratio might be provided by eggs, for example), we can calculate the optimal stoichiometric ratio:

$$n_{\text{LYS}} = 7.0 \text{ g} \times \frac{1 \text{ mol LYS}}{146.19 \text{ g}} = 0.048 \text{ mol LYS}$$

$$n_{\text{ARG}} = 6.0 \text{ g} \times \frac{1 \text{ mol ARG}}{174.2 \text{ g}} = 0.034 \text{ mol ARG}$$

$$\text{The ratio is } \frac{\text{LYS}}{\text{ARG}} = \frac{0.048}{0.034} = \frac{1.4}{1}$$

And since 1.4 is about 1-2/5 or 7/5,

$$\frac{7/5}{1} = \frac{7}{5}$$

This exact amino acid ratio may not be found in any particular rat protein, but it is the average ratio for all the proteins in the rat. The (partial) chemical equation is



That is, the stoichiometric ratio $S(\text{LYS}/\text{ARG}) = 7 \text{ mol LYS} / 5 \text{ mol ARG}$.

b. Let's see what the mole ratio is in the corn diet.

$$n_{\text{LYS}} = 2.4 \text{ g} \times \frac{1 \text{ mol LYS}}{146.19 \text{ g}} = 0.016 \text{ mol LYS}$$

$$n_{\text{ARG}} = 5.0 \text{ g} \times \frac{1 \text{ mol ARG}}{174.2 \text{ g}} = 0.029 \text{ mol ARG}$$

$$\text{The ratio is } \frac{\text{LYS}}{\text{ARG}} = \frac{0.016}{0.029} = \frac{0.55}{1}$$

Clearly, LYS is the limiting amino acid because the ratio initial amounts of LYS to ARG (0.55:1) is much less than the required stoichiometric ratio (1.4 : 1).

General Approach to Limiting Reactant Problems

From the corn diet example you can begin to see what needs to be done to determine which of two reagents, X or Y, is limiting. We must compare the stoichiometric ratio $S(X/Y)$ with the actual ratio of amounts of X and Y which were initially mixed together.

The corresponding general rule, for any reagents X and Y, is

$$\text{If } \frac{n_X(\text{initial})}{n_Y(\text{initial})} \text{ is less than } S\left(\frac{X}{Y}\right), \text{ then X is limiting.} \quad (3.3.1.1)$$

$$(3.3.1.2)$$

$$\text{If } \frac{n_X(\text{initial})}{n_Y(\text{initial})} \text{ is greater than } S\left(\frac{X}{Y}\right), \text{ then Y is limiting.} \quad (3.3.1.3)$$

Of course, when the amounts of X and Y are in exactly the stoichiometric ratio, both reagents will be completely consumed at the same time, and neither is in excess.

We can verify that LYS is limiting by calculating the amount of LYS that would be required if all the ARG reacted, using a stoichiometric ratio:

$$n_{\text{ARG}} \xrightarrow{S(\text{LYS}/\text{ARG})} n_{\text{LYS}}$$

$$0.029 \text{ mol ARG} \times \frac{7 \text{ mol LYS}}{5 \text{ mol ARG}} = 0.041 \text{ mol LYS}$$

Similarly, if all the LYS reacts, the required amount of ARG is

$$0.016 \text{ mol LYS} \times \frac{5 \text{ mol ARG}}{7 \text{ mol LYS}} = 0.018 \text{ mol ARG}$$

This is much less than the amount present, so ARG is the **Excess Reactant**.

c. We can easily use the molar masses to convert the amounts of each amino acid to the masses.

Let's set up a table:

	7 LYS	+ 5 ARG	other amino acids →	Protein + water
M (g/mol)	146.19	174.2		
m (g)	2.4	5.0		
n (mol)present	0.016	0.029		
if all ARG reacts	-0.041	-0.029		
if all LYS reacts	-0.016	-0.011		
Remaining amount	0	0.018		
Remaining mass (g)	0	3.14		

When the reaction ends, 3.14 g of the original 5.0 g (63%) remains, and will be metabolized to urea and excreted. Of the entire diet of 2.4 g LYS + 5.0 g ARG, we see that 3.14 g or 42% is wasted. What a waste of food and the resources to produce it! Notice that the corn serving will also have to be larger than the serving of optimal diet for the same amount of protein, because corn has only 5 g/kg ARG, while the optimal diet has 6.0 g/kg. The optimal diet might be provided by meat or eggs, but they are environmentally demanding and present health concerns.

Example 2: Complementary Amino Acids

A subsistence bean diet may also lead to protein malnutrition, as inspection of Table II above shows. Although the Lysine content is much greater than in beans, the methionine (MET) and Cystine (CYS) levels are low, as are the Phenylalanine (PHE) and Tyrosine (TYR).

But suppose beans and corn are eaten in the same day, in a diet that mixed equal masses of corn and lentils. Now recalculate the limiting amino acid in a diet of $2.4 + 6.3 = 8.7$ g of LYS and $5.0 + 6.97 = 11.97$ g of ARG.

b. In this case, how much of the excess amino acid is wasted?

Solution

$$n_{\text{LYS}} = 8.7 \text{ g} \times \frac{7 \text{ mol LYS}}{146.19 \text{ g}} = 0.060 \text{ mol LYS}$$

$$n_{\text{ARG}} = 11.97 \text{ g} \times \frac{1 \text{ mol ARG}}{174.2 \text{ g}} = 0.069 \text{ mol ARG}$$

Now we see that the ratio of amounts present is

$$0.060 \text{ mol LYS} / 0.069 \text{ mol ARG} = 0.869,$$

which is still less than the stoichiometric ratio,

$$7 \text{ mol LYS} / 5 \text{ mol ARG} = 1.4$$

So LYS is once again the limiting amino acid. Recalculating the values in a table as above, we get

	7 LYS	+ 5 ARG	other amino acids →	Protein + water
M (g/mol)	146.19	174.2		
m (g)	8.7	11.97		

n (mol)present	0.060	0.069		
if all ARG reacts	-0.080	-0.069		
if all LYS reacts	-0.060	-0.043		
Remaining amount	0	0.027		
Remaining mass (g)	0	4.61		

In this case, 4.61 g of the excess ARG remains from a 11.97 g portion, or 38% of the ARG. The total diet is 8.7 g LYS + 11.97 g ARG = 20.7 g, and only 22% of that is wasted, a big improvement over the corn alone case above.

In order to design a proper diet, complementary protein foods would have to be chosen from tables of amino acid contents of foods.

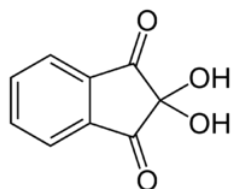
Is Nixtamalization an Anthropological Counterargument?

In Central America, field corn or "maize" is traditionally boiled in lime (CaO) to loosen the hulls and soften the kernels in the preparation of tortilla flour. The process is shown beautifully in a Culinary Institute of America film. The Southern US breakfast "grits" are also made this way^[14].

But this process converts some of the already limiting tryptophan to 2-aminoacetophenone, exacerbating the poor nutritional, and survival value, of the corn. But the nixtamalization also makes niacin in the kernels more bioavailable, reducing the incidence of Pellagra, and more than offsetting the loss of amino acids if plenty of beans are also included in the diet. The beans & corn diet, even with nixtamalization, has survival value for cultures.

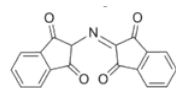
Example 3: Amino Acid Detection

To detect an amino acid (even in a fingerprint in forensic chemistry), the *ninhydrin* test is often used.



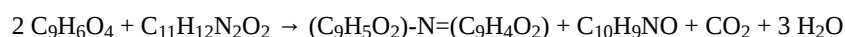
Ninhydrin

In the ninhydrin test, two ninhydrin molecules (C₉H₆O₄, shown to the left) become linked by the N attached to the first carbon of the amino acid chain, producing the blue/purple ion shown at right.



Blue/Purple Product^[15]

The balanced chemical equation is:



If 2.00 mg of ninhydrin (Nin) is used to detect 2 mg of TRP, has enough ninhydrin been added to react with all the TRP? Which is the limiting reagent, and what mass of H₂O will be formed?

Solution

The stoichiometric ratio connecting Nin and TRP is

$S\left(\frac{\text{Nin}}{\text{TRP}}\right) = \frac{2 \text{ mol Nin}}{1 \text{ mol TRP}}$ The initial amounts of Nin and TRP are calculated using appropriate molar masses (160.13 g mol⁻¹ for Nin and 204.23 g mol⁻¹ for TRP:

$$n_{\text{Nin}}(\text{initial}) = 2.00 \times 10^{-3} \text{ g} \times \frac{1 \text{ mol Nin}}{178.14 \text{ g}} = 1.12 \times 10^{-5} \text{ mol Nin} \quad (3.3.1.4)$$

$$(3.3.1.5)$$

$$n_{\text{TRP}}(\text{initial}) = 2.00 \times 10^{-3} \text{ g} \times \frac{1 \text{ mol TRP}}{204.23 \text{ g}} = 9.79 \times 10^{-6} \text{ mol TRP} \quad (3.3.1.6)$$

Their ratio is $\frac{n_{\text{Nin}}(\text{initial})}{n_{\text{TRP}}(\text{initial})} = \frac{1.123 \times 10^{-5} \text{ mol Nin}}{9.79 \times 10^{-6} \text{ mol TRP}} = \frac{1.15 \text{ mol Nin}}{1 \text{ mol TRP}}$. Since this ratio is smaller than the 2:1 stoichiometric ratio, you have insufficient Nin to react with all the TRP, so Nin is the limiting reagent. To ensure detection, it would be better to add excess Ninhydrin.

b) The amount of water product formed in a reaction may be calculated via an appropriate stoichiometric ratio from the amount of a reactant which was *consumed*. Some of the excess reactant TRP will be left over, but all the initial amount of Nin will be consumed. Therefore we use $n_{\text{Nin}}(\text{initial})$ to calculate how much H_2O can be obtained

$$n_{\text{Nin}} \xrightarrow{S(\text{water/Nin})} n_{\text{water}} \xrightarrow{M_{\text{water}}} m_{\text{water}} \quad m_{\text{water}} = 1.12 \times 10^{-5} \text{ mol Nin} \times \frac{3 \text{ mol water}}{2 \text{ mol Nin}} \times \frac{18.016 \text{ g}}{\text{mol water}} = 3.02 \times 10^{-4} \text{ g water}$$

This is 0.302 mg water.

These calculations can be organized as a table, with entries below the respective reactants and products in the chemical equation. Calculations are shown for each possible case, assuming that one reactant is completely consumed and determining if enough of the other reactants is present to consume it. If not, that scenario is discarded.

	2 C ₉ H ₆ O ₄	+ C ₁₁ H ₁₂ N ₂ O ₂ →	(C ₉ H ₅ O ₂)-N= (C ₉ H ₄ O ₂)	+ C ₁₀ H ₉ NO	+ CO ₂	+ 3 H ₂ O
m (g)	0.0020	0.0020				
M (g/mol)	178.1	204.23	303.3	159.2	44.0	18.0
n (mol)	1.12 × 10 ⁻⁵	9.79 × 10 ⁻⁶	--	--	--	--
if all C ₉ H ₆ O ₄ reacts	-1.12 × 10 ⁻⁵	-5.62 × 10 ⁻⁶				
if all C ₁₁ H ₁₂ N ₂ O ₂ reacts	-1.96 × 10⁻⁵	-9.79 × 10⁻⁶				
Actual Reaction Amounts	-1.12 × 10 ⁻⁵	-5.62 × 10 ⁻⁶	5.62 × 10 ⁻⁶	5.62 × 10 ⁻⁶	5.62 × 10 ⁻⁶	1.68 × 10 ⁻⁵
Actual Reaction Masses	-0.0020	-0.00115	1.71 × 10 ⁻³	8.93 × 10 ⁻⁴	2.47 × 10 ⁻⁴	3.02 × 10 ⁻⁴

As you can see from the example, in a case where there is a limiting reagent, *the initial amount of the limiting reagent must be used to calculate the amount of product formed*. Using the initial amount of a reagent present in excess would be incorrect, because such a reagent is not entirely consumed.

The concept of a limiting reagent was used by the nineteenth century German chemist Justus von Liebig (1807 to 1873) to derive an important biological and ecological law. **Liebig's law of the minimum** states that the essential substance available in the smallest amount relative to some critical minimum will control growth and reproduction of any species of plant or animal life. When a group of organisms runs out of that essential limiting reagent, the chemical reactions needed for growth and reproduction must stop. Vitamins, protein, and other nutrients are essential for growth of the human body and of human populations. Similarly, the growth of algae in natural bodies of water such as Lake Erie can be inhibited by reducing the supply of nutrients such as phosphorus in the form of phosphates. It is for this reason that many states have regulated or banned the use of phosphates in detergents and are constructing treatment plants which can remove phosphates from municipal sewage before they enter lakes or streams.

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