A Toast To An Enzyme

XXXXXXX XXXXX

February 22, 2007

CH 370

Original Amino Acid Sequence: `fakakelgatecinpkdfkk`

Entire Protein Sequence: >Chicken

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1   rsddhvvtga lampfpiilg heaagviesv gekvtslkpg daviplfvpq cgecrsclst
61  kgnlcikndl sssptglmad gttrftckgk aihhfvgtst fteytvvhet aaakidsaap
121 lekvcligcg fstgygavlq takveagstc avfglggvgl svvmgckaag asriiavdin
181 kdkfakakel gatecinpkd fkkpihevlt emtgqgvdys fevigrietm taalaschnn
241 ygvsvivgvp paaqkisfdp mlifsgrtwk gsvfggwksk davpklvady mkkkfvldpl
301 ithtlpftki negfdllrtg ksirsvlvl
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Name of Protein: Avian Alcohol Dehydrogenase

Source Organism: Chicken (Gallus gallus)

Functional Class: dehydrogenase (reductase)

When thinking of enzymes in the body that humans cannot possibly live without, a number of enzymes might come to mind—pepsin, lysozyme and a-amylase because of their function in food digestion[1] or DNA Polymerases because of their function in DNA replication and maintenance.[2] In fact, our bodies are full of thousands of proteins and enzymes whose functions are depended upon on a daily basis. Upon researching a 20 amino acid sequence, belonging to a much longer chain representing an enzyme, another enzyme extremely important to the function of not only humans, but many eukaryotes and prokaryotes, was "uncovered." This enzyme, especially, is undoubtedly an enzyme that the stereotypical college student could not live without. Alcohol dehydrogenase is the enzyme that our bodies use to decompose all types of alcohols—including those found in many everyday foods, weekend party beers, or congratulatory glasses of champagne—into other compounds our bodies can use. The specific protein described below, however, was the alcohol dehydrogenase present in a less celebratory organism—the chicken.

The first step in determining the enzyme to which the 20 amino acid sequence belonged involved the use of the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information website.[3] The sequence was entered into the program and a lengthy list of the best possible matches for the sequence and the protein it is included in was produced. The complete amino acid sequence could be determined by simply choosing a protein that was on the list, denoted by an extremely small Expectation (E) value. In the case of the sequence chosen, the expectation value was zero, indicating a perfect match.

Upon reviewing the list of proteins, mostly alcohol dehydrogenases belonging to a number of different organisms, it was concluded that the peptide given belonged to an alcohol dehydrogenase or ADH. Some of the organisms that this enzyme is present in include chickens, horses, yeast, chimpanzees, ostriches, *Shewanella putrefaciens*, and humans, to name a few. The sequence encoding the protein for the class IB alcohol dehydrogenase found in chickens was chosen for further research. From the BLAST program, the avian alcohol dehydrogenase contained a link and the following complete sequence of amino acids for the protein was determined and is shown in FIGURE 1 below. The original amino acid sequence given is highlighted in red starting with amino acid number 184.

The chicken, whose scientific name is Gallus gallus, belongs to the avian class and is a vertebrate—like humans and horses—which accounts for the striking similarity of its alcohol dehydrogenase sequence with those of humans and horses. The chicken, presently being a very domesticated animal, was descended from the Red Junglefowl from Southeast Asia but is now spread all over the world.[4]

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  1 rsddhvvtga lampfpiilg heaagviesv gekvtslkpg daviplfvpq cgecrsclst
 61 kgnlcikndl sssptglmad gttrftckgk aihhfvgtst fteytvvhet aaakidsaap
121 lekvcligcg fstgygavlq takveagstc avfglggvgl svvmgckaag asriiavdin
181 kdkfakakel gatecinpkd fkkpihevlt emtgqgvdys fevigrietm taalaschnn
241 ygvsvivgvp paaqkisfdp mlifsgrtwk gsvfggwksk davpklvady mkkkfvldpl
301 ithtlpftki negfdllrtg ksirsvlvl
```

FIGURE 1. Complete amino acid sequence of Avian Alcohol Dehydrogenase (Chickens).[3]

The general function of the protein alcohol dehydrogenase is the breakdown of potentially toxic alcohols in the organism's body—such as ethanol and methanol—into aldehydes or ketones.[5] For example: ethanol + NAD^+ $\xrightarrow{\text{Alcohol Dehydrogenase}}$ acetaldehyde + NADH + H^+. Without an ADH, these toxic compounds would always be present and their breakdown products not available for use by the organism. Depending on the

organism, of course, this function varies in its specificities. These functions ultimately depend upon the respective structures of the protein within each organism as well.

Before understanding the differences in structure of alcohol dehydrogenase in several organisms, it is important to understand that this enzyme works with the help of a coenzyme or cofactor, in many cases. For chickens, the enzyme requires two zinc ions per subunit of the homodimer that serve to hold the hydroxyl group of the alcohol substrate in place during the enzymatic reaction.[5] The illustration of the interaction between the enzyme alcohol dehydrogenase and the zinc ions, as well as the necessary nicotinamide adenine dinucleotide (NAD+) cofactor, is included in Figure 2. Nicotinamide adenine dinucleotide is required as a two electron and proton (H^- or hydride) carrier in the alcohol dehydrogenase reaction of all organisms. The structure of NAD^+ related to its function in the alcohol dehydrogenase reaction is shown in Figure 3.

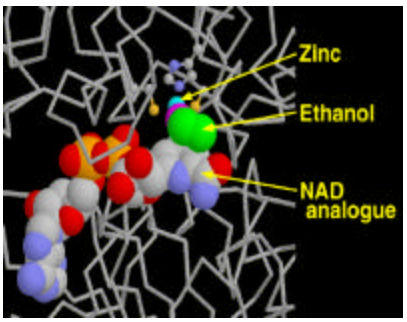

FIGURE 2. Portion of Alcohol Deyhydrogenase Enzyme shown interacting with its cofactors (Nicotinamide adenine dinucleotide and Zinc) and substrate (Ethanol).[6]

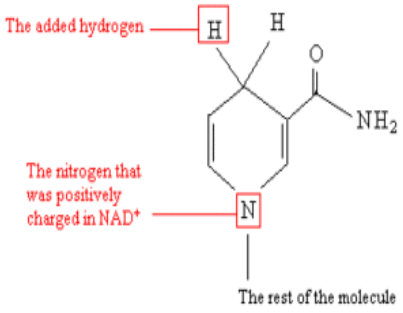

FIGURE 3. Structure of Nicotinamide Adenine Dinucleotide.[8]

The reason these cofactors and coenzymes are "allowed" to fit into the enzyme's structure is written in the amino acid sequence of the enzyme—alcohol dehydrogenase's primary structure. It has been determined that of the total 329 amino acids that compose the enzyme, the amino acids responsible for interacting with the nicotinamide adenine dinucleotide which, in turn, allows for proper enzyme functioning, include: Arginine 47,

Serine 48, Histidine 51 and Isoleucine 269 and Arginine 271.[7] This interaction allows the proton transfer to occur through NAD^+, using zinc as a hydrogen acceptor from the substrate as well.[8] The zinc ion, as shown in the Figure 5, is associated with 4 cysteine residues in each subunit, interacting with the sulfhydryl (-SH) side chains of the amino acids. This occurs in each subunit of the enzyme dimer. These regions of each subunit of the enzyme constitute its active site, where various alcohols are bound and chemically reacted upon.

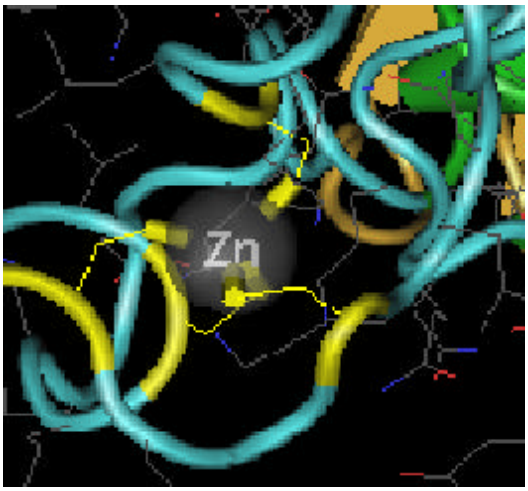

FIGURE 4. Zinc ion coordination to four cysteine residues (shown in yellow) of the alcohol dehydrogenase enzyme.[9]

Directly related to alcohol dehydrogenase's primary structure is its secondary structure. Using the Cn3D Version 4.1 Program provided on the National Center for Biotechnology Information website, the three-dimensional structure of the enzyme alcohol dehydrogenase was determined. This structure is shown in Figure 5 below. This figure also illustrates the enzyme's quaternary structure, as it shows the two subunits of the enzyme interacting. One subunit is colored in blue, and the other is colored in purple.

FIGURE 5. Three dimensional structure of alcohol dehydrogenase shown with zinc cofactors. The enzyme is a dimer of identical subunits, containing alpha helices and beta sheets as part of its secondary structure.[9]

After determining the sequence, structure and function of the enzyme to which the twenty amino acid sequence belonged, another BLAST search was performed using the entire amino acid sequence and a number of matches with small expectation values, on the order of 10^{-180}, were returned. These matches came from a variety of organisms including the following: *Pseudomonas fluorescens PfO-1*, horse, *Shewanella putrefaciens 200*, potato, chimpanzee, the African clawed frog, and the human. These sequences were aligned with the sequence from the chicken and, using the CLUSTAL W multiple sequence alignment program from the National Center for Biotechnology Information website, the results in Figure 6 were returned. Only a small portion of the entire amino acid sequence is in the figure, but it is enough to illustrate the highly conserved and identical amino acids present in this broad variety of organisms, ranging from bacteria to humans. Each **(*)** below the sequence denotes an identity and each **(:)** or **(.)** denotes a highly conserved, and a less highly conserved amino acid, respectively. The importance of these identical and highly conserved regions of the enzymes' primary structures serves as an explanation for the common general reaction that alcohol dehydrogenase performs.

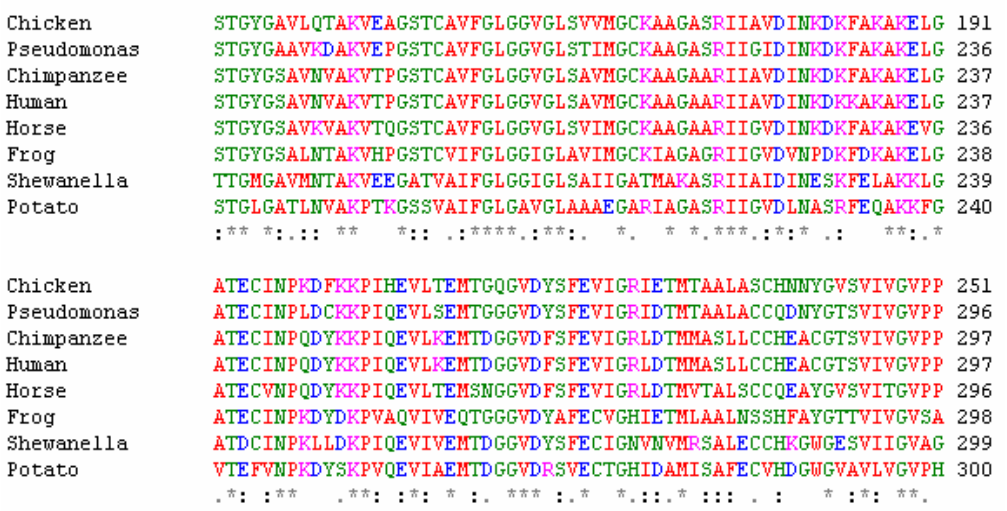

FIGURE 6. Clustal W Multiple Sequence Alignment of a subset of amino acids in eight types of alcohol dehydrogenase from various organisms.

As far as the comparison between the chicken and human sequences, many similarities occur due to the fact that the chicken alcohol dehydrogenase is most closely related to the human alcohol dehydrogenase of class I. Amino acids necessary for coenzyme and substrate binding are very highly conserved, if not identical between chickens and humans. Table 1[7] summarizes these sequences.

Region	Position	Chicken	Mammalian Class I
Coenzyme Binding	47	Arg	Arg/Gly/His
	48	Ser	Ser/Thr
	51	His	His
	269	Ile	Ile
	271	Arg	Arg/Gln
Substrate Binding	48	Ser	Ser/Thr
	57	Leu	Leu/Met
	93	Phe	Phe/Ala
	116	Leu	Leu/Val
	140	Phe	Phe
	141	Val	Val/Leu
	306	Met	Met
	318	Val	Val/Ile

TABLE 1. Residue Comparisons between the chicken liver enzyme and Class I of the mammalian enzyme.[7]

To illustrate the importance of the consistent amino acid sequence in the alcohol dehydrogenases belonging to all organisms, the horse liver alcohol dehydrogenase is a prime example. Extensive research has been carried out on this enzyme and the three dimensional structures of the enzyme's active site pocket, NAD^+ binding domain, and catalytic site have been determined.[10] Cysteine 46, Histidine 67 and Cysteine 174 bind the required zinc cofactor (very similar to the four cysteine residues in the chicken enzyme). Likewise, Arginine 47 binds the NAD^+ cofactor and Serine 48 binds the substrate alcohol. Arginine, due to its positive charge is able to bind the pyrophosphate group of the NAD, limiting the freedom of amino acid mutations to not affect enzyme function. Something like the replacement of the Serine 48 with Threonine (which applies to the *Shewanella putrefaciens* sequence used in the multiple sequence alignment) severely reduces catalytic activity of alcohol dehydrogenase. This, however, is made up for by the replacement of Arginine 47 (in horses, chickens, humans, etc.) with Histidine in the bacteria. Histidine restores active center space and, therefore, the enzyme's catalytic activity.[10] These small changes resulting in loss of catalytic activity illustrate the importance of something as basic as the amino acid sequence of an enzyme.

It is in these small variations between species where alcohol dehydrogenase gains an interesting function in the yeast species. To begin with, the chicken contains only one known type of alcohol dehydrogenase, while humans have three classes of the enzyme and yeast has five types. Clearly, the size of the organism is not a predictor of the enzyme's abundance. The different capabilities of each organism, however, predict the necessity for different types of alcohol dehydrogenases, even within the same organism. While chicken and human liver enzyme break down ethanol into acetaldehyde using

NAD^+ and zinc as cofactors—which might sound boring when compared to bacteria and yeast, but is extremely necessary—many yeast species have the same enzyme but it is able to carry out a reverse-type reaction as well, and produce alcohol through a process called fermentation. The acetaldehyde that is converted from pyruvate in glycolysis in bacteria is converted to ethanol to complete the fermentation process.[5] *Saccharomyces cerevisiae* is the most common type of prokaryote used in the beer brewing process. The only reason for this is the presence of the yeast's own type of alcohol dehydrogenase. As shown above using the Clustal W Multiple Sequence Alignment, the sequences belonging to the chicken and the two bacterial species (*Shewanella putrefaciens* and *Pseudomonas fluorescens*) are comparable and show amino acid conservation when studied. Yeast and other bacterial alcohol dehydrogenases, however, contain an extra function that allows for the conversion of sugars to alcohol, a pseudo-reverse mechanism of the general alcohol dehydrogenase mechanism of many other organisms. It is no surprise, then, to know that bacterial and yeast enzymes contain four, rather than two, identical subunits and require two zinc ions per subunit in order to function properly. The point in the brewing process once the malt is created requires the addition of either *Saccharomyces cerevisiae* or some other bacteria capable of this last step in the fermentation process. Ethanol, the common alcohol found in all alcoholic drinks, is the product of this alcohol dehydrogenase reaction.[5] Expectedly, however, the yeast and bacteria can also carry out the "normal" alcohol dehydrogenase reaction, as alcohols present in these organisms can be toxic at certain levels as well.

As illustrated above, alcohol dehydrogenase is a very handy and very necessary enzyme for a variety of organisms. The chicken liver alcohol dehydrogenase to which the

original twenty amino acid sequence belonged proved to be an enzyme that simply converts toxic alcohols into less toxic aldehydes, using NAD^+ and zinc ions as cofactors. This function is comparable to the enzyme's function in other organisms such as humans, chimpanzees, and horses, to name a few. More notable differences exist between the chicken liver alcohol dehydrogenase and that of bacteria and yeast—specifically, an important functionality that creates the same compound which is broken down by the human, chicken and horse versions of the same enzyme. Consequently, alcohol dehydrogenase clearly illustrates the uniformity, yet natural variation in enzyme functionality among a wide range of organisms.

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