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Life by chance? Studies on folate co-enzymes add weight to that impossibility

Ian Macreadie

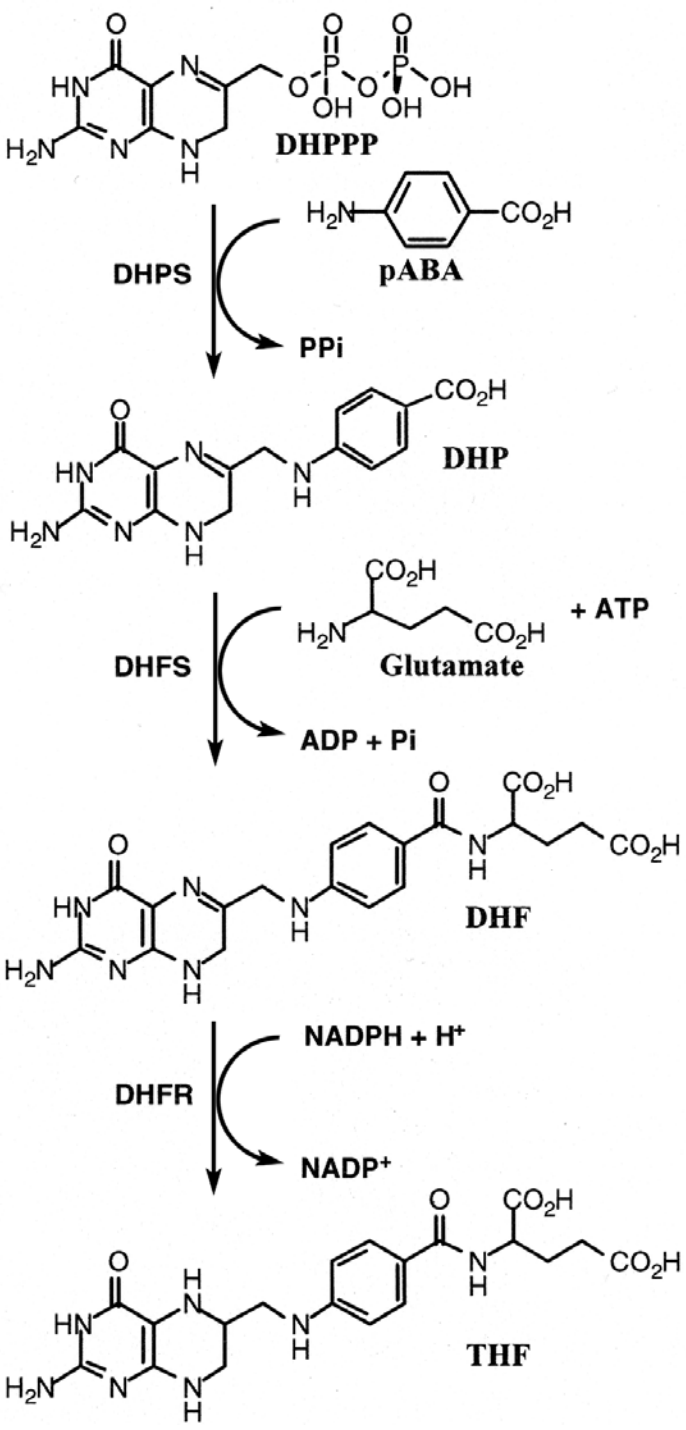
Life depends on folate

It is evident from our knowledge of biochemistry and molecular biology that there is a universal requirement for folates. No wild organisms survive without folate; thus there is no evidence of any organism that started without folate dependence or has attained an ability to dispense with folate. All life, from carnivores to plants to bacteria, has this dependence. In humans, their absolute importance is noted by health authorities[1] which have issued notification of a recommended daily allowance (RDA). For pregnant women, the RDA is increased to account for the growth requirement of the developing embryo. Modern diets which frequently lack the vegetables that are high in folate are under this level, so producers of processed foods, such as breakfast cereals, provide additional folic acid to supplement their products. This supplementation is supported by the National Health and Medical Research Council of Australia recommendations.[2] With insufficient folate supplementation, there can be disastrous consequences for a child developing *in utero*. Most notable are neural tube defects such as *spina bifida*.

What are the roles of folates? Folate, also known as vitamin B9, is actually a complex collection of molecules that do not occur by chance. Folates are coenzymes that are required by apoenzymes (proteins) to make an enzyme. Enzymes play a critical role in the chemical reactions that take place in life. Without enzymes, there is no known way that biochemical reactions could occur. Their purpose is to lower the free energy required to produce a product or an intermediate in the reaction. The way in which enzymes perform such processes is extremely complex. For example, the process to simply reduce dihydrofolate (DHF) to tetrahydrofolate (THF) requires the addition of two hydrogens to DHF (see figure). This cannot happen spontaneously, so all cells have the enzyme dihydrofolate reductase (DHFR) to carry out this reaction. Some of the complexity of the reaction can be see in a web movie.[3] For this reaction to occur, DHFR itself requires the coenzyme nicotinamide adenine dinucleotide phosphate (NADPH), which becomes oxidised in the reaction to make THF. The oxidised $NADP^+$ can be recycled by the cell to regenerate NADPH.

Folates are cofactors (or coenzymes) in numerous reactions leading to the synthesis of numerous amino acids for protein synthesis and purines for DNA synthesis, indicating just how essential folates are. These folate cofactors comprise several specific variations of a basic structure. The variations include THF, formyl THF, formino THF, methyl THF and methylene THF, and each are co-factors of a specific enzyme. In addition, because of the inherent instability of these compounds, they invariably have several glutamate residues added to them so they become impermeable to the cell membranes.[4] Thus if they are required to cross a membrane to be taken up by a cell or to be used within a sub-cellular compartment, the poly-glutamate has to be removed and then added again after the membrane has been crossed.[5] This is an essential process, and a specific enzyme is required to detach the glutamates, while another enzyme is required to add them on.

Fast growing cells have very high requirements for folates, and without them, cell growth does not occur. How so many cell types from such a range of organisms all depend on the same folate cofactors for these reactions is a testament to the fact that no intermediate life forms occur. It is easier to accept that all life was made to utilize folate in the same way.



The biochemical pathway leading to the production of tetrahydrofolate (THF). From the top: the first reaction involves the condensation of dihydropterin pyrophosphate (DHPPP) with p-amino benzoate (pABA), catalysed by dihydropteroate synthase (DHPS). The reaction produces dihydropteroate (DHP) and releases pyrophosphate (PPi). Dihydrofolate synthase (DHFS) then aids the addition of glutamate to the DHP formed from the preceding reaction. That reaction requires energy from a high-energy phosphate bond in adenosine triphosphate (ATP). The product, dihydrofolate (DHF) is then reduced by dihydrofolate reductase (DHFR) to produce THF and numerous derivates (not shown) that are the co-factors for several reactions in all cells. The reduction of DHF requires proteins and a coenzyme, reduced nicotinamide adenine dinucleotide phosphate (NADPH) which becomes oxidized to $NADP^+$. The enzymes DHPS, DHFS and DHFR are far more complex than the other chemicals shown here. In size they are two orders of magnitude larger.

The origin of folates

A further element of complexity arises from the origin of folates. Folates are complex molecules that do not occur naturally. Their source is biochemical synthesis arising from pathways that exist in plants[6] and many microbial cells, but not in animals. The total absence of these pathways in animals provides an interesting example of how dependent we are upon other life forms for our survival. We might be 'evolutionarily fitter' if we could synthesize our own folate, but there is no evidence that we ever will, or that we have ever done so in the past.

Now that we have a complete knowledge of our genetic blueprint, it is relatively easy to consider questions regarding what old or new emerging genes may be present in an organism. One has to be cautious about such analyses, since it is not usually possible to conduct an experiment to test ideas. However, in the case of the folate synthesis pathway, it is clear that genes for enzymes such as a dihydropteroate synthase are absent from genomes of animals. If animals evolved (ultimately) from microbes, one might wonder why they lost the ability to synthesize folates, making themselves wholly dependent on external sources.

The uniqueness of folate synthesis to microbes (and plants) presents a major opportunity for therapeutic intervention in the treatment of microbial infections. Some of the biggest breakthroughs in the treatment of infectious diseases have been achieved by targeting folate synthesis through the use of a class of antibiotics known as sulfa drugs, as discussed below. Similarly, folate utilization in microorganisms has also been the target of antibiotics. However, as noted below, this pathway is also present in humans. Therefore, drugs selective for the infectious agent and not the host have to be chosen. Inhibition of folate utilization in humans is also frequently targeted in diseases with uncontrolled cell growth such as cancer, arthritis and psoriasis treatments.

Evolution and sulfa drug resistance

The closest we come to any 'evolution' is when existing genetic information transfers between species or mutates. A well-known example is the so-called 'evolution of drug resistance'. Molecular biology has now established the causes of this process. Drug resistance can occur due to something as simple as a point mutation in an existing gene. For example, in the pathway shown in the figure, resistance to sulfa drugs (which are analogs of *p*-amino benzoate (pABA)) may occur due to point mutations in the gene that encodes the enzyme dihydropteroate synthase (DHPS). Sulfa drugs are our oldest chemically synthesized antibiotics[7] and they are still in use today, being some of the last remaining affordable drugs for malaria treatment in Africa. They function by molecular mimicry of pABA (shown in figure).

Some forty years ago, it was found that they compete with pABA causing depletion of folate and reduction of growth.[8] In malarial sulfa drug resistance, it has been shown that mutations lead to a change in DHPS that causes an alteration in the competition between the pABA and sulfa drug. The change to DHPS means that the enzyme has less affinity for the sulfa drug compared to pABA.[9] Mutations leading to sulfa drug resistance do not result in an improved species (although resistance does confer an advantage in the presence of a drug).

It is interesting that despite the long history of the sulfa drugs and quite a substantial understanding of how they work, we continue to discover new aspects to their action. For example, recent work in my laboratory[10] has shown that sulfa drugs exert an additional effect. Sulfa drugs are metabolised to a sulfa-containing folate analog that competes with DHF and this also leads to reduced growth of the microbe. Such a discovery will obviously lead to further new insights into drug resistance.

As another example from the figure, folate analogs inhibit the essential enzyme DHFR by binding to it and blocking its action. Resistance can again arise through point mutations that disrupt that binding of the drug to DHFR. Alternatively, mutations or gene amplification, resulting in many copies of the gene per cell, may increase the amounts of cellular DHFR, and thus increase drug resistance. No new information gets added in this process, so it does not represent an example that evolutionists desperately require. The evolutionists would agree with my point here, but they would argue that the explanation is because the process of evolution and hence the generation of new information takes too long to measure. That means we cannot test their model, so the concept of life by chance remains a theory that is weak and not supported by any legitimate example.

As a final example, DHFR is also used as a selectable marker in recombinant DNA technology. Thus cell lines can be transformed with a foreign DHFR gene and transformants containing multiple copies of the DHFR can be selected because they now have resistance to the drug methotrexate. Although this provides an example of inter-species gene transfer it still utilizes information that was pre-existing. It is not clear whether DHFR gene transfer may also occur in the wild. However, there are numerous examples of where horizontal gene transfer between microbial species appears to be a mechanism for microbial 'evolution'.[11] Such gene transfer has led to major problems in the spread of multiple-drug resistance in bacteria. However, these examples do not provide support for the evolution of the genetic information: in all cases preexisting sequences are utilised.

Conclusion

This review gives a small glimpse of recent breakthroughs in our understandings of the biochemistry and molecular biology of folates. It also points to life being intricately complex and demonstrates one aspect of how much animals can depend on plants for their nutrition. Further, it addresses a central tenet of molecular biology, which teaches that the genetic blueprint and its direction of the production of proteins which are necessary to replicate the blue print, belong to a closed system that can be viewed as self-sustaining. In addition, the irreducible complexity of folate synthesis and utilization pathway defies Neo-Darwinian story telling. Knowledge of the basis of antibiotic resistance provides no evidence for an upward progression in genetic information as required by the evolutionary scenario for microbe to man transition.

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