Deciphering Functions of Intracellular Formaldehyde - Linking Cancer and Aldehyde Metabolism

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Formaldehyde, the simplest aldehyde, is an environmental pollutant and human toxin. Acute exposure to exogenous formaldehyde can cause irritation, nausea, renal failure, cardiac arrhythmia, and coma. Chronic formaldehyde exposure correlates with increased cancer incidence, in particular of nasopharyngeal cancer and leukaemia. In addition to exogenous sources, formaldehyde is produced endogenously in cells; eukaryotic pathways producing formaldehyde include xenobiotic metabolism and enzyme-catalysed *N*-methyl demethylation of the *N*-methylated histone and DNA components of chromatin, as well as of RNA. Thus, endogenously produced formaldehyde may have biological roles; there have been very few studies to understand such functions at the biochemical level and even fewer connecting the biochemistry of formaldehyde with physiology.

The carbonyl group of formaldehyde is electrophilic, reacting efficiently with nucleophiles, including water; in aqueous solution, formaldehyde is predominantly in its hydrated form, ($H_2C(OH)_2$). It has long been known that reactions of formaldehyde with biologically relevant nucleophiles have potential for complexity, such as the formation of oligomeric, cyclised, and disproportionated products, including via Mannich and Cannizzaro type reactions.[1, 2] Many, but not all, of formaldehyde's reactions, including hydration, are readily reversible with the degree of reversibility depending on the nucleophile, conditions, and nature of the reaction product.

Formaldehyde's toxicity is proposed to result from its reaction with nucleophiles in cells, i.e. with nucleic acid bases and nucleophilic amino acid side-chains (such as the cysteinyl thiol and lysyl N^{ε}-amino groups). Reactions with small molecules are important in formaldehyde metabolism and may be relevant to its pathophysiological effects. Formaldehyde detoxification involves its reaction with nucleophilic thiols: in plants, animals, and some bacteria,

formaldehyde reacts with glutathione to give *S*-hydroxymethylglutathione, which is the substrate for an NAD-dependent dehydrogenase (ADH5 in humans). The product, *S*-formylglutathione, is then subjected to enzymatic hydrolysis, giving formate, which is less reactive than formaldehyde, and recycling glutathione (Figure 1). A similar pathway in mycobacteria uses mycothiol instead of glutathione.

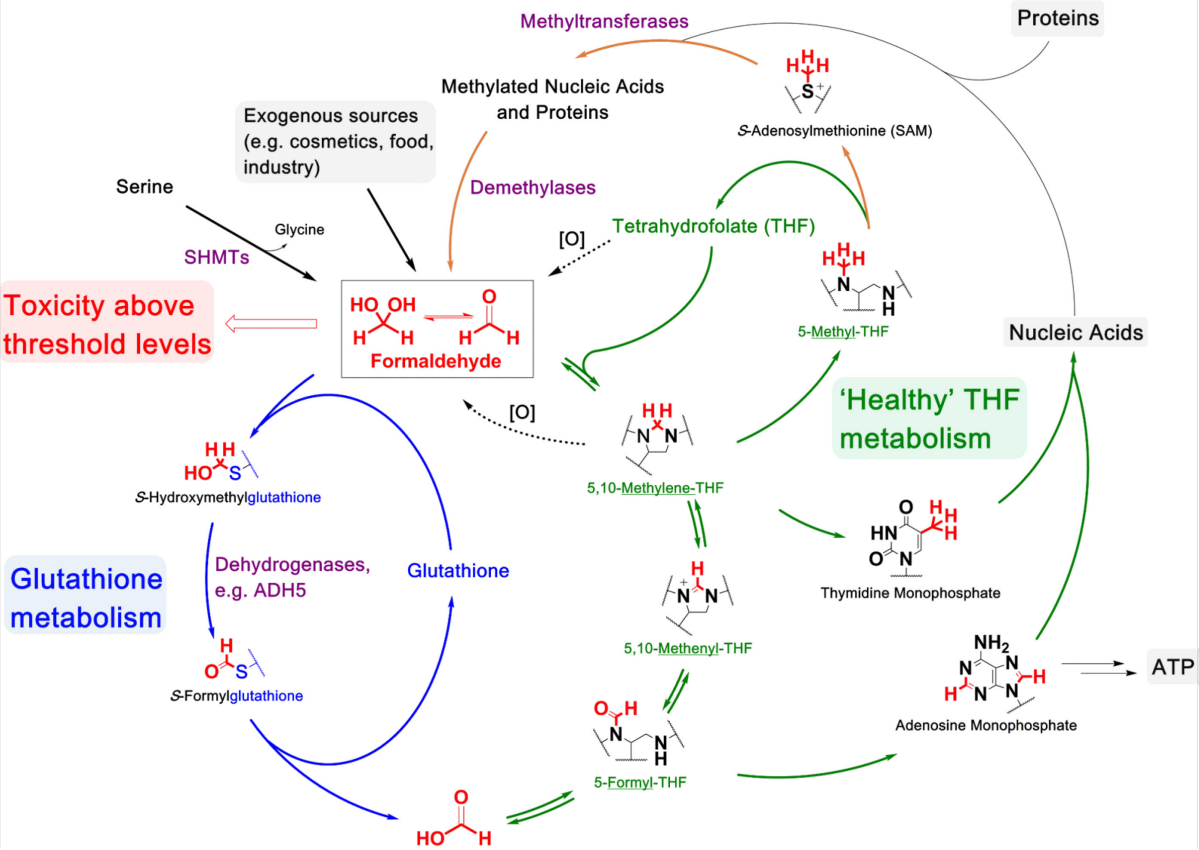

Figure 1. Recent work has shown how formaldehyde links the glutathione and tetrahydrofolate (THF) 'metabolic systems' (only selected elements of these are shown). HCHO is toxic above threshold levels and is detoxified via its reaction with the cysteinyl thiol of glutathione. HCHO can also act as a C1 precursor for THF-mediated metabolism. THF and some of its derivatives are susceptible to oxidation, releasing formaldehyde (dashed arrows). SHMT, serine hydroxymethyltransferase; ADH5, alcohol dehydrogenase 5.

Insights into the mechanisms by which elevated levels of formaldehyde and acetaldehyde cause toxicity have emerged from ongoing studies from K.J. Patel and co-workers concerning the origins of Fanconi Anaemia (FA). FA is a rare genetic disorder leading to an increased cancer risk, which is characterised by mutations causing deficiencies in a specific DNA

damage repair pathway. In the breakthrough work, robust evidence linking the FA-associated DNA repair pathway and impaired aldehyde metabolism has been accrued. The research demonstrates that a functional glutathione-dependent formaldehyde metabolism pathway is required for survival of FA models (Rosado *et al*).[3] The observations suggest that elevated levels of endogenous formaldehyde lead to DNA damage, which is not tolerated in FA repair-deficient cells. The initial work has been supported by subsequent studies with ADH5-deficient FA mice (Pontel *et al*),[4] which manifest toxic phenotypes including kidney failure, bone marrow dysfunction, and liver cancer. The same symptoms are observed in human DNA cross-link repair deficient syndromes; DNA cross-linking is induced by drugs such as cis-platin and electrophiles, notably including formaldehyde.

The identification of links between FA and aldehydes stimulated efforts to identify physiologically relevant sources of cellular formaldehyde in mammalian cells (Burgos-Barragan *et al*).[5] Given the relatively high levels of formaldehyde in blood (20-100μm), the Patel group hypothesised that the ubiquitous folate-utilising C1 metabolic cycle, which is crucial in nucleotide/nucleic acid and amino acid/protein biosynthesis (Figure 1), is a source of endogenous formaldehyde.

5,10-Methylenetetrahydrofolate, which is formed by reaction of tetrahydrofolate (THF) with formaldehyde, fragments to give formaldehyde and tetrahydrofolate (THF); it was initially proposed that addition of THF to cells would sequester formaldehyde and so reduce its concentration. However, it was observed that addition of THF was cytotoxic, especially to cells deficient in ADH5 and in FA models.

Sensitivity to added THF was tested in cells deficient in both ADH5 and C1 metabolism genes, including serine hydroxymethyltransferases 1/2 (which enable formation of 5,10-methylenetetrahydrofolate), mitochondrial folate transporter (which regulates mitochondrial THF levels), and folylpolyglutamate synthase (which is crucially involved in folate homeostasis). Diminished C1 metabolism is toxic; however, supplementation with glycine, hypoxanthine, or thymidine enables cell growth. All the tested cells types were similarly THF sensitive, suggesting that THF toxicity is independent of 'direct' C1 metabolism. It was also noted that deletion of ADH5 exacerbated the growth defect in the C1 metabolism-deficient cells without supplementation.

Studies on the oxidative decomposition of THF (and derivatives) reveal it reacts to release formaldehyde, the production of which is promoted or inhibited by hydrogen peroxide or ascorbate, respectively. Assessment of the toxicity of THF derivatives, folate, dihydrofolate, 5-methyltetrahydrofolate and 5-formyltetrahydrofolate, using C1 metabolism/ADH5-deficient

cell lines implies that only the derivatives susceptible to oxidative formaldehyde release are cytotoxic.

Whilst the folate derivative associated toxicity appears to be principally due to formaldehyde release, it was observed that addition of either formaldehyde or THF rescued growth in C1 metabolism-deficient cells (when not appropriately supplemented). The THF rescue effect was dependent on the presence of active ADH5, implying a role for the glutathione-dependent metabolism. Mass spectrometric analysis of cells treated with ^{13}C-formaldehyde revealed that a significant proportion of cellular formate is derived from ADH5-dependent oxidation of formaldehyde (blood formate levels are decreased in ADH5-deficient mice). Interestingly, incorporation of ^{13}C into deoxyadenosine, adenosine triphosphate, and thymidine was observed. Incorporation of the ^{13}C label into the nucleotides was most prevalent in C1 metabolism-deficient cells and inhibited by loss of ADH5; though some incorporation was observed in ADH5-deficient cells. These observations define ADH5-dependent links between formaldehyde metabolism and the C1 metabolic cycle – as well as being toxic, formaldehyde can act as a C1 precursor, including to mediate nucleotide synthesis (via formate). Upregulating formaldehyde levels (e.g. by drug or diet therapy) in combination with ADH5 inhibition may be a way to selectively target cancer cells with defective DNA repair pathways, such as in BRCA mutant cancers.

The Patel group's work has thus identified *physiologically relevant* links between formaldehyde metabolism and DNA damage repair / genotoxicity. Formaldehyde can act as both a genotoxin and as a benign metabolically relevant C1 metabolite. The balance between these roles is presumably altered by context-dependent factors, including concentration, localisation, pH, and, as hinted at in the work, redox state. The findings provide a mechanistic rationale for formaldehyde genotoxicity, and reveal the potential of altered levels of formaldehyde, and other aldehydes including acetaldehyde, to regulate multiple cell functions in health and disease. Folate is widely used as dietary supplement because its deficiency causes birth and neural defects. This, coupled with the widespread societal use of the alcohol precursors of aldehydes, and of formaldehyde/formaldehyde derivatives, means the results may have implications far beyond academic work on genetic diseases.

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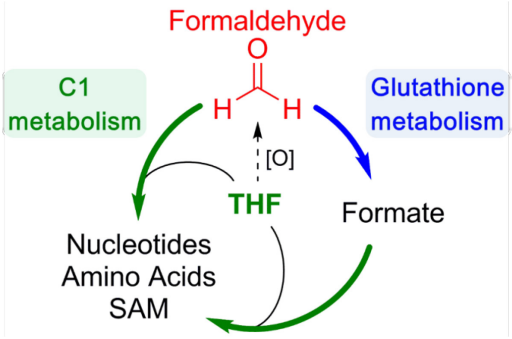

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