Glyphosate
An Enzymologist's Perspective

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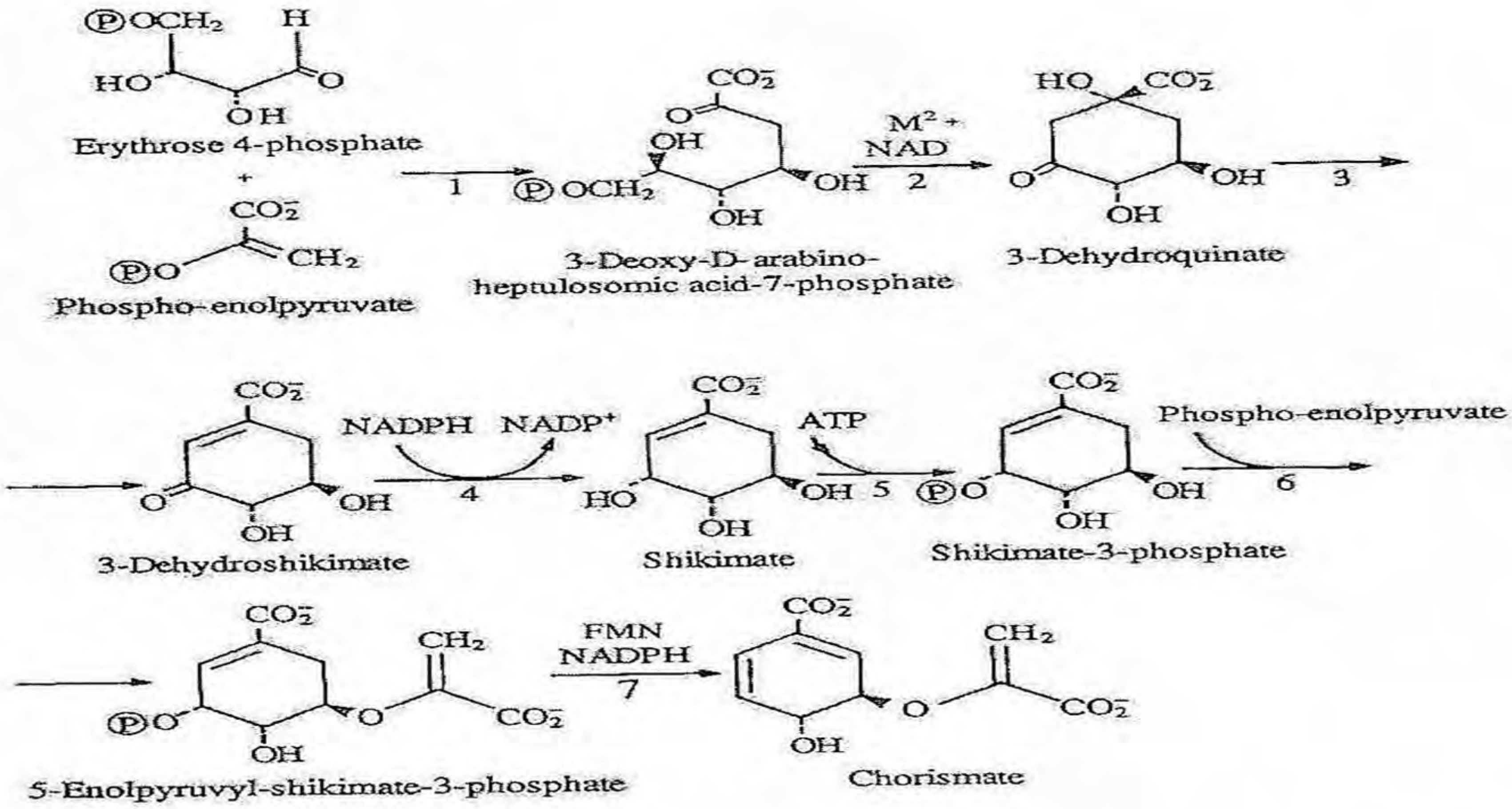
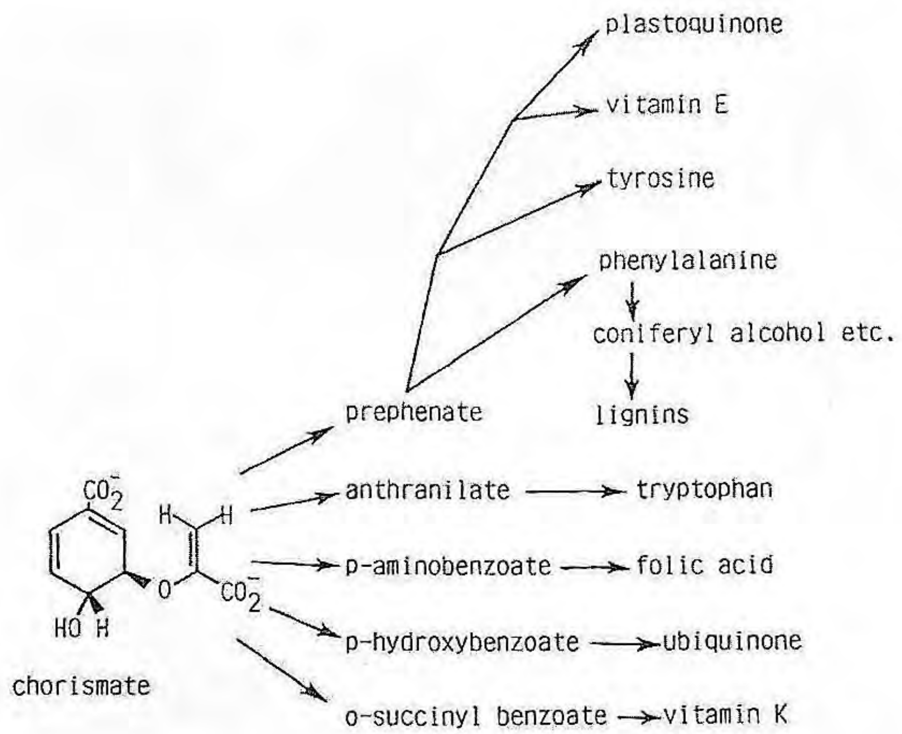
Early History

- **John Franz discovered the herbicidal properties of Glyphosate in 1970**
- **It was patented and brought to the market place as a broad spectrum, post-emergence herbicide by Monsanto in 1974 under the trade name Roundup**
- **The compound emerged from studies of potential water-softening agents - it was not a rationally designed herbicide**
- **Glyphosate acts by inhibiting the shikimate pathway which is used by plants to synthesise the 3 essential aromatic amino acids phenylalanine, tyrosine and tryptophan and a host of other important aromatic compounds**

The Shikimate Pathway



The Products of the Shikimate Pathway in Plants



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Glyphosate – Biological Context

- **The shikimate pathway is essential for bacteria and fungi as well as plants**
- **Besides its herbicidal activity glyphosate has significant antimicrobial activity**
- **Many soil microorganisms can degrade glyphosate into harmless metabolites so that it does not persist in the soil**
- **The shikimate pathway does not occur in vertebrates or invertebrates**
- **The enzymes of the shikimate pathway are mechanistically very similar in plants and microorganisms**

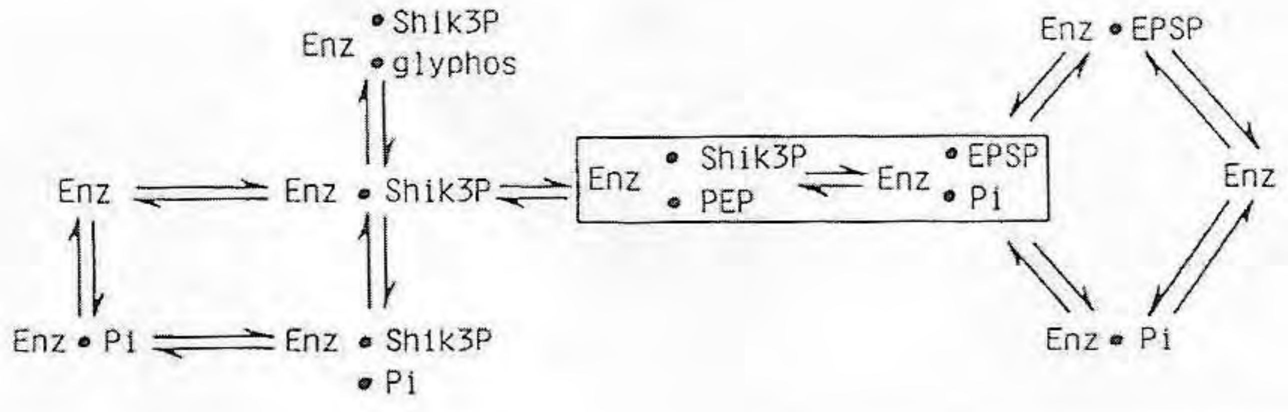
The Specific Site of Action of Glyphosate

- **In 1980 Hans Steinrucken & Nic Amrhein reported that glyphosate inhibited 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) the 6th enzyme of the shikimate pathway**
- **Their work was done on impure preparations of a bacterial enzyme**
- **At the time the plant enzyme was not available**
- **EPSPS had only been purified and characterised from the fungus *Neurospora crassa***
- **The first detailed kinetic study on the *N. crassa* enzyme by Martin Boocock revealed why glyphosate was such an effective inhibitor of EPSPS**
- **Later work by David Mousdale confirmed that the *Pisum sativum* enzyme had similar kinetic properties**

Glyphosate inhibition of EPSPS

- **The kinetic patterns for EPSPS inhibition by glyphosate indicate a compulsory order sequential mechanism in which either phosphoenolpyruvate or glyphosate bind to an enzyme:shikimate-3-phosphate complex**
- **This type of inhibition, involving a ternary dead end complex, explains the biological effectiveness of glyphosate**
- **In poisoned plants there is a build up of shikimate 3-phosphate and shikimate because the shikimate pathway is blocked**
- **The only way to compete out the inhibition would be through an elevated level of phosphoenolpyruvate; this is very unlikely to occur *in vivo* because of the multiple metabolic uses for phosphoenolpyruvate**

Kinetics of Inhibition of EPSPS by Glyphosate



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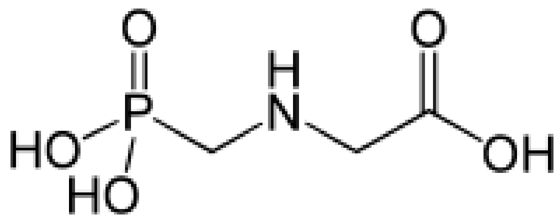
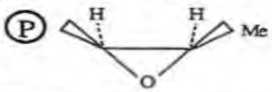
Glyphosate

N-(phosphonomethyl)glycine



- **A modified amino acid**
- **The neutral form of the molecule is shown**
- **In solution the phosphonate and carboxylate groups would be negatively charged and the nitrogen positively charged**
- **Not chemically reactive**
- **Biodegradable**

Glyphosate is an isolated Toxophore (Boocock 1983)

Structure	Trivial name	Estimated K_I'/K_M' (PEP)
Ⓟ $CH_2\ NH\ CH_2\ CO_2H$	glyphosate	0.32
Ⓟ $CH_2\ NH\ CH_3$		> 200
Ⓟ $CH_2\ NH_2$		> 30
$H_2N\ CH_2\ CO_2H$	glycine	> 100
$Me\ CO\ CO_2H$	pyruvate	> 100
$Me\ CH(OH)CO_2H$	L - lactate	> 100
Ⓟ $CH_2\ O\ CH_2\ CO_2H$		> 60
Ⓟ $CH_2\ S\ CH_2\ CO_2H$		> 180
Ⓟ $CH_2\ NH\ CO\ CCl_3$		> 50
$Me{-}PO(OH){-}CH_2\ NH\ CH_2\ CO_2H$		> 50
Ⓟ $CH_2\ N(Me)CH_2\ CO_2H$		> 90
Ⓟ $CH_2\ NH\ CH(Me)\ CO_2H$		> 150
Ⓟ $CH_2\ NH\ CH_2\ CH_2\ CO_2H$		> 50
Ⓟ $CH_2\ CH_2\ NH\ CH_2\ CO_2H$		> 120
(Ⓟ $CH_2)_2\ N\ CH_2\ CO_2H$	glyphosine	> 4.0
Ⓟ $CH_2\ CH(NH_2)\ CO_2H$		> 200
Ⓟ $O\ CH_2\ CH(NH_2)\ CO_2H$	phosphoserine	> 20
Ⓟ $CH_2\ CH_2\ CH(NH_2)\ CO_2H$		> 50
	fosfomycin	> 100

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Roundup Success Stimulated Herbicide Research

- The early success of Roundup greatly stimulated research not only on the enzymes of the shikimate pathway but also on the enzymes of other essential biosynthetic pathways in plants
- Many companies and their academic collaborators sought to rationally design better herbicides that blocked these biosynthetic pathways
- Initially research was hampered by both the lack of detailed knowledge of the potential target enzymes and the availability of the substrates
- Despite huge efforts over more than a decade which produced a great deal of knowledge of these enzymes no serious competitor for glyphosate resulted

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Roundup Ready Crops

- **While their competitors struggled in vain to produce a rival to glyphosate, the rapid advances in molecular biology during the 1980's encouraged Monsanto to modify crops to be resistant to the herbicide**
- **Their approach was to insert into the crop species a mutant form of EPSPS that was resistant to glyphosate**
- **Today "Roundup Ready" varieties of major crops such as soybean, maize and cotton are grown very widely in the Americas, Asia and Australia**

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Advantages/Disadvantages of Glyphosate

ADVANTAGES

- Very low mammalian toxicity
- Efficiently taken up and transported around the plant
- Degraded to harmless products by soil microorganisms
- Effective at low application rates and ideal for precision agriculture
- Facilitates low tillage cultivation

DISADVANTAGES

- Some weeds have become resistant to glyphosate
- The relative populations of soil organisms are perturbed when glyphosate is used persistently
- Seeds for the resistant crop plants cannot be saved by the farmer but have to be purchased each year

Conclusions

- A serendipitous discovery
- Proved to be an “ideal” enzyme inhibitor
- Has very low mammalian toxicity and is not persistent in the soil
- The development of engineered resistant crop species proved straight forward
- BUT like antibiotics herbicides must be used with care to avoid undesirable consequences for the environment – this may require restrictions on their overuse
- ALSO there are concerns that the agrochemical companies control access to the seeds for the resistant crop plants

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The Shikimate Pathway as a Target for Herbicides

John R. Coggins

In "*Herbicides and Plant Metabolism*"

A. D. Dodge (editor)

Society for Experimental Biology Seminar Series 38

Cambridge University Press (1989)

Gives a useful view of the field at the end of the 1980's

My research on glyphosate and the shikimate pathway enzymes was supported by the SERC (now the BBSRC) and by ICI Plant Protection (Jealott's Hill) now part of Syngenta