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Competitive electron transfers from a tyrosyl side-chain and peptide bond in the photodegradation of *N*-tosyl α-aminomethylamides: an insight into photosynthesis and photodamage in the biological oxidation of water?

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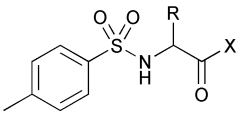
Photo-excited *N*-tosyl derivatives of phenylalanyl- and, more particularly, *O*-methyltyrosylmethylamides undergo electron transfer from aryl to tosyl groups whereas the photo-degradation of aliphatic analogues is initiated by electron transfer from the peptide bond, suggesting the latter as one possible reason for the rapid turnover of the D1 protein in biological water oxidation when the essential mediating role of tyrosine 116 in the PSII complex is inhibited.

The photochemical oxidation of water by green plants has been studied intensely for many years.[1] The structure of the protein-based complex (PSII) in the photosynthetic unit has now been uncovered and major features of the mechanism identified.[2]

An electron is transferred from a photoexcited chlorophyll pair (P680), relayed through a series of acceptors and used ultimately in the reduction of carbon dioxide. $P680^{\cdot+}$ is reduced by a nearby tyrosine residue in the D1 protein as the first of a sequence of electron and proton transfers that allows, ultimately, a molecule of dioxygen and four protons to be formed from two molecules of water. The tyrosine (Tyr 116) plays an essential role in an exquisite design that prevents an otherwise catastrophic photodegradation of the components. Even so, sacrificial breakdown and resynthesis of the surrounding D1 protein occurs continually, its lifetime in the illuminated leaf being typically 0.5 h.[3]

Several mechanisms of photodamage in PSII have been proposed,[4] including ones implicating the highly oxidising $P680^{\cdot+}$ (at 1.2V, perhaps the highest in biology)[5] which the design otherwise requires for the oxidation of water. But the process remains poorly understood.[1]

In a study of the photolysis of *N*-tosyl α-aminomethylamides (**1**) by product analysis, we have obtained results that are best explained by initial electron transfer from the peptide bond to excited tosyl group except for phenylalanyl (**2**) and methyltyrosyl (**3**) derivatives where part or all of the products derive from oxidation in the side-chain. Main-chain oxidation would seem thus to be an ever present competitor in protein photochemistry and may initiate one degradation route for the D1 protein.



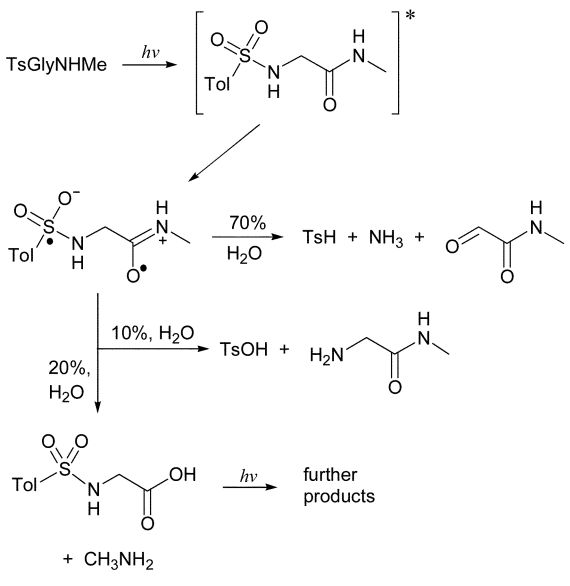
X	NHMe	OMe	OH
R	**1**		
$PhCH_2$	**2**	**7**	**8**
$MeOC_6H_4CH_2$	**3**		**6**
H	**4**		**5**

Photolyses were carried out under nitrogen with 10^{-2} mol dm^{-3} solutions of *N*-tosylaminomethylamides[6] in 60% aqueous acetonitrile in quartz tubes and using a carousel surrounding a 400 W medium pressure arc as previously described.[7] Products were identified by diagnostic chromatographic comparison with standards and quantified by HPLC[8] (aldehydes and ketones as stereoisomeric DNP derivatives, NH_3 and amines as AccQ.TagR® derivatives (by fluorescence detection)).

At 20% conversion, the deep yellow photolysate of TsGlyNHMe (**4**) afforded the following product distribution (%): NH_3, 70; TsH, 60; TsOH, 10; HGlyNHMe, 10; $MeNH_2$, 20; TsGlyOH, 14; MeCOCHO, 70. An interpretation of these results analogous to that already proposed for TsGlyOH (**5**) photolysis[9] provides a similar internally consistent explanation, where the degradation is initiated by intramolecular electron transfer from amide to tosyl groups (Scheme 1) and continued by plausible rearrangements and bond cleavages in the charge-separated intermediate (Schemes 2 and 3).

While results from the yellow photolysates of other aliphatic amino acid and dipeptide derivatives follow a similar pattern,[10] the product distribution in the colourless photolysate of Ts(Me)TyrNHMe (**3**) is dominated by MeNHCOCHO (> 90%), clearly not obtainable *via* a pathway analogous to Scheme 1 and requiring loss of the side-chain. We suggest that, in this case, transfer from the electron-rich methoxyphenyl group initiates the degradation, leading to side-chain cleavage (Scheme 4).

This is also observed with the corresponding acid (**6**) to a lesser extent (19% $OCHCO_2H$ at 30% degradation) and as a competitive pathway in the photolyses of *N*-tosylphenylalanyl methyl ester (**7**) and methyl amide (**2**) (4% $OCHCO_2Me$ and 20% OCHCONHMe, respectively, the latter also confirmed in this case by LC-MS),[11] but not the free acid (**8**). So the contribution of electron transfer from the side-chain changes consistently with changes in structure.



Scheme 1

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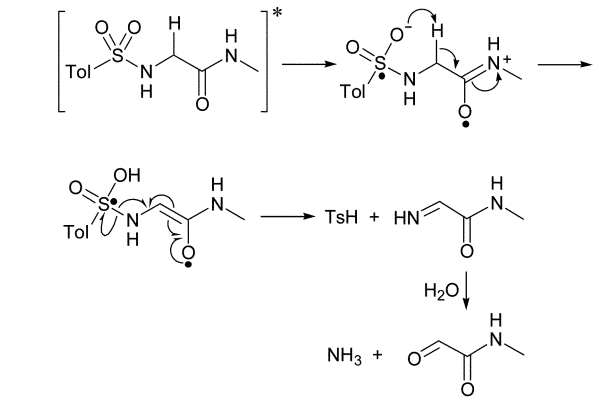
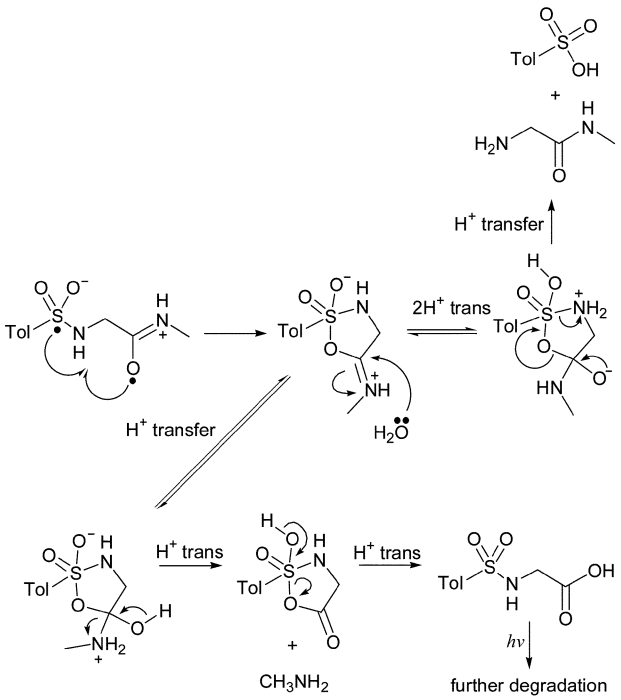
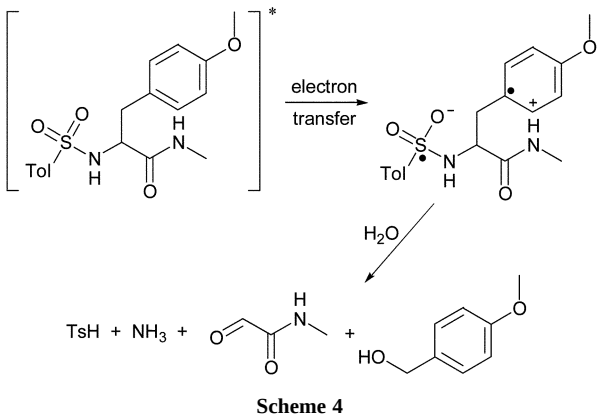


Scheme 2



Scheme 3



Scheme 4

Further observations in these experiments lend support to our proposals. The rates of photolysis were closely similar, indicating a common photoactive chromophore and benzyl alcohol was confirmed in the the photolysate of **2** by gas chromatography. Moreover, unlike those of the other methyl amides, the photolysate of **3** remains colourless while others become deep yellow or orange which, on the basis of other evidence, we have ascribed to the formation of a highly conjugated metastable photoisomer arising from charge separation in the main chain.[12]

The analogy we see in these results with the redox behaviour in the water oxidising complex of PSII assumes a similarity in the oxidising capabilities of $P680^{\cdot+}$, a product of complete photoinduced charge separation, and those of the photoexcited state of the tosyl chromophore, which are typical of the electronically excited states of ground-state acceptors.[13] Peptide bond oxidation dominates in the present series, the notable exception being a tyrosyl derivative, so may similarly play an important role in photodamage of protein within the PSII complex.[14]

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