On the Interaction of Flavins with Phosphine-derivatives

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Flavin-phosphine adducts, kinetics, light absorption, structure

The interaction of flavinium salts with triphenylphosphine is described. The complexes which can be isolated in high yields in the crystalline state contain one mole triphenylphosphine per mole flavin. Triphenylphosphine is covalently bound to the N(5)-atom of the isoalloxazine ring system. Dissociation constants and rate constants have been determined spectrophotometrically. Evidence for the structure of the complex was obtained from light absorption, infrared and nuclear magnetic resonance studies. The similarities between the flavin-triphenylphosphine adduct and the earlier described flavin-sulfite adduct are described and the possible biological relevance of these complexes is discussed.

The biochemists still depend largely on model studies for the postulation of structures of the intermediates observed with flavoprotein dependent reactions. Therefore, recently many scientists have become active in the field of flavin model studies and studies for the postulation of structures of the intermediates. However, many of these reactions are light catalyzed and, therefore, can not be directly correlated to the flavoprotein dependent biological reactions since most of them are dark reactions.

Recently, we described the interaction of free and some protein-bound flavins with a common substrate, namely sulfite. These studies revealed the interesting fact that the flavoprotein oxidases are capable to form a sulfite addition product, whereas the flavoprotein dehydrogenases do not interact with sulfite. In this context, the studies described below were undertaken to search for the possible interaction of flavins with other nucleophilic reagents.

Materials and Methods

The syntheses of the flavinium salts employed in this study were described elsewhere. The solvents and chemicals used were of analytical grade. The light absorption studies have been performed either on a Cary 16 or on a Durrum prism-grating recording spectrometer, both equipped with thermostated cell holders. Cells of 1 cm light path have been used.

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unsubstituted 1,5-dihydroflavin derivative as might be concluded from the light absorption spectrum of the product (Fig. 1) because N(5)-unsubstituted 1,5-dihydropflavins are extremely sensitive towards molecular oxygen. Moreover, the flavinphosphine complex formation is quantitatively reversible leading to its parent compounds, i.e. flavinium salt and triphenylphosphine oxide. This is demonstrated in Fig. 1. The spectral course of this reaction shows isosbestic points at 320, 287 and 248 nm. However, when more concentrated solutions (>10^{-2} M) of the complex were prepared and allowed to stand at room temperature a deep red colored intermediate was formed rather slowly. After several days, the solution was colored yellow. The light absorption spectrum of the reaction mixture showed that besides the oxidized flavin also triphenylphosphine oxide had been formed. This indicates that the flavin-phosphine adduct solution had been oxidized to the flavoquinonium level and that the phosphine molecule was still covalently attached to the flavin. The subsequent hydrolysis reaction yields then the uncomplexed flavin and triphenylphosphine oxide. No experiments under anaerobiosis have been done yet. This reaction is now under further investigation. This reaction has not been observed with the flavin-sulfite adduct.

The light absorption spectrum of a solution of crystalline flavin-adduct is given in Fig. 1. In contrast to the flavin-sulfite addition product which shows a well defined light absorption spectrum with a maximum in the 310–330 nm region, the flavin-phosphine adduct exhibits a rather diffused light absorption spectrum extending beyond 450 nm with shoulders at 328, 298 and 248 nm. In fact, this spectrum resembles very much that of the N(5)-unsubstituted 1,5-dihydroflavin derivative in aqueous solution. The light absorption characteristics of a few flavin-triphenylphosphine complexes are given in Table I.

The equilibrium constant of reaction (1) was determined spectrophotometrically for a few flavinium salts in acetonitrile solutions.

\[ K = \frac{[\text{flavin}][P(C_6H_5)_3]}{[\text{flavin-P}(C_6H_5)_2\text{-adduct}]} \]

The results are summarized in Table I. Similar results were obtained when the spectral data were treated according to BENESI and HILDEBRAND.

The rate of the formation of the flavin-triphenylphosphine complex was found to be of pseudo first order. When, e.g. 4 × 10^{-5} acetonitrile solution of the flavin derivative shown in eqn. (1) was reacted with 4 × 10^{-2} M, 2 × 10^{-2} M or 4 × 10^{-3} M triphenylphosphine in acetonitrile, plots of log(A − A_{\text{final}}) at 380 nm vs time were linear. The rate constants were, respectively, 1.03 min^{-1}, 0.51 min^{-1} and 0.10 min^{-1} at these triphenylphosphine concentrations. In Table I are also listed the second order rate constants of the triphenylphosphine complexes of various flavinium salts.
Table I. Some properties of various flavin-phosphine adducts.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>$\varepsilon$ (mM$^{-1} \cdot$ cm$^{-1}$)</th>
<th>$K_c$</th>
<th>$k_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>295 (sh) 277 (sh) 225</td>
<td>7.60 9.75 41.0</td>
<td>8.55 X 10$^{-5}$ M 40.9 M$^{-1}$ min$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>(C$_6$H$_5$)$_3$P + ClO$_4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH$_3$</td>
<td>328 298 (sh) 225</td>
<td>3.40 4.15 26.2</td>
<td>5.70 X 10$^{-4}$ M 25.6 M$^{-1}$ min$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>(C$_6$H$_5$)$_3$P + ClO$_4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH$_3$</td>
<td>325 295 225</td>
<td>4.78 5.55 35.8</td>
<td>1.38 X 10$^{-4}$ M 14.7 M$^{-1}$ min$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>(C$_6$H$_5$)$_3$P + ClO$_4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH$_3$</td>
<td>too instable to be measured</td>
<td></td>
<td>3.05 X 10$^{-3}$ M 8.50 M$^{-1}$ min$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>(C$_6$H$_5$)$_3$P + ClO$_4$</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

a All measurements were done with acetonitrile solutions at 25 °C. b sh = shoulder, c $K$ = dissociation constant, d $k$ = phosphine complex formation rate constant.

Support for the proposed structure of the flavin-triphenylphosphine complex shown in eqn. (1) comes from elemental analysis, infrared and nuclear magnetic resonance data obtained from the crystalline compound. Thus, the elemental analysis revealed that one mole triphenylphosphine is bound per mole flavin. The most characteristic absorptions of the infrared spectrum of the crystalline complex (using KBr pellet technique) are located at 3125, 2940, 1724, 1649 and 1096 cm$^{-1}$, respectively, and are assigned to NH(3), CO(4), CO(2) and ClO$_4^-$.

The corresponding absorptions of the parent compound of the triphenylphosphine complex appear at 3200, 3035, 1750, 1710 and 1100 cm$^{-1}$. The nuclear magnetic resonance spectrum of the complex in deuterated dimethylsulfoxide (internal standard was tetramethylsilane) exhibits the following absorptions at 7.71 and 7.26 ppm (15H, triphenyl residue), 6.73 and 6.23 ppm (2H, CH-aromatic), 4.68 ppm (2H, NCH$_2$ - 1), 4.12 ppm (2H, NCH$_2$ - 10), 2.03 ppm (3H, CH$_3$ - 8), 1.74 ppm (3H, CH$_3$ - 7). These results are in agreement with the postulated structure.

The above described results show that flavins exhibit a greater reactivity towards sulfite than towards triphenylphosphine, i.e. the neutral flavin molecule forms complexes with sulfite but not with triphenylphosphine. The protonated, respectively, the N-1-alkylated flavin species reacts about 10 times faster with sulfite than with triphenylphosphine. The lower reactivity of flavins towards triphenylphosphine is partly due to the bulkiness of the phosphine molecule.

In a previous paper, we have shown that a linear relationship exists between the oxidation-reduction potential of the flavin derivatives and their reactivity towards sulfite. A similar relationship exists probably also for the triphenylphosphine-adduct reaction. This relationship reflects the electron density at the flavin "active site", i.e. N(5), C(4a) double bound, where the covalent addition (nucleophilic attack) occurs. To explain the difference in reactivity of flavoproteins towards sulfite we have postulated that the flavoprotein oxidases possess a positively charged group in the vicinity of the prosthetic group, whereas the flavoprotein dehydrogenases possess a negatively charged group. Support for this idea comes from several papers where evidence is presented for anion binding sites of various flavoprotein oxidases such as...
D-amino acid oxidase, L-amino acid oxidase and glycollate oxidase.

The different reactivity of flavoprotein towards sulfite could be explained postulating for the flavoprotein oxidases, the positively charged group be located close to the N(1) atom which is the most basic center of the prosthetic group (cf. Scheme A, where X might be S or NH$_2$). The degree of the hydrogen bridge formation would thus be one among other factors determining the reactivity of the protein towards nucleophiles, e.g. coenzyme-substrate interaction. A possible interaction of the substrate with the N(5)-atom of the prosthetic group requires in plane interaction. Therefore, the positively charged group at N(1) of the prosthetic group is not identical with the substrate binding site, but it might well be involved in the binding of inhibitors. Thus, binding of e.g. benzoate to D-amino acid oxidase to the positively charged group placed close to N(1)-atom of the prosthetic group would allow a maximum of interaction between the $\pi$-system of the coenzyme and that of the inhibitor which manifests in the perturbation of the flavin light absorption spectrum. Studies concerning the possible interaction of flavoproteins with phosphine derivatives are now in progress.

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