Acid-Base Titrations of Schiff Bases in Deuteriomethanol as Studied by $^1$H NMR

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The Schiff bases of salicylaldehyde and pyridoxal 5'-phosphate with amino compounds were studied by $^1$H NMR in CD$_3$OD by using a glass electrode for the control of acidity. This study makes a new contribution for the elucidation of the structures of pyridoxylidene Schiff bases and gives additional data on the $^1$H NMR of the compounds that have not been available from the related studies in aqueous solutions.

KEY WORDS: Pyridoxal 5'-phosphate; salicylaldehyde; Schiff bases; amino acids.

1. INTRODUCTION

Pyridoxal 5'-phosphate (PLP) functions as the coenzyme in a large family of enzymes involved in the primary metabolic reactions of amino acids. The coenzyme is bound to the apoprotein through an $\varepsilon$-amino group of a lysine residue with a Schiff base linkage. In the course of the catalytic act this linkage is changed to a new one with the substrate in so-called transimination reaction (for a review, see Ref. 1). Various ionic and hydrophobic interactions keep PLP in a proper orientation at the active site of the enzymes. The protonation equilibria of the coenzyme substrate complex play a crucial role in the catalysis of PLP-dependent enzymes. The PLP reactions can be modelled in solution reactions by systems using PLP, substrate and buffer or metal ion catalysts and hence this group of enzymes is subject of an active investigation of enzyme mechanisms. A priori it is evident that the apoprotein moiety of PLP-enzymes provides a special ordered chemical milieu for the coenzyme and this milieu probably varies considerably.
from one enzyme to another.

The knowledge of the chemical structures of PLP Schiff bases inside the enzymes is usually based upon spectroscopic model studies in aqueous solutions. These structures are well documented.\(^4\) In aqueous solutions polar tautomers of the Schiff bases prevail while in less polar solvents different tautomers can be found.\(^4\) The complexity of the "solvent" for the Schiff bases inside the enzymes makes it evident that the model studies in aqueous solutions cannot alone describe all aspects of the enzyme catalysis.

The non-aqueous studies of PLP-Schiff bases have certain experimental advantages due to the fact that the Schiff base equilibrium involves water and therefore a high excess of amine must be used in aqueous studies.\(^7\) On the other hand, evaluation of the acidity may be more difficult in non-aqueous studies and the limited solubility of PLP Schiff bases may present difficulties. Different ionic forms have been studied by adding predetermined amounts of acid or base to the Schiff bases (see Ref. 5 and references therein) but these studies are somewhat problematic since the protolytic equilibria of the titrant should also be known in the solvent. We have approached the equilibria in organic solvents by measuring an operational pH (later termed pH*) with a glass electrode in spectrophotometric titrations of Schiff bases.\(^6\) The present study was conducted to achieve \(^1\)H NMR data of the compounds since in aqueous solutions they are limited in a quite narrow pH range. A related study by \(^31\)P NMR yielded useful data for assignment of the phosphorus resonances from PLP enzymes.\(^7\)

The \(^1\)H NMR studies of the Schiff bases have to be done in deuterated solvent. The glass electrode is rather reliable in methanol solution and, hence, CD\(_3\)OD is the solvent of choice. To the knowledge of the authors, the theory of pH (pD*) is not well developed in it. However, since quite close correlation of the titration behaviour of Schiff bases in CH\(_3\)OH and CD\(_3\)OD was observed here and since an operational property is considered we have utilized the same equation for CD\(_3\)OD solutions as developed for CH\(_3\)OH.\(^8\) The poor solubility of PLP Schiff bases in methanol restricted the selection of titratable compounds therefore more soluble Schiff bases of salicylaldehyde (SA) were studied.

2. MATERIALS AND METHODS

2.1. Chemicals

Tetradeuteromethanol and salicylaldehyde were purchased from