U22

Interaction between the Tyrosyl Free Radical and the Antiferromagnetic Iron Center in Ribonucleotide Reductase

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Ribonucleotide reductase catalyzes the reduction of ribonucleotides to their corresponding deoxyribonucleotides. The enzymes from *E. coli*, from *E. coli* after infection with bacteriophage T4, and from mouse fibroblast 3T6 cells have all been shown to be of similar type, containing a tyrosyl free radical essential for enzyme activity [1–5]. The free radical is situated in one of the two subunits of the enzyme, denoted B2 in the bacterial enzyme, β_2 in the T4-induced enzyme, and M2 in the mammalian enzyme. Proteins B2 and β_2 have in addition been shown to contain an antiferromagnetically coupled pair of high-spin ferric iron atoms exhibiting a temperature-dependent paramagnetism [4]. A similar iron pair stabilizing the M2 radical is postulated also for the mammalian enzyme.

The EPR spectra of the radicals at 77 K and below show slight differences between the various enzyme species. These differences have been ascribed to small differences in the angle of the aromatic ring in relation to the β methylene group of tyrosine [2]. The variations probably reflect differences in the polypeptide chain around the radical site. However, the differences observed are small and the major geometrical properties of the protein around the radical are obviously conserved, which may be important for the radical stability and enzyme function.

The temperature dependence of the EPR spectra of the radicals was studied from 10 K up to room temperature. With increasing temperature gradual changes were observed in spectral linewidth (broadening), signal amplitude (decrease), double integral of the spectrum, representing apparent signal intensity (decrease), and microwave saturation. The temperature dependences of these changes were significantly different for the radicals residing in proteins of different origins (B2, β_2 or M2). Taken together the results strongly indicate a significant interaction between the tyrosyl free radical and the temperature-dependent magnetic moment of the antiferromagnetically coupled iron pair. A tentative evaluation is presented using the dipolar coupling model proposed by Leigh for the EPR line shape in a system of two interacting spins [6]. This has to be combined with results of microwave saturation of the radicals showing that the product $T_1 \cdot T_2$ is smaller in M2 than in B2. The influence of a weaker antiferromagnetic coupling in the M2 iron pair and a shorter iron pair–radical distance will be discussed.

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U23

Influence of Solvent and Ligand-Structure on the Extent of Intramolecular Stacking Interactions in Mixed Ligand Complexes

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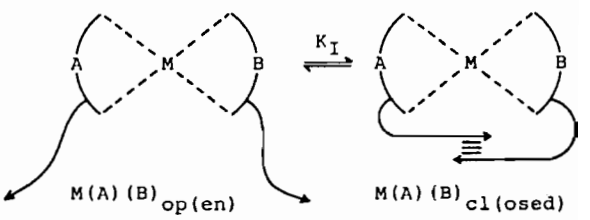
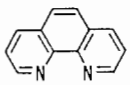
The importance of aromatic-ring stacking for the creation of certain structural arrangements in large bio-molecules has often been emphasized (*e.g.* [1]). However, the fact that at low concentrations stacking interactions between smaller molecules like amino acids and nucleotides can be promoted by the formation of a metal ion-bridge has only recently been recognized [2]. For example, the stacking between the indole moiety of tryptophanate (Trp^-) and the purine system of adenosine 5′-triphosphate (ATP^{4-}) is facilitated in ternary $M(ATP)(Trp)^{3-}$ complexes [3, 4]. Similarly, the hydrophobic interaction between the isopropyl moiety of leucinate (Leu^-) and the purine residue of ATP^{4-} is also promoted in $M(ATP)(Leu)^{3-}$ complexes [4]. Based on the stability constants of the complexes and ^{1}H-NMR shift experiments the percentage of $M(A)(B)^{3-}_{cl}$ was estimated for both types of ternary complexes:



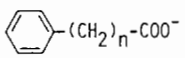
The formation of the species $M(ATP)(Trp)_{cl}^{3-}$ was first shown [5] in 1974; its occurrence was subsequently confirmed by studies in several laboratories using different methods [6].

To learn more about the factors which govern the position of the intramolecular equilibrium (1), we have now used the following simple systems, since with them structural alterations are easily achieved:



Phen

Cu^{2+}



n = 0 to 5

$R-(CH_2)_n-COO^-$

In 50% aqueous dioxane (I = 0.1, 25 °C) the stacking interaction is most pronounced for n = 1; *i.e.* for the ternary complex $Cu(Phen)(C_6H_5-CH_2-COO)^+$ about 60 percent exists in the closed form. If phenylacetate is replaced by 2-(β-naphthyl)acetate the concentration of the closed isomer increases to about 80 percent. Ligands $R-(CH_2)_n-COO^-$ with n = 0 or n > 1 form ternary complexes with a less pronounced intramolecular stacking interaction. Variation of the solvent composition also leads to a change in the percentage of $[Cu(Phen)(C_6H_5-CH_2-COO)^+]_{cl}$:

solvent	H_2O	30% diox.	60% diox.	90% diox.
% closed isomer:	48	56	64	48

The observation of a maximal degree of formation for the closed isomer in about 60% aqueous dioxane is very surprising, because the stability of binary adducts like $(Phen)(R-COO^-)$ decreases with increasing dioxane concentration. Hence, two opposite effects must be operating in the presence of metal ions. This puzzling result is of interest for biological systems, because in these the water activity may also be altered, *e.g.*, at the surface or in grooves of proteins. Hydrophobic interactions are important, *e.g.*, in adduct formation between carboxypeptidase A and the inhibitor β-phenylpropionate [7]. Presently we are repeating the experiments in several ethanol/water mixtures to provide a broader generalization.

Acknowledgement. Research supported by the Swiss National Science Foundation.

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U24

Intramolecular Ionic Interactions in Ternary Amino Acid Complexes [1]

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The so-called 'noncovalent' interactions [4] between biomolecules are not only crucial for the structural organization of high molecular weight biological systems, but also determine to a large extent the structure of amino acid and nucleotide containing low molecular weight metal ion complexes in solution. For example, the *intra*molecular equilibria between different isomers of mixed ligand complexes are well established for aromatic-ring stacking and hydrophobic interactions [3, 5, 6]. Ionic interactions between oppositely charged side-chains of two amino acids coordinated to the same metal ion are also known [7], but the extent of this interaction has so far hardly been characterized [6]. Therefore, the ionized forms of the following two amino acids were selected for such a study:

$(CH_3)_3\overset{+}{N}-CH_2-CH_2-CH_2-\underset{\underset{NH_2}{|}}{CH}-COO^-$

DL-δN-Trimethylornithine (=TMO)

$^-O_3S-CH_2-CH_2-\underset{\underset{NH_2}{|}}{CH}-COO^-$

DL-Homocysteic acid (=HC)

In the $(CH_3)_3\overset{+}{N}$-residue the positive charge is somewhat shielded, but the advantage of this residue is that no hydrogen bonds can be formed with ^-O_3S-, as would have been the case with the more common $H_3\overset{+}{N}$- group. In addition, the latter group and the use of a carboxylate group (instead of the sulfonate residue in HC) would have led to additional