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

M. SAHLIN, A. EHRENBERG, A. GRÄSLUND and L. PETERSSON

Department of Biophysics, University of Stockholm, Arrhenius Laboratory, S-106 91 Stockholm, Sweden

and B.-M. SJÖBERG and L. THELANDER

Department of Biochemistry, Karolinska Institute, Box 60400, S-104 01 Stockholm, Sweden

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

HELMUT SIGEL*, KURT H. SCHELLER, ULRICH K. HÄRING, and RAMAN MALINI-BALAKRISHNAN

Institute of Inorganic Chemistry, University of Basle, CH-4056 Basle, Switzerland

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 $M(A)(B)_{cl}^{3-}$ was estimated for both types of ternary complexes: