LOCUS OF THE CATALYTIC SITES OF UDP-GLUCOSE DEHYDROGENASE IN THE NATIVE ENZYME HEXAMER AS DELINEATED BY FLUORESCENCE ENERGY TRANSFER

James S. Franzen and Paul Marchetti, *Department of Biological Sciences, Faculty of Arts and Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260*

David S. Feingold, *Department of Microbiology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261 U.S.A.*

UDP-glucose dehydrogenase is comprised of six identical subunits arranged in a hexagonal manner. The half-sites reactivity behavior of the enzyme (Franzen et al., 1976) suggests that the array has 32 symmetry, i.e., it is a trimer of dimers. Since the catalytic site thiol groups are biphasically alkylated, one can prepare enzyme which has three catalytic sites covalently blocked with a fluorescent donor and three with an acceptor. Peptide mapping of tryptic digests of enzyme so modified shows that < 5% of the fluorophores are on noncatalytic site peptides. The AEDANS group has been used as a donor with either fluorescein, eosin, or nitrobenzoxadiazole as the acceptor. The extent of incorporation was determined from the radioactivity and/or optical absorbance of the modified protein. Energy transfer efficiencies were evaluated routinely from the size of donor fluorescence quenching, after it was certain that sensitized fluorescence data gave comparable results.

The observed transfer efficiency is an average of the efficiencies of transfer from each of the three donors to the acceptors on the same hexamer. To account for the collective transfers, it is first assumed that the individual subunits are spheres and that the catalytic sites are on their surfaces. If each sphere is likened to a geographical globe, and the hexagonal array of globes is assembled with 32 symmetry, the location of any surface point on one globe by angles of latitude (ϕ) and longitude (θ) fixes the like points on the other globes and determines all of the intersite distances. We further assume that the labeling of native enzyme with donor proceeds randomly with respect to which dimer in a trimer of dimers is modified, but that once one subunit of a dimer is modified, the other is refractory (half-of-the-sites reactivity). Therefore the fully modified enzyme, ED_3A_3, will be a 1:3 mix of the two forms drawn schematically in Fig. 1. Fig. 1 also defines the four possible donor acceptor distances. These distances are related to the angles ϕ and θ, for unit radius subunit spheres, by the equations $\alpha = 2\,[(1 - \cos(\theta + 60^\circ)\cos\phi)^2 + \sin^2\phi]^{1/2}$, $\beta = 2[(2 - \cos\theta\cos\phi)^2 + \sin^2\phi]^{1/2}$, $\delta = 2[(1 - \cos(\theta - 60^\circ)\cos\phi)^2 + \sin^2\phi]^{1/2}$, and $\gamma = [3\{4(1 - \cos\theta\cos\phi) + \cos^2\phi\}]^{1/2}$. Efficiencies were calculated for ED_3A_3 at 15° intervals of ϕ and θ by the equation, $E = 0.25\,(E_{\alpha\beta\gamma} + E_{\alpha\beta\delta} + E_{\alpha\gamma\delta} + E_{\alpha\delta\delta})$, where $E_{ijk} = [1 + \rho^{-6}(i^{-6} + j^{-6} + k^{-6})^{-1}]^{-1}$ and $\rho = R_0$/subunit radius. The subunit radius was estimated from its mass and density. R_0 was calculated for each donor acceptor pair from the donor labeled enzyme quantum yield, measured overlap integrals, and an orientation factor of 2/3. Inspection of the resulting ϕ -θ array of calculated efficiencies revealed which surface regions of the hexamer gave efficiencies within experimental error of the observed efficiency. From Fig. 2 one concludes that the catalytic sites are neither in the central region of the hexamer nor in the intersubunit bonding domains. They are most likely to be found on the periphery of the aggregate or alternate poles of adjacent subunits. This basic conclusion is not abrogated by the choice of reasonable R_0 values other than $R_0(2/3)$.

Received for publication 19 November 1979.

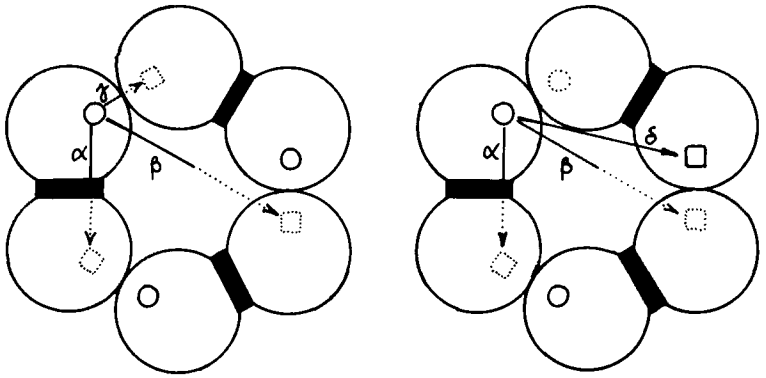

Figure 1 Schematic representation of catalytic site locations on UDPGDH hexamer having 32 symmetry: Form I (left), Form II (right). The intersubunit domains marked by black bars are those across which half-of-the-sites reactivity activity occurs. Circles indicate catalytic sites labeled with donors: squares are used for those with acceptors. Dotted lines or symbols represent positions below the central plane of the molecule. α, β, γ, and δ are the distances between one of the donors and the three acceptors. The position of the catalytic site in this drawing corresponds to the location $\phi = 45°$ and $\theta = 60°$ ($\phi = \theta = 0°$ defines a point on the surface of each sphere on the line of centers between the center of the sphere and the center of the array).

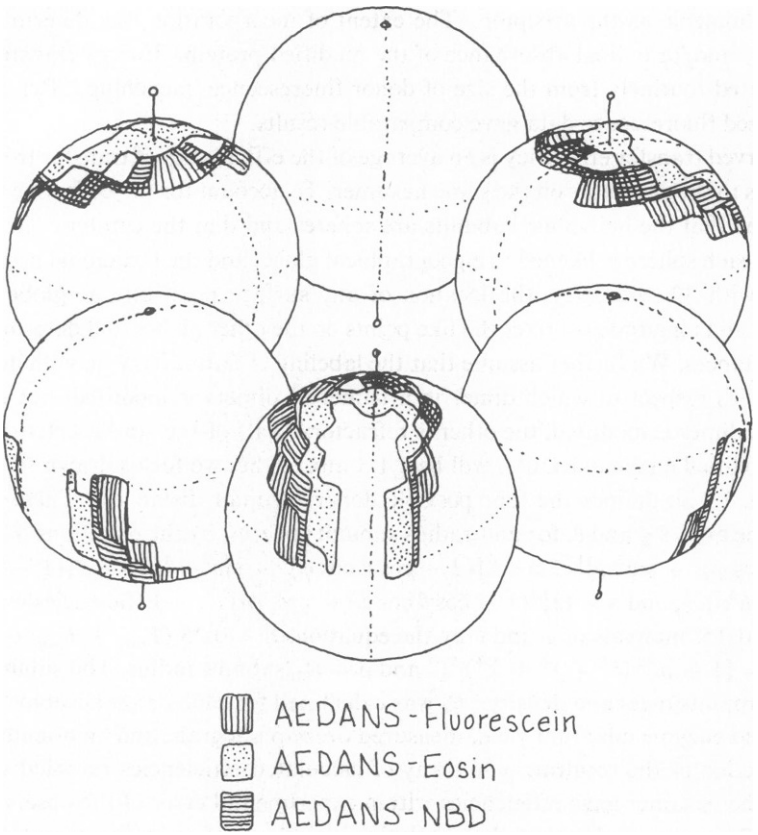

Figure 2 Probable location of catalytic sites of UDPGDH as determined by fluorescence energy transfer. The observed transfer efficiencies for the three donor-acceptor pairs of ED_3A_3 in which S-AEDANS served as the donor were 0.44 ± 0.05, 0.32 ± 0.03, and 0.32 ± 0.03 for fluorescein, eosin, and NBD as acceptors, respectively. The marked regions on the spheres correspond to areas where the calculated efficiencies fall in the range of the observed efficiencies.

REFERENCES

Franzen, J. S., R. Ishman, and D. S. Feingold. 1976. Half-of-the-sites reactivity of bovine liver uridine diphosphoglucose dehydrogenase toward iodoacetate and iodoacetamide. *Biochemistry.* **15**:5665–5671.

CHROMIUM (III)-NUCLEOTIDE COMPLEXES AS PARAMAGNETIC PROBES FOR CATALYTIC SITES OF PHOSPHORYL TRANSFER ENZYMES

Raj K. Gupta, *Institute for Cancer Research, Philadelphia, Pennsylvania 19111 U.S.A.*

Cr^{3+}-nucleotides, as exchange-inert, paramagnetic analogs of Mg^{2+}-nucleotides, are useful paramagnetic probes for nuclear relaxation studies to determine intersubstrate distances between phosphoryl donor and acceptor substrates on phosphoryl transfer enzymes (1–6) and, for enzymes with an additional metal ion site (7–9), to estimate the intermetal distance between the enzyme- and nucleotide-bound metal ions (10). A basic assumption of this approach, that Cr^{3+}-nucleotides bind at the Mg^{2+}-nucleotide sites in a structurally similar manner, can be tested by observing kinetic inhibition patterns in the presence of Cr^{3+}-nucleotides (2) and, where possible, by their ability to activate partial reactions (1, 2, 7) and to participate as substrates in phosphoryl transfer reactions (9, 11, 12).

Intersubstrate distances are determined from the magnitudes of paramagnetic effects of Cr^{3+}-nucleotides on the longitudinal nuclear relaxation rates ($1/T_1$) of various nuclei (^{13}C, ^{31}P, ^{1}H) of the non-nucleotidyl substrates in the ternary enzyme·substrate·Cr^{3+}-nucleotide complexes via the following equation (see, e.g., reference 13):

$$\frac{1}{T_{1M}} = \frac{2S\,(S+1)\,\gamma_I^2\,g^2\,\beta^2}{15r^6}\left(\frac{3\tau_{c1}}{1+\omega_I^2\,\tau_{c1}^2} + \frac{7\tau_{c2}}{1+\omega_s^2\,\tau_{c2}^2}\right). \tag{1}$$

For $\omega_s^2\tau_{c2}^2 \gg 1$, the first term in Eq. 1 predominates and the dipolar correlation time τ_{c1} may be obtained from magnetic field-dependence of $1/T_1$ of water protons in the same enzyme complex in the region of ω_I dispersion. This approach is valid since τ_{c1} for both substrate and water relaxations is dominated by τ_S^{Cr}, the electron spin relaxation time of Cr^{3+}. Distance calculations via Eq. 1 neglect electron delocalization and anisotropy in the magnetic moment of Cr^{3+}. They also require rapid exchange of substrates out of the paramagnetic environment and assume no outer sphere effects. The validity of these assumptions can be verified by observing magnetic field and temperature dependencies of paramagnetic effects, by comparing paramagnetic effects on $1/T_1$ and $1/T_2$, and by displacing substrates with analogs.

The intermetal distance may be estimated from the de-enhancement of the paramagnetic effect of enzyme-bound Mn^{2+} on $1/T_1$ of water protons caused by the presence of Cr^{3+} in the enzyme·Mn^{2+}·ATPCr^{3+} complex. This de-enhancement arises from a reduction in the τ_S of Mn^{2+} (τ_S^{Mn}) due to cross-relaxation of Mn^{2+} and Cr^{3+} electron magnetic moments. From the magnitude of extra relaxation of Mn^{2+} caused by Cr^{3+}, $\Delta(1/\tau_S^{Mn})$, the Cr^{3+}-Mn^{2+} distance may be estimated according to Eq. 2.

$$\Delta\left(1/\tau_S^{Mn}\right) = \frac{2}{15}\frac{S^{Cr}\,(S^{Cr}+1)\,\gamma_{Cr}^2\,\gamma_{Mn}^2\hbar^2}{r^6}\,\tau_S^{Cr}. \tag{2}$$