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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.

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

TABLE I

PARAMAGNETIC EFFECTS OF CHROMIUM (III)-NUCLEOTIDES

Complex	Nucleus	T^{-1}_{1M}	τ_S	Distance
		(s^{-1})	($\times 10^{10} s$)	(Å)
	^1H	218 ± 26		7.9 ± 0.5
PK·Mg^{2+}·ATPCr·Pyr	^{13}C (C = 0)	67 ± 7	1.5	6.1 ± 0.3
	^{13}C (-COO^-)	71 ± 11		6.1 ± 0.4
	^{31}P	510 ± 33		5.9 ± 0.4
PK·Mg^{2+}·ADPCr·PEP	^1H_D	326 ± 43	3.5	8.5 ± 0.6
	^1H_U	159 ± 93		9.6 ± 1.3
	^{31}P	490 ± 130		6.6 ± 0.7
HK·ADPCr·G6P	$^1H_\alpha$	440 ± 80	7	8.9 ± 0.8
	$^1H_\beta$	250 ± 75		9.7 ± 1.1
CK·ADPCr·P-creatine	^{31}P	700 ± 100	5	6.0 ± 0.5
AK·ATPCr·AMP	^{31}P	400	5	6.6 ± 0.6
PK·Mg^{2+}·ATPCr·PEP HK·ATPCr·G6P CK·ATPCr·P-creatine	^{31}P	≤25		

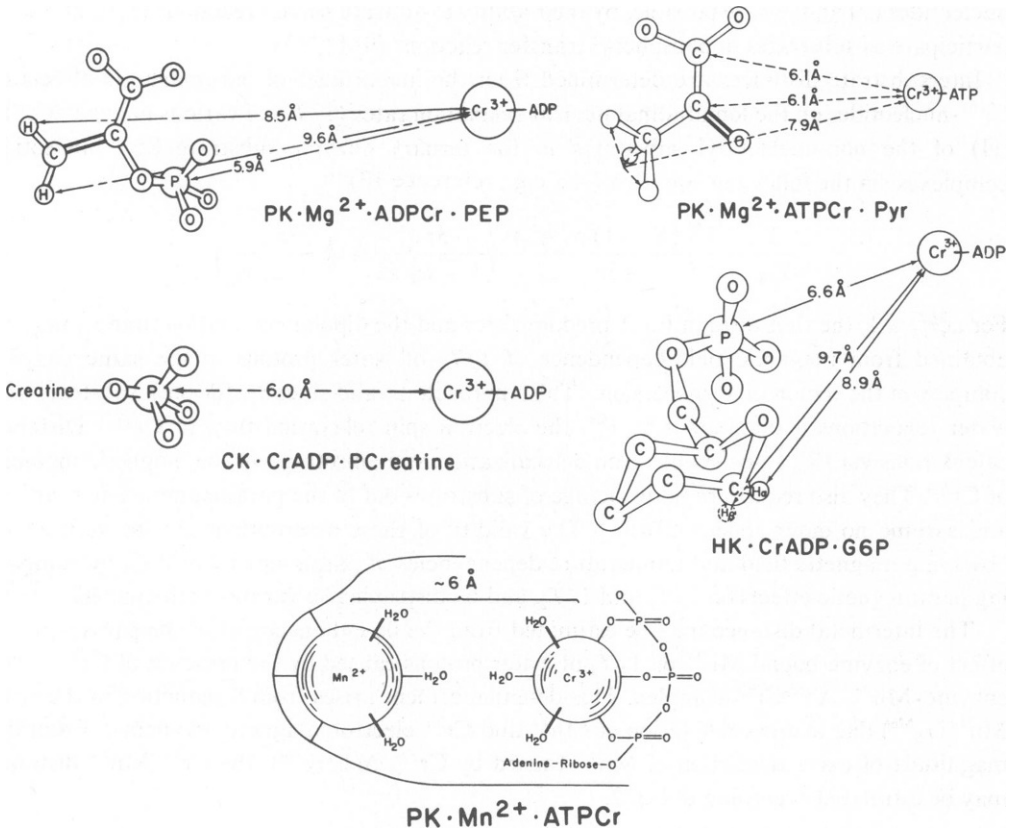

Figure 1 Intersubstrate and intermetal distances in several ternary enzyme·substrate·Cr^{3+}-nucleotide complexes.

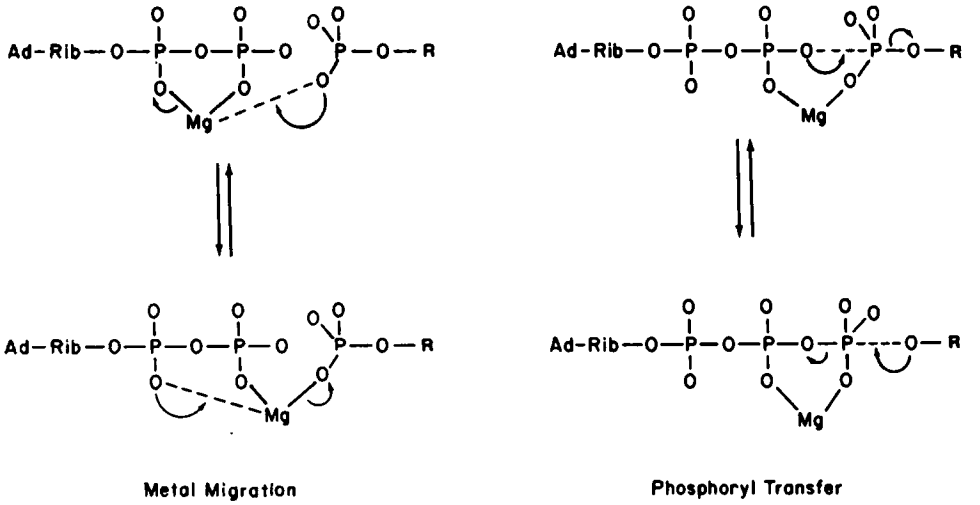

Figure 2 A stepwide mechanism for kinase reactions.

Application of the above techniques to pyruvate kinase (PK), hexokinase (HK), creatine kinase (CK), and adenylate kinase (AK) has yielded a wealth of new information towards elucidation of the structures of enzyme-substrate and enzyme-product complexes and of the role of metal ions in enzyme-catalyzed phosphoryl transfer reactions. The observed paramagnetic effects and the resulting intersubstrate and intermetal distances are summarized in Table I and Fig. 1. The intersubstrate distances are consistent with direct phosphoryl transfer from donor to acceptor molecules and indicate no need for intermediate phosphorylation of the enzyme for the kinases studied. The intermetal distance on PK reveals that both essential metal ions are located at the active site in close proximity, with their hydration spheres in molecular contact.

Kinetic studies indicate that CrADP is not a substrate for PK, HK, or CK, although it replaces MgADP at the active site (2–5). Cleland and co-workers (11, 12) have, however, shown that an isomer of β,γ-bidentate CrATP is a slow substrate for HK, CK, and AK, and we have shown substrate activity for the Δ-isomer of β,γ-bidentate CrATP in a single turnover on PK (9). The NMR-derived Cr^{3+}-nuclear distances and the results of kinetic studies indicate that coordination of the transferred phosphoryl group by the nucleotide-bound metal is essential for phosphoryl transfer. The phosphorus atom of the non-nucleotidyl phosphoryl donor substrate is 5.9–6.6 Å from the metal in CrADP on all kinases so far studied. Kinetic studies indicate that reversible coordination of the transferred phosphoryl group must precede phosphoryl transfer. Considerable metal-migration (3.3 ± 0.3 Å) from 6.3 ± 0.4 Å to an inner sphere distance of ~3.0 Å would, therefore, be necessary before phosphoryl transfer to ADP can occur or after phosphoryl transfer from ATP occurs. A stepwise mechanism for the catalysis of phosphoryl transfer is thus indicated by our NMR and kinetic data on kinases (Fig. 2).

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DIFFUSION-ENHANCED LANTHANIDE ENERGY TRANSFER STUDIES OF PROTEIN PROSTHETIC GROUPS

C. F. Meares, S. M. Yeh, and L. S. Rice, *Chemistry Department, University of Calfiornia, Davis, California 95616 U.S.A.*

A long-lived luminescent solute in aqueous solution (e.g., $^5D_4$ terbium, $\tau \simeq 10^{-3}$ s) can donate its excitation energy to a chromophore such as a protein prosthetic group by, e.g., the radiationless dipolar mechanism of Förster. However, in contrast to the usual energy-transfer experiment, a donor with a 10^{-3} s lifetime can diffuse extensively through the solution and, in a time scale short compared to its excited lifetime, sample all permitted locations with respect to chromophoric acceptors. As recently indicated by Thomas et al. (1) energy transfer in this rapid-diffusion limit can permit direct measurement of the allowed distance of closest approach of small solute molecules to chromophores which may be buried within proteins or membranes.

The rate of radiationless energy transfer by the dipolar mechanism from an excited donor to a stationary acceptor a distance, r, away is

$$k_T = \frac{1}{\tau_0}\left(\frac{R_0}{r}\right)^6 \text{s}^{-1}, \tag{1}$$

where τ_0 is the excited donor's lifetime in the absence of acceptor, and R_0 is the distance at which the rate of energy transfer equals the rate of donor de-excitation in the absence of acceptor (2). If the donor and/or acceptor diffuse significantly during the donor lifetime, Eq. 1 no longer holds (3). If diffusive motion is rapid enough so that $\sqrt{6D\tau} >> s$ (where the diffusion coefficient $D = D_d + D_a$, τ is the experimental lifetime of the donor, and s is the average separation between acceptors), then the energy acceptors are effectively uniformly distributed in space (1). In this rapid-diffusion limit, the rate of energy transfer by the dipolar mechanism from an excited donor to the surrounding acceptors is found by integrating Eq. 1 over all space which the acceptors are allowed to occupy with respect to a given donor. As