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Saturation transfer in living systems

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N.m.r. studies of living systems can be used to obtain kinetic rates *in vivo*, in addition to providing information about metabolite levels and their time dependences. This is possible through the use of magnetization transfer techniques, which rely on the fact that a nuclear spin will remain in a given quantum state for a period of the order of its spin lattice relaxation time, T_1, thus enabling perturbation of the nuclear spins in one molecular species and then observation of the transfer of that perturbation to another species, provided that the transfer takes place in a time of the order of T_1. These times are about 1s in most systems, thus allowing the measurement of rates in the range of $0.1\text{–}10^{-1}$ s by this method. These techniques were introduced by Forsen & Hoffman in a series of papers exploring their use in relatively simple chemical systems (Forsen & Hoffman 1963, 1964; Hoffman & Forsen 1969). An early biological application was to cytochrome c, by Gupta & Redfield (1970). The techniques have been applied to *Escherichia coli* to obtain an ATP synthesis rate (Brown *et al.* 1977) and to frog muscles and perfused rat hearts to obtain the exchange rates of ATP and phosphocreatine (PCr), this reaction being catalysed by creatine phosphokinase (CPK) in these systems (Brown *et al.* 1978).

Theory

To illustrate the effect in a simple system, consider the Bloch equations, modified to include chemical exchange (McConnell 1958), for the z components of the magnetization of two exchanging species, A and B:

$$\frac{\mathrm{d}}{\mathrm{d}t}M_z^{\mathrm{A}} = -\frac{M_z^{\mathrm{A}}-M_0^{\mathrm{A}}}{T_1^{\mathrm{A}}} - k_1 M_z^{\mathrm{A}} + k_2 M_z^{\mathrm{B}}$$

and

$$\frac{\mathrm{d}}{\mathrm{d}t}M_z^{\mathrm{B}} = -\frac{M_z^{\mathrm{B}}-M_0^{\mathrm{B}}}{T_1^{\mathrm{B}}} + k_1 M_z^{\mathrm{A}} - k_2 M_z^{\mathrm{B}},$$

where k_1 and k_2 are the rate constants for A going to B and vice versa, respectively, and T_1^{A} and T_1^{B} are the individual spin lattice relaxation times of A and B in the *absence* of exchange. At equilibrium, $k_1 M_0^{\mathrm{A}} = k_2 M_0^{\mathrm{B}}$. Now, if a low power r.f. field is continuously applied at the resonant frequency of B, ω_{B} (which is assumed to be distinct from ω_{A}), with just suffiicient power to saturate the B spins, the equation for M_z^{A} will become

$$\frac{\mathrm{d}}{\mathrm{d}t}M_z^{\mathrm{A}} = -\frac{M_z^{\mathrm{A}}-M_0^{\mathrm{A}}}{T_1^{\mathrm{A}}} - k_1 M_z^{\mathrm{A}}.$$

If the system is being observed with 90 ° pulses every T seconds, then the observed signal at ω_{A} will be the amount that M_z^{A} has recovered from the last 90 ° pulse by the time the next one arrives. This is simply

$$M_z^{\mathrm{A}}(T) = [M_0^{\mathrm{A}}/(1+k_1 T_1^{\mathrm{A}})]\,(1-\exp\{-T/T_1^{\mathrm{sat.}}\}),$$

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where $T_1^{\text{sat.}} = T_1^A/(1+k_1 T_1^A)$. Thus, provided that $T \gg T_1^{\text{sat.}}$, the M_z^A observed under these conditions will be reduced by a factor of $1+k_1 T_1^A$ from that observed when the B spins are not being saturated. The relative change in magnetization, $\Delta M/M_0^A$, between these two conditions is then $k_1 T_1^A/(1+k_1 T_1^A) = k_1 T_1^{\text{sat.}}$. A measurement of the apparent 'T_1' during saturation gives $T_1^{\text{sat.}}$, hence k_1 can be obtained simply as

$$k_1 = \frac{\Delta M}{M_0^A} \frac{1}{T_1^{\text{sat.}}}.$$

Clearly, a similar procedure involving the saturation of A would yield k_2.

Several points should be mentioned. First, if T is too short, very little effect due to exchange is observed during saturation since, if $T \ll T_1^{\text{sat.}}$,

$$M_z^A(T) = M_0^A \, T/T_1^A$$

and is therefore independent of k_1. Thus, for reliable estimates of $\Delta M/M_0$ and hence of k_1 and k_2, the magnetization must have sufficient time to recover to its steady state. Secondly, if $k_1 T_1^A$ is too small, there will be very little change in M_z^A, and signal to noise considerations will usually prevent the measurement of k_1. If $k_1 T_1^A$ is too large, on the other hand, the measurement of $T_1^{\text{sat.}}$ becomes very difficult, and again a reliable measurement of k_1 is usually not possible. In this instance however, one can use inversion transfer techniques (Dahlquist *et al.* 1975; Alger & Prestegard 1977; Brown & Ogawa 1977; Campbell *et al.* 1978) to measure these rate constants. Finally, the equations for more complicated reaction schemes are considerably more complex and thus the application of these straightforward formulae to the data obtained from something as complex as even a cell, let alone an organ, should be carried out with great care. For example, in multiple reactions, what is essentially measured by $\Delta M/M_0$ is the ratio of the lifetime of the species to its free T_1. In spite of this *caveat*, however, this technique has the advantage that it is one of the few capable of providing unidirectional rates in living systems.

Experiments

Figure 1 shows the application of saturation transfer to the determination of the ATP synthesis rate in a suspension of aerobic *E. coli* at 25 °C. The spectra shown are of ^{31}P nuclei at 145 MHz and consist of 4000 accumulations of 60° pulses every 0.17 s. The cellular suspension contained *ca.* 5×10^{11} cells/ml. It was oxygenated by having O_2 bubbled through it and was respired on endogenous carbon sources. The reader is referred to the original paper for further details (Brown *et al.* 1977). In the lower spectrum (*b*) in figure 1, the terminal phosphate of ATP (ATP_γ) was saturated by a low power r.f. field. In the upper spectrum (*a*), the frequency of this field was moved as indicated by the arrows. The difference spectrum (*c*) is shown at the bottom. As can be seen, the internal phosphate signal is reduced when the ATP_γ is saturated. This transfer is eliminated if the cells are treated with DCCD, an ATPase inhibitor, thus indicating that the dominant pathway between ATP and $P_i^{\text{in.}}$ is due to the membrane-bound ATPase. With use of the T_1 of $P_i^{\text{in.}}$ measured in the presence of DCCD, 0.4 s, as that of the unexchanging $P_i^{\text{in.}}$, a synthesis rate of 0.8 s^{-1} is obtained. Note that this rate is the unidirectional synthesis rate rather than the net synthesis rate (i.e. the flux), which would be measured by changes in the ATP level. In fact, in these experiments it takes several minutes for the ATP to decay after the O_2 is turned off, which suggests that the flux here is indeed much slower.

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Turning to experiments on the CPK system in muscle, which exchanges a phosphate moiety between PCr and ATP, we find, in the two systems thus far studied (Brown *et al.* 1978), qualitatively different results. In the simpler system, resting frog gastrocnemii at 4 °C, the ratio of the exchange rates obtained by saturation transfer agrees well with the observed ratio of the concentrations of ATP and PCr, as expected. In the instance of perfused rat heart, the simple two species exchange model is clearly wrong, as the ratio of the forward to backward rates disagrees with the observed concentration ratios by more than a factor of three.

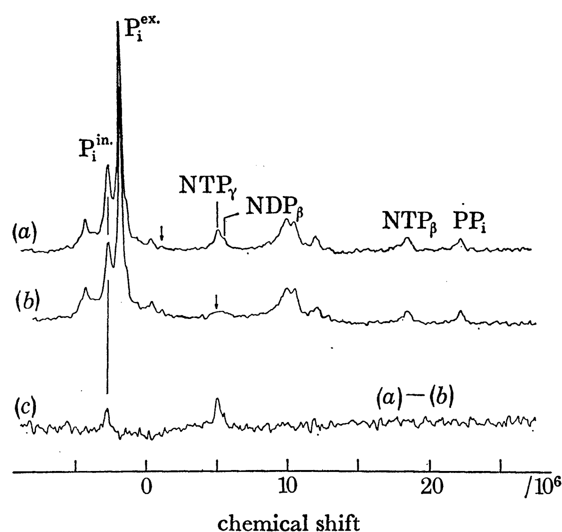

FIGURE 1. ^{31}P n.m.r. spectra of respiring suspension of *E. coli* at 25 °C. Low power r.f. was applied in *a* and *b* at frequencies indicated by the arrows.

The results from frog muscle correspond to an exchange rate between ATP and PCr of 1.6 mmol s^{-1} kg^{-1} (wet mass), at 4 °C in the resting state. Since this is not substantially faster than the observed rate of hydrolysis of ATP (0.8 mmol s^{-1} kg^{-1}) during contraction in these muscles, it is clear that any assumptions regarding the ability of CPK to remain in equilibrium at all times need to be carefully examined. Preliminary measurements by Dr David Gadian, Dr Joan Dawson and myself, for example, appear to indicate that during contraction the forward flux from PCr to ATP remains at 1.6 mmol s^{-1} kg^{-1}, while the reverse rate from ATP to PCr drops to about 0.8 mmol s^{-1} kg^{-1}. Note that this is consistent with the ATP concentration remaining constant, as the ATP is also being hydrolysed at 0.8 mmol s^{-1} kg^{-1}.

In the rat heart the situation is less clear. A naive application of the formulae derived above implies a flux from PCr to ATP of about 8 mmol s^{-1} kg^{-1}, while the reverse flux from ATP to PCr is of the order of 2 mmol s^{-1} kg^{-1}. As neither concentration is observed to change with time, this cannot possibly be correct. Clearly a model where PCr is in simple exchange with ATP, which is then coupled to energy producing and using reactions must be ruled out. Further experiments and more complex analyses are required to elucidate exactly what is taking place in heart. It seems clear howver, that these measurements, even in their present form, provide important constraints for any model attempt to explain high energy phosphate flow in heart.

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In conclusion, the use of saturation transfer in three separate systems has provided the kinetic one way rates across enzymically catalysed reactions in a totally non-invasive way. By observation of how these rates vary under different metabolic conditions it should thus be possible to develop molecular models of their controlling mechanisms.

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