total description of specificity for a two-substrate enzyme with a sequential mechanism. (As Barnsley points out [4], this comparator is inapplicable to a ping–pong mechanism, where the separation of the partial reactions and absence of a ternary complex are reflected in the absence of the $\phi_{AB}/[A][B]$ term [5,6].

I entirely accept the case [1,4] for also retaining the separate specificity constants $k_{cat.}/K_m$, i.e. $1/\phi_A$ for A and $1/\phi_B$ for B, so long as we do not pretend that they tell the whole story. Their applicability for all concentrations of the substrate in question is a useful feature, shared in fact by the proposed 'new' constant $1/\phi_{AB}$, provided that the concentration of the second substrate is low. On the other hand, it is difficult to see why, as Cornish-Bowden suggests [1], specificity constants for A should also be generalized for all values of [B]; his 'apparent specificity constants', defined at arbitrary experimental concentrations of the second substrate, are not constants at all, and are as likely to cause confusion as apparent Michaelis constants have done in the past.

It is not clear whether Barnsley's superscripted velocity ratios [4] are intended to refer to saturating or arbitrary concentrations of the fixed substrate, but either way they would fall foul of one of the criticisms above.

Perhaps the real conclusion from this exchange is that we are all trying too hard to force multi-substrate kinetics into a descriptive mould devised for one-substrate enzymes. The least ambiguous way to present comparisons either between substrates or between mutants is via a complete set of initial-rate parameters. This involves more measurements than some protein engineers are keen to perform, but they are already implicit in the determination of the 'combined specificity constant' $1/\phi_{AB}$.

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Is there an alternating site co-operativity between the two subunits of lamb liver 6-phosphogluconate dehydrogenase?

It has recently been proposed that in the homodimeric enzyme mercuric ion reductase both subunits function in catalysis, but the steps are staggered and the subunits reverse roles after the first part of the reaction [1]. This hypothesis is based on the following facts: (i) each active site of the enzyme is composed of amino acid residues from both subunits, and (ii) the binding of an effector to one active site induces conformational changes involving both subunits and co-operativity between the two subunits [1].

We now present evidence that a similar hypothesis could be applied to the action mechanism of lamb liver 6-phosphogluconate dehydrogenase [6-phosphogluconate:NADP oxidoreductase (decarboxylating), EC 1.1.1.44], a dimer of equal subunits [2], each containing one binding site for 6-phosphogluconate (6PG) and $NADP^+$ [2,3]. The two active sites are on opposite sides of the dimer molecule, but each 6PG binding site is made up of residues from both subunits [4]. In the presence of 6PG, the covalent binding of one molecule of a $NADP^+$ analogue to only one of the subunits makes the other subunit unable to bind even the adenylic moiety of the coenzyme, and thus inactive. This indicates that the simultaneous binding of 6PG and a coenzyme analogue, through an intersubunit communication, induces asymmetry in the dimer and half-site reactivity [5,6]. The half-site reactivity of this enzyme is further confirmed by stopped-flow experiments [7] which indicate formation of only one NADPH molecule per enzyme dimer during the first turnover.

When 6-phospho-2-deoxygluconate (d6PG) is used as substrate, it is possible to isolate 6-phospho-3-keto-2-deoxygluconate (3kd6PG) as an intermediate of the oxidative decarboxylation [5]. In the absence of NADPH, the enzyme is unable to catalyse the decarboxylation of this intermediate [5]. NADPH also activates the enzyme-catalysed exchange reaction of ^3H between medium and ribulose 5-phosphate, the ultimate product of the oxidative decarboxylation of 6PG [8]. In both reactions NADPH does not have a redox role [5,9], but could instead, through the basic pyridine nitrogen, influence the ionization of an amino acid residue involved both in the decarboxylation and ^3H exchange reactions at the substrate binding site.

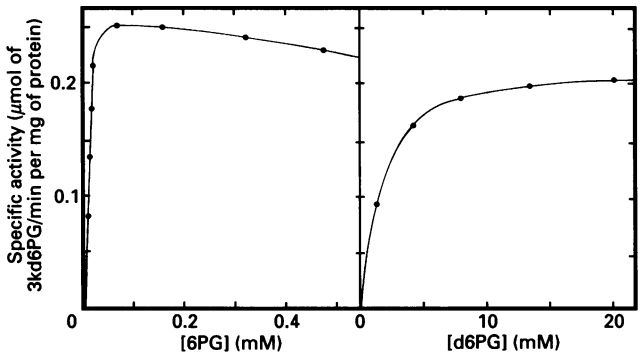

Figure 1 Kinetics of decarboxylation of 6-phospho-3-keto-2-deoxygluconate in presence of 6-phosphogluconate or 6-phospho-2-deoxygluconate

3kd6PG was prepared as previously described [5] and its concentration determined with a method specific for β-ketoacids [10]. The reaction mixture contained 18 μM 3kd6PG, 0.0124 mg/ml lamb liver 6-phosphogluconate dehydrogenase (specific activity 35 units/ml), 50 mM triethanolamine/HCl buffer (pH 7.5) and 6PG or d6PG, at the concentrations indicated. The enzyme was prepared as previously reported [11].

Table 1 Effect of 6PG, d6PG and NADPH concentrations on decarboxylation rates of 3kd6PG

Abbreviation: n.d., not determinable.

	6PG	d6PG	NADPH
K_m for enzyme (μM)	7 (12)	1420 (5)	0.22 (12)
Highest specific activity (μmol of 3kd6PG decarboxylated/min per mg of protein)	0.26	0.2	0.052*
Concentration giving 50% activation (μM)	9	1250	n.d.

* Corrected for reduction to d6PG.

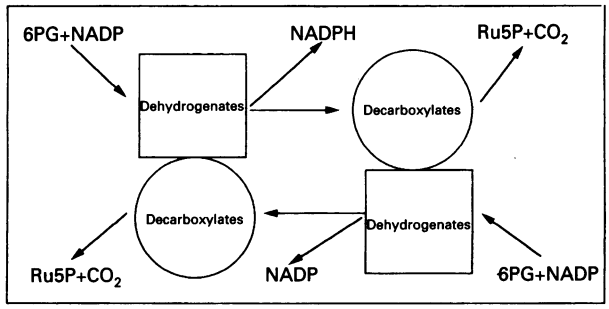

Figure 2 Hypothetical scheme of the mechanism of action of 6-phosphogluconate dehydrogenase

Abbreviation: Ru5P, ribulose 5-phosphate.

It has now been found that 6PG or d6PG also activate the enzymic decarboxylation of the keto intermediate (Figure 1); 50% of the highest rate of decarboxylation is obtained when 6PG or d6PG are present at concentrations almost equal to their K_m for the enzyme (Table 1). At concentrations higher than 0.07 mM, 6PG produces a lower activating effect (Figure 1a), due to competition with 3kd6PG for the same protein site [5]. 6-Sulphogluconate, 6-phosphogalactonate, sorbitol 6-phosphate, ribulose 5-phosphate (even at a 50 mM final concentration) and, of course, 3kd6PG do not activate the decarboxylation reaction, indicating that an activator must contain six carbon atoms, a carboxyl at C-1, a phosphate group at C-6, and hydroxyls at C-3 and C-4 (with the same configuration as in 6PG), while the presence of hydroxyl at C-2 is not essential.

Since each subunit has only one substrate binding site, these results indicate that the binding of 6PG (or d6PG) to one site, through a conformational change involving both subunits, activates the decarboxylation of the 3kd6PG bound to the other site.

If decarboxylation, the rate-limiting step of oxidative decarboxylation [13], is triggered by 6PG, then, while one subunit is involved in decarboxylation, the other subunit can catalyse the oxidation of 6PG by $NADP^+$; thus both subunits are catalytically active at the same time, one in the redox and the other in the decarboxylation reaction. The 3kd6PG, formed from 6PG in the subunit which catalysed the redox reaction, must now be decarboxylated. The more simple (and likely) hypothesis is that decarboxylation is catalysed by the same subunit, while 6PG is bound and oxidized by the other subunit. According to this hypothesis, the two equal subunits reverse their role during each turnover of oxidative decarboxylation: each subunit alternately catalyses the redox and decarboxylation reactions (as illustrated in Figure 2), and during catalysis there is a fluid functional asymmetry triggered by intersubunit communications and positive co-operativity [6,14]. The validity of this hypothesis could be tested by determining whether the release of NADPH precedes or follows the decarboxylation. An alternative, less likely, hypothesis foresees the dimer with one permanently catalytic subunit and the other with only a regulatory role; in this case the turnover number would be lower.

NADPH also activates (Table 1) the decarboxylation of 3kd6PG, probably bound to the other subunit [5], but at a 5-fold lower rate; the two activating effects (by 6PG and NADPH) are not additive. NADPH and 6PG, even if they induce the conformational changes required for decarboxylation, have different effects on the enzyme; indeed, 6PG (tested at concentrations ranging from 0.004 to 4 mM) does not activate the ^3H exchange reaction between the medium and ribulose 5-phosphate, a reaction activated instead by NADPH. The mechanism of NADPH-induced decarboxylation is also different: if NADPH activates decarboxylation, this does not allow the simultaneous binding of 6PG and $NADP^+$ [6] (and thus the redox reaction) in the other subunit. In this case, there is not the alternating site co-operativity, and only one of the two subunits is catalytically active.

The finding that activation of decarboxylation by NADPH is lower than that by 6PG raises the question whether the level of cellular NADPH could determine the reaction mechanism and thus the turnover, regulating the activity of the enzyme.

It would be interesting to check if this hypothesis of alternating sites is also valid for other homodimeric and homopolymeric enzymes.

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An Avrami analysis of the effects of serum and human albumin on calcium hydroxyapatite crystal growth

The nucleation and growth of hydroxyapatite (HAP) from low supersaturated aqueous solutions is of considerable interest to the biomedical field. Both the calcification of tissue and that of implants are serious problems that are poorly understood. The areas of biocompatibility and biomimetic processing are also keenly interested in the nucleation and growth kinetics, and the effect of proteins in moderating the precipitation reaction.

The recent paper by Garnett and Dieppe [1] presented results showing that albumin and other serum proteins significantly slowed the growth of HAP from low supersaturations. The results are explained in terms of the specific adsorption of the proteins, and the subsequent lowering of the surface growth rate. The data presented, though, can be analysed using fairly simple models to suggest that other mechanisms may be possible.

The modelling of the nucleation, growth and agglomeration of particles from a solution has been described for a number of various conditions in some detail [2–6]. All are based on the population distribution function and include various approximations for creation and annihilation of particles and agglomerates. Most of these later models are so complicated and include