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The effect of the presence of globular proteins and elongated polymers on enzyme activity

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Abstract

We have studied the effect of a crowded (macromolecular) solution on reaction rates of the decarboxylating enzymes urease, pyruvate decarboxylase and glutamate decarboxylase. A variety of crowding agents were used including haemoglobin, lysozyme, various dextrans and polyethylene glycol. Enzyme reaction rates of all three enzymes show two different types of effect that separate the globular proteins from the polysaccharides/polymers. Increasing concentration of globular proteins caused a dramatic rise in enzyme activity up to 30% crowding concentration then the activity decreased, whereas the polymers caused a concentration dependent decrease in activity. The viscosities of the globular proteins were low compared to the polymers. The increased activity with proteins may be due to the decreased amount of free water, which leads to higher effective concentration of substrates, or to an increased oligomeric state by self-association. The lower activities of all enzymes with all agents at high concentrations may be explained by a decrease in rates of diffusion. An increase in protein crowding (decrease in cell water content) may contribute to pathological conditions including cataract and Alzheimer's disease.

Keywords: Enzyme; Decarboxylase; Globular; Protein; Polymer; Crowding

1. Introduction

The compact nature of living cells differs greatly from experimental media in cuvettes. Dilute protein concentrations approximately 1 g/l are usually required to measure catalytic activity to prevent aggregation and light scattering, whereas living cells have high protein content; erythrocytes ~350 g/l, cytoplasm (*Escherichia coli*) ~340 g/l [1] and human lens ~340 g/l [2]. To produce a more physiological environment in which to study enzyme reactions, globular proteins or elongated polymers could be added to the enzyme and reactants to recreate the intracellular milieu. Within the cell, the medium is not homogeneous, but highly compartmented, containing a variety of macromolecular particles, such as ribosomes, microtubules, intermediate filaments, membrane boundaries and nucleic acids. Such a medium is referred to generically as crowded rather than concentrated. The term macromolecular crowding, as applied to biological systems, describes the fact that the total concentration of macromolecules inside cells is so high that a significant proportion of the volume is physically occupied and, hence, unavailable to other molecules [3].

By adding globular proteins and polymer dextrans to a reaction mixture, the available volume to solute is decreased. This exclusion of volume to neighbouring macromolecules reduces the configurational entropy and increases the free energy of a solution, thereby increasing the chemical potential of each species of macromolecule present in that solution [4]. The energy needed to initiate a reaction is lowered and this is thought to affect reaction rates. Crowding of enzymes that exist in more than one state of self-association (e.g. monomer, dimer, trimer etc.) may increase self-association and, where different aggregation states differ in specific activity would alter the overall catalytic activity of the enzyme [5].

In general, the addition of globular proteins or polymer dextrans to the reaction vessel is expected to stabilise native proteins, leading to a decreased mean size relative to less-compact non-native structures and to favour the formation of

Abbreviations: PEG, polyethlyene glycol; MES, 2-Morpholinoethanesulfonic acid

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functional complexes of native proteins. This would therefore increase the reaction rate. However, the addition of globular proteins or polymer dextrans will reduce the diffusion mobility of enzymes and reactants and thereby the encounter rate. There is a balance between extra particle concentration within a reaction vessel and reaction rate. If the concentration is too high then a saturated mixture can occur leading to decreased association rates. The latter is thought to be associated with protein aggregation seen in amyloid disease, which has a significant decrease in water content [4].

Previous experiments in our laboratory have demonstrated that resealing certain enzymes within ghost erythrocyte cells, a more physiological environment, resulted in huge increases in enzyme activity [6]. Exploration of enzyme activity in high concentrations of globular proteins, the main crowding macromolecule in vivo, is difficult because absorbance by the protein interferes with most enzyme assays. Here we have investigated the enzyme activity of urease in solutions containing globular protein and polymer dextran, and the effect of globular protein or polymer dextrans on enzymes catalysing decarboxylation reactions, the removal of a terminal carbon atom and liberating it in the form of CO_2. If the terminal carbon is labelled then the reaction rate can be determined by the amount of labelled $^{14}CO_2$ liberated within a sealed vessel. The globular proteins and polymers should be inert and not too viscous. Haemoglobin conforms to these requirements, as does lysozyme. Polysaccharides such as dextran (10, 70 and 120 kDa), polyethylene glycol (8 kDa) and Carbopol 941 (an acrylamide polymer with low viscosity but high molecular weight, 1250 kDa) were also investigated.

The reaction rate of the enzymes dramatically increased in the presence of haemoglobin, lysozyme and bovine serum albumin, but not with polymers such as dextran and polyethylene glycol. The effect of increasing the concentration globular protein beyond 30% caused an equally dramatic decrease in enzyme activity. Although many consequences of increased protein/polymer concentration on equilibria have been determined [7], these experiments show direct determination of enzyme activity. The possible effect of oligomeric state on enzyme activity is discussed.

2. Experimental

2.1. Materials

Urease was from Jack bean (EC 3.5.1.5), pyruvate decarboxylase was from bakers yeast (EC 4.1.1.1) and glutamate decarboxylase (type V) was from *E. coli* (EC 4.1.1.15). Lysozyme (from chicken egg white), haemoglobin (substrate powder from bovine), dextran (from *Leuconostoc mesenteroides*), polyethylene glycol and the enzymes were from Sigma (Poole, UK). [1-^{14}C] Urea, [1-^{14}C] pyruvic acid and [^{14}C] glutamic acid were from Amersham Pharmacia Biotech, UK Carbopol 941 (a polymer of acrylic acid) was from BF Goodrich Company, Ohio, USA. Soluble lens protein was from bovine lenses [8] and prepared by homogenizing the lenses in 0.05 M sodium phosphate buffer (pH 6.7), centrifuging the suspension at 22,400×*g* for 10 min at 4 °C, then dialysing the supernatant against 4 changes of distilled water for 24 h at 4 °C, followed by freeze-drying. All polymers were of the highest grade possible with no buffer salts.

2.2. Urease assay

Urea, 30 mg was dissolved in 10 ml potassium phosphate buffer (0.1 M) pH 7.0 to give a final concentration of 0.05 M containing 1 μCi [1-^{14}C] Urea. Prior to starting the reaction urease (2.5 mg) was dissolved with the urea in the reaction mixture and the solution mixed. Samples (1 ml) of the solution were added to individual vials containing 0–60 mg crowding agent to provide final concentrations of 0, 10, 20, 30, 40, 50 and 60% (w/v) and shaken until dissolved. The contents were then transferred into Warburg flasks. Filter papers, cut into squares and soaked in 40% NaOH, were placed in the middle wells. The flasks were stoppered and incubated over 3 h with gentle shaking at room temperature. Filter papers were taken out and placed in 3 ml-scintillation fluid so the counts of labelled $^{14}CO_2$ absorbed by the NaOH filter paper could be determined. The filter papers were replaced every hour. All results are shown as % of control. Each experiment was repeated at least nine times. Plotted error bars represent standard deviations.

2.3. Pyruvate decarboxylase

The incubation buffer was 10 mM pyruvate, (0.5 mM) thiamine diphosphate (cocarboxylase) and (5 mM) $MgCl_2$ in 10 ml 250 mM MES buffer (pH 6.5). Pyruvate decarboxylase (0.6 mg) was dissolved into the reaction mixture with 1 μCi [1-^{14}C] pyruvate. The crowding agents were prepared as above and the rate of formation of labelled $^{14}CO_2$ was monitored as above.

2.4. Glutamate decarboxylase

Glutamate (5 mM) was dissolved in 10 ml sodium acetate buffer (250 mM, pH 5) with (2 mM) pyridoxal 5-phosphate. Glutamate decarboxylase (3 mg) was added to the solution with 1 μCi [^{14}C]-glutamic acid. The crowding agents were prepared as above and the rate of release of labelled $^{14}CO_2$ was monitored as above.

2.5. Viscosity measurements

Viscosity is measured by sensing the torque required to rotate a spindle at a constant speed while immersed in fluid. The torque is proportional to the viscous drag on the spindle and thus to the viscosity of the fluid. It is therefore a ratio of the shear force applied and the rate of shear. A Contraves Low-Shear 30 rheometer was used in which the sample fluid fills the annular space between a stainless steel bob suspended from a torsion wire and the outer wall of the stainless-steel sample cup. Rotation of the cup induces a drag on the bob and a digital display gives the restoring force applied by a servo system, which is necessary to keep the bob stationary [9]. All readings were taken at room temperature.