Hypothesis

PEROXISOMAL RESPIRATION AND ENERGY CONSERVATION

Possible relationships between heat production, thermoosmosis and conformational changes

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1. Introduction

Peroxisomes (microbodies) are cytoplasmic oxidative organelles, characterized morphologically by a single limiting membrane and a finely granular matrix, and biochemically by their content of H_2O_2-producing oxidases and H_2O_2-decomposing catalase [1–4]. Peroxisomes have been found in species belonging to all kingdoms of eukaryotes. They have been described in protists, fungi, plants, and animals, but not necessarily in all cell types or in all developmental stages [2–4]. However, the discovery of a smaller kind of peroxisome (microperoxisome) in over 50 mammalian cell types [5–8] is contributing to the view that the peroxisome is a ubiquitous eukaryotic organelle.

Far from being a 'fossil' organelle [1], the peroxisome is now known to play a dynamic role in eukaryotic cell and tissue metabolism [4]. Oshino et al. [9] have observed peroxisomal H_2O_2 production when rat livers were perfused with urate and glycolate (substrates for urate oxidase and L-α-hydroxy acid oxidase, respectively) and demonstrated that this constituted nearly half of the total liver respiration. Thus they concluded that biological oxidations of considerable physiological significance are possible in the peroxisome. Although high (nonphysiological) concentrations of urate and glycolate were used in their perfusion experiments their results could be interpreted to represent maximal respiratory capacities of the peroxisome [9]. Our knowledge of the endogenous substrates of the peroxisome is still very limited. The discovery of the peroxisomal fatty acyl-CoA oxidase [10–13] and the peroxisomal β-oxidation pathway that this novel oxidase initiates and regulates [10,12, 14,15], is beginning to clarify the cellular functions of the peroxisome. The rat liver fatty acyl-CoA oxidase oxidizes medium to long-chain length acyl-CoAs (C_8–C_{18}) in the presence of O_2, producing H_2O_2 [11,12]. The contribution of the peroxisome to the total liver fatty acid oxidation in normal rats varies from 10–30%, depending on age, sex and on the fatty acid that is being oxidized; its chain length and degree of unsaturation being major factors [12,16]. Constant (steady state) production of H_2O_2 in the peroxisome is now established as a normal part of liver metabolism and is, in fact, a physiological event in living eukaryotic organisms [4].

2. Peroxisomal respiration, heat production and thermogenesis

The generation of H_2O_2 by peroxisomal H_2O_2-producing oxidases has been much too readily dismissed as the formation of a toxic and wasteful byproduct, that is rapidly removed from the scene by the action of catalase [17,18]. Indeed, de Duve [1,18,19] has claimed that the respiratory chain of peroxisomes ($O_2 \rightarrow H_2O_2 \rightarrow H_2O$, catalyzed by H_2O_2-producing oxidases and catalase) is of a very primitive nature, since it carries no provision for the conservation of energy and catalyzes an essentially wasteful form of respiration. Thus it can be (and has been) argued that the first oxidative step in the peroxisomal β-oxidation system, that is catalyzed by the fatty acyl-CoA oxidase, as well as all the other oxidations

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catalyzed by peroxisomal H_2O_2-producing oxidases, is totally wasteful insofar as energy conservation is concerned [20]. However, this may not be so. It should be pointed out that peroxisomal respiration could, despite its seemingly wasteful nature, be energetically useful to the cell.

I would like to submit that part of the energy of peroxisomal respiration is conserved in the form of heat and in the formation of a temperature gradient across the peroxisomal membrane. The formation of H_2O_2 by H_2O_2-producing flavoprotein oxidases and the action of catalase on the H_2O_2, releases heat (ΔH for $RH_2 + O_2 \rightarrow R + H_2O_2$ is -20 kcal/mol, and ΔH for $H_2O_2 \rightarrow H_2O + \frac{1}{2}O_2$ is -30 kcal/mol [21]) into the peroxisol (inner liquid portion or matrix of the peroxisome). The peroxisomal membrane serves as a thermal barrier (lipids are good heat insulators) separating the relatively hotter peroxisol from the cytosol, thus creating a temperature gradient. Once a temperature differential has been established, heat will flow across the peroxisomal membrane. Since the mass of the cytosol is much greater than the mass of the peroxisol, any increase in its (cytosolic) temperature will be immediately dissipated throughout the cell. Therefore the temperature of the cytosol will always remain relatively lower than the peroxisol, thereby maintaining a temperature differential across the peroxisomal membrane. The steady state heat flow out of the peroxisome and into the cytosol could be used in the heating of the cell, organ and/or organism (chemical thermogenesis).

During active peroxisomal respiration, ~1.3 μmol $H_2O_2 \cdot min^{-1} \cdot g\ liver^{-1}$ is produced in the peroxisomes [9]; which translates to $\sim 6.5 \times 10^{-2}$ cal $\cdot min^{-1} \cdot g\ liver^{-1}$. If the water content of the liver is assumed to be 69%, peroxisomal respiration could raise the temperature of the liver at ~0.1 K $\cdot min^{-1} \cdot g\ liver^{-1}$. It should be pointed out however, that this calculated rate of heat production by peroxisomes represents the maximal rate at which peroxisomes could raise the temperature of the liver, and does not represent a normal, physiological rate. Nevertheless, maximal rates of heat production by peroxisomes could be achieved under certain physiological stresses, i.e., cold adaptation. There is some evidence that the number of liver and brown adipose tissue peroxisomes is elevated in rats exposed to cold [22–24]. In cold-adapted rats, the total peroxisomal activity of both catalase and β-oxidation in brown adipose tissue was increased 10-fold over normal rats [25].

3. Temperature gradients, thermoosmosis and conformational changes

By imposing a temperature gradient across the peroxisomal membrane, we can expect a mass-transfer process based on the theory of non-equilibrium thermodynamics [26]. When a temperature gradient causes the flow of a solvent (water) across a membrane, it is called thermoosmosis. Thermoosmosis has been studied and interpreted in detail for several synthetic semipermeable membranes [27–29]. However, except for Spanner's [30] speculation on the significance of thermoosmosis on active water transport in plants, very little is known about thermoosmosis in biological membranes.

The known equation for thermoosmotic pressure based on the thermodynamics of irreversible processes is given by:

$$\Delta P/\Delta T = -Q^*/V_w T \qquad (1)$$

where V_w is the molal volume of water, and Q^* is the total heat of transport for the discontinuous system [26,29]. In order to be able to determine the thermoosmotic pressure ($\Delta P/\Delta T$) across the peroxisomal membrane, Q^* must be approximated. The heat of transport (Q^*) can be calculated from a knowledge of the temperature dependence of the membrane permeability and is given by:

$$10\, Q^*/RT^2 = \ln (1.034 Q_{10}) \qquad (2)$$

where Q_{10} is the temperature coefficient of permeability [30]. In animal cells a fairly typical value for Q_{10} for water would be between 1.0 and 2.6 [30,31]. Using the smaller value of Q_{10}, and substituting it into eq. (2) we would obtain a value for Q^* of 637 cal/mol at 310 K. Substituting this value into eq. (1), the thermoosmotic pressure across the peroxisomal membrane is calculated to be $\sim -4.78 \times 10^6$ dyn/cm^2 K or -4.6 atm/K at 310 K. The negative sign indicates that the pressure develops on the low temperature side [29], thus water will flow from the peroxisol to the cytosol. Assuming that the temperature differential across the peroxisomal membrane is in the order of 1.0–0.01 K, peroxisomes could develop pressures equivalent to 4.6–0.046 atm across the membrane. In other words, a temperature differential of 0.1 K across the peroxisomal membrane could cause the transport of water out of the peroxisome at the same

rate as a pressure difference of ~0.5 atm.

Under active peroxisomal respiration, the thermo-osmotic pressure created by the temperature differential across the peroxisomal membrane could 'actively pump' (transport) water out of the peroxisome, dehydrating it. As the peroxisome dehydrates or condenses (shrinks) two events take place:

(i) It becomes hypertonic;
(ii) The conformation of the peroxisomal membrane and its intrinsic proteins will change, probably leading to a collapse of the thermoosmotic pressure.

Since the peroxisome has become hypertonic, water will then diffuse into the peroxisome, restoring it to its orthodox (expanded) conformation and re-establishing the thermoosmotic pressure. Thus the peroxisome could continually occillate in conformation between an orthodox and a condensed configuration. This conformational change could result in energy-dependent shifts in the number or location of weak bonds (hydrogen bonds, hydrophobic interactions) maintaining the three-dimensional conformation of the peroxisomal membrane or its intrinsic proteins. Therefore, the 'energized' conformational state could be used in the transport of ions and metabolites across the peroxisomal membrane. Finally, the changes in the conformation of the peroxisome could lead to the formation of high energy compounds. No one has yet looked for conformational changes specifically associated with active peroxisomal respiration. Peroxisomes however, have been widely described as having many sizes and with highly convoluted and continually varying shapes [4]. These size and shape variations could have resulted from the dehydration and rehydration of the peroxisome (as above), leading to the formation of smaller (shrunken), enlarged (swollen), or irregularly shaped peroxisomes.

4. Conclusions

The production of heat and high temperatures by the action of catalase on H_2O_2 in biological systems is not without precedent. One of the most impressive examples illustrating this is the defensive chemical spray of the bombardier beetle. This hot discharge is sprayed from a special reaction chamber at 100°C. A reaction mixture of 25% H_2O_2 and 10% hydroquinone is stored in a reservoir and reacts explosively when it comes into contact with the catalase and peroxidases found in the reaction chamber [31,32]. Like the reactor gland of the bombardier beetle, the respiratory chain of the peroxisome is highly exergonic (ΔH of −50 kcal/mol). Therefore, it is possible that enough heat could be generated through the production and destruction of H_2O_2 within the peroxisome, to establish temperature differentials in the other of 1.0–0.01 K across the peroxisomal membrane. As postulated, temperature differentials of this magnitude could then perform:

(i) Chemical thermogenesis;
(ii) The mechanical work of conformational changes in the peroxisomal membrane and/or its intrinsic proteins.

The partial conservation of energy by the establishment of a temperature gradient across a membrane need not only be applicable to the peroxisome; mitochondrial respiration can also generate a temperature differential across its inner membrane. Thus, the conformational changes (orthodox–condensed) observed in mitochondria during active respiration [33,34] could be explained in terms of thermo-osmosis.

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