An enzymic 'latch' on a global carbon store

A shortage of oxygen locks up carbon in peatlands by restraining a single enzyme.

Historically, northern peatlands have removed carbon dioxide from the atmosphere faster than it has been re-released, so they now contain 20–30% of the world's soil carbon stock[1] (the equivalent of over 60% of the atmospheric carbon pool[2]). Here we show that the anaerobic conditions in peatlands prevent the enzyme phenol oxidase from eliminating phenolic compounds that inhibit biodegradation. This indicates that oxygen limitation on a single peatland enzyme may be all that prevents the re-release of a major store of global carbon into the atmosphere, with potentially serious implications for future global warming.

Mechanisms proposed to account for the slow decomposition rates in peatlands include the effects on microbial metabolism of low oxygen availability, low pH, low nutrient supply and low temperatures. But decomposition can be highly efficient in anaerobic environments such as the rumen or man-made sewage digesters. Likewise, it can remain relatively inefficient in fens that are nutrient-rich and whose pH is around neutral, or in mangrove systems that are far from cold[3].

One feature common to all of these wetlands, however, is the ubiquity of phenolic compounds. These have attracted intense interest as potent inhibitors of enzymes[4] and, with the exception of phenol oxidase, few enzymes are able to degrade these recalcitrant materials[5]. But the *in situ* activity of phenol oxidase is severely constrained[6]: it requires bimolecular oxygen, even though it exists in an essentially anaerobic environment. Also, the activity of all the major biodegradative hydrolase enzymes is depressed in peatlands[7], although they normally retain high activity in anaerobic environments such as the rumen[8] or anaerobic sludge digest[9]. We propose that the low rate of biodegradation in peatlands is due to oxygen constraints on phenol oxidase, which allow phenolic materials to accumulate and inhibit these pivotal hydrolase enzymes.

We compared enzyme activities under oxygen-saturated and oxygen-free conditions and found that phenol oxidase was the only enzyme to increase in activity under the more aerated conditions (7-fold increase in activity; Table 1). Evidence of a link between phenol oxidase activity and oxygen availability has been found in a Florida wetland, where phenol oxidase activity was detectable only under aerobic conditions[5]. Experimental supplementation with phenol oxidase resulted in a 27% drop in phenolic compound concentrations within 18 hours (Table 1). Lower water-tables, which are associated with increased aeration, also cause a sharp drop in phenolic concentrations[10].

Table 1 Effects on enzyme activities

	Control	*Manipulated*
Effect of oxygen on enzyme activity		
Sulphatase	66 ± 2.3	35 ± 1.4
Phosphatase	571 ± 2.4	387 ± 7.9
β-Glucosidase	237 ± 2.3	177 ± 12
Phenol oxidase	615 ± 93	4,350 ± 27
Effect of increasing phenol oxidase abundance		
Phenolics (μg l^{-1})	1,985 ± 55.4	1,444 ± 9.9
β-Glucosidase	1,677±280	10,111±380
Effect of phenolic removal on hydrolase activity		
Sulphatase	579 ± 36	849 ± 43
Phosphatase	3,707 ± 25	4,369 ± 180
β-Glucosidase	1,723 ± 120	2,183 ± 180
Xylosidase	116 ± 2.5	134 ± 5
Chitinase	243 ± 14	296 ± 3.5

Phenol oxidase activity (nmol 2-carboxy-2,3-dihydroindole-5,6-quinone formation min^{-1} per g peat), hydrolase activities (nmol methylumbelliferone formation min^{-1} per g peat) and phenolic compound concentrations (μg l^{-1}) are reported as mean ± s.e.

We determined the effect of reduced dissolved phenolic concentrations on hydrolase activities after removing them selectively by using crosslinked *N*-vinyl-2-pyrrolidone[11]. We found that the activity of hydrolases in peat samples exposed to phenolic-free waters was significantly higher than the corresponding activity in untreated waters containing phenolic compounds at just 2.40 mg l^{-1} (mean) (Table 1). It has been shown that hydrolases are strongly inhibited (over 80%) in the presence of higher phenolic concentrations[12] or after longer exposure to phenolic materials[4].

Supplementing aerobic peat samples with additional phenol oxidase led to a significant increase in the activity of hydrolase enzymes (Table 1) in response to the increased removal of inhibitory phenolic compounds. We also showed by doing a regression analysis of data from a 3-month field survey ($r = 0.61$, $P < 0.05$) that every doubling in phenol oxidase activity was accompanied by an approximate doubling in CO_2 production.

Taken together, our findings support the idea that oxygen constraints on a single enzyme, phenol oxidase, can minimize the activity of hydrolytic enzymes responsible for peat decomposition. This has profound implications in the context of climate change as a feedback to the process of intensified carbon loss. Increased peat aeration, as a result of droughts predicted by certain climate-change models[13], has the potential to eliminate a critical mechanism restricting the re-release of CO_2 to the atmosphere. As such, phenol oxidase could be considered to represent a fragile 'latch' mechanism holding in place a vast carbon store of 455 gigatonnes.

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Deafness

Cross-modal plasticity and cochlear implants

Hearing in profoundly deaf people can be helped by inserting an implant into the inner ear to stimulate the cochlear nerve. This also boosts the low metabolic activity of the auditory cortex[1], the region of the brain normally used for hearing. Other sensory modalities, such as sign language[2], can also activate the auditory cortex, a phenomenon known as cross-modal plasticity. Here we show that when metabolism in the auditory cortex of prelingually deaf children (whose hearing was lost before they learned to talk) has been restored by cross-modal plasticity, the auditory cortex can no longer respond to signals from a cochlear implant installed afterwards. Neural substrates in the auditory cortex might therefore be routed permanently to other cognitive processes in prelingually deaf patients.

Glucose metabolism can be stimulated in the primary auditory cortex and in the auditory-association cortex of prelingually

deaf patients[3]. In a study of a long-term prelingually deaf patient, the auditory-association cortex was active while watching sign language[2] but the primary auditory cortex was not. But after this patient received a cochlear implant, the primary auditory cortex was activated by the sound of spoken words, whereas adjacent language areas were not. This phenomenon has been attributed to auditory-to-visual cross-modal cortical plasticity[2,4].

We examined and compared glucose metabolism in the auditory and related cortices in 15 prelingually deaf patients before cochlear implantation (mean age, 6 ± 4 years old; age range, 2.2 to 20.3 years); 7 boys and 8 girls) with that of 17 young normal adults (23 ± 3 years old) by using F-18 fluorodeoxyglucose positron emission tomography (PET) and statistical parametric mapping methods. We found that the degree of hypometabolism before the operation was related to the amount of improvement in hearing capability after cochlear implantation (6 subjects received an implant in the right ear, 4 in the left).

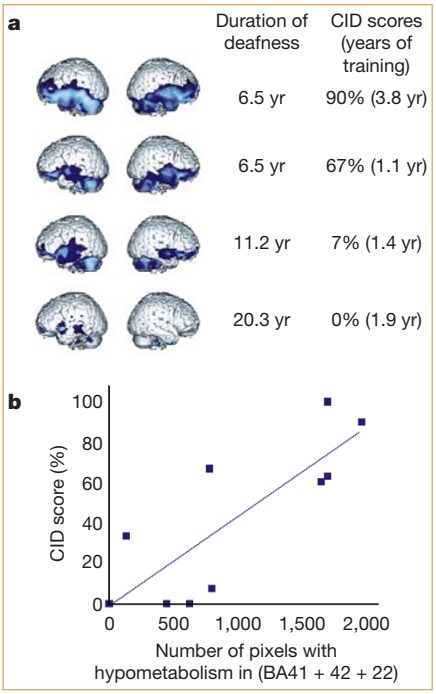

Figure 1 Correlation between hearing response in the prelingual deaf after cochlear implantation and pre-operative hypometabolism in the auditory cortex. **a,** In four representative patients, areas of hypometabolism (shown in blue) on a surface-rendered PET image are displayed with the duration of deafness (age), duration of hearing training, and the CID scores. The 20-year-old patient (bottom row) showed restored metabolism in the auditory and related cortex. She had no hearing capability (CID score, 0%), despite intensive training for 1.9 years. **b,** Scatter plot of CID speech-perception scores and the extent of the hypometabolic area in the primary auditory cortex (BA41) and the auditory-association cortex (BA42, BA22) of both hemispheres in prelingually deaf patients. The pre-operative extent of the hypometabolic areas showed significant correlation with the CID scores after cochlear implantation.

Metabolism fell in the superior temporal (Brodmann area (BA) 41, BA 42, BA 22) or inferior frontal (BA 44 or 45) areas. As the child grew older and the duration of deafness increased, the extent of the hypometabolic area was reduced in the superior temporal and inferior frontal regions (Fig. 1a).

We examined speech-perception performance in a Korean version of the CID (Central Institute for the Deaf) tests during postoperative hearing training, which lasted from 8 months up to 3 years and 8 months, depending on the patient's responsiveness. After cochlear implantation, there was a positive correlation ($r = 0.81$, $P < 0.005$, $n = 10$) between the size of the hypometabolic area in BA 41, 42 and 22 and the hearing-capability score (range was from 0 to 100%; average was 42%) (Fig. 1b). In a multiple regression analysis that included duration of deafness, duration of implant use, and degree of metabolism (measured as the number of hypometabolic pixels) as predictors, the hearing-capability score was predicted independently and most strongly by the degree of metabolism (results not shown).

In our prelingually deaf patients who performed poorly with their cochlear implants, the auditory cortex was probably incapable of perceiving auditory signals from the implants. The realm of afferent neural networks of other sensory systems, such as the visual or somatosensory system, might have increased[4]. Alternatively, higher cognitive functions such as the interpretation of sign language[2] or lip-reading[5] could have occupied the relatively under-utilized areas of the auditory cortex. The increased neuronal activity of the auditory cortex, manifesting as an increase in blood flow[6] and glucose metabolism[1,3], implicates cross-modal plasticity as the cause of the continuing lack of hearing response in these prelingually deaf patients.

A similar phenomenon can occur in the congenitally blind[7], in whom Braille reading activates the visual cortex. When the visual cortex is disturbed by transcranial magnetic stimulation, Braille reading becomes less efficient[7,8]. Cross-modal plasticity is functionally relevant in congenitally blind patients, but not in patients with acquired blindness[9].

We have demonstrated the prognostic importance of auditory-to-visual cross-modal plasticity in prelingual deaf patients with the cochlear implants. If cross-modal plasticity restores metabolism in the auditory cortex before implantation, prelingually deaf patients will show no improvement in hearing function, even after successful implantation and concentrated rehabilitation. The resting cortical metabolism of untreated prelingually deaf patients represents a usurping by cross-modal plasticity, which deters the improvement of hearing capability and the restoration of normal function in the auditory temporal cortices after cochlear implantation.

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Molecular engineering

Networks of nanotubes and containers

We have constructed complex two-dimensional microscopic networks of phospholipid bilayer nanotubes and containers in which we are able to control the connectivity, container size, nanotube length, and angle between the nanotube extensions. Containers within these networks can be chemically differentiated and materials successfully routed between two containers connected by a common nanotube. These networks will enable model systems to be devised for studying confined biochemical reactions[1–3], intracellular transport phenomena[4] and chemical computations[5].

Our method is based on the propensity in liposomes to undergo complex shape transitions as a result of mechanical excitation. It complements earlier micromanipulation protocols for the formation of lipid nanotube networks[6]. As the starting material for generating networks, we used either multilamellar liposomes (5–25 lamellae) prepared by rotary evaporation[7], or unilamellar liposomes formed by a dehydration/rehydration technique[3].

We have been able to produce networks from a wide variety of liposomes of different lipid composition with controlled charge, wettability and functionality. In this instance, we used liposomes either from type XVI-E L-α-phosphatidylcholine isolated from fresh egg yolk or from type II-S acetone-purified soybean lecithin in physiological saline buffer. Liposomes were transferred to borosilicate coverslips coated