presence of E2, REA, and coactivator SRC-1 compete for the binding to the ER. ProTα causes a dissociation of REA from ER and shifts the ER status to the SRC-1-binding, or transcription-active, form (Fig. 1B).

Cytosol functions

Switch on/off of Nrf2-Keap1 mechanisms

ProTα possessing an NLS at the C-terminus is localized in the nucleus in most cells. The nuclear import of these molecules with an NLS is mediated by importin α. As the nucleus-to-cytosol export of ProTα is presumed to occur in a passive diffusion due to its smaller size,[11] it is natural that ProTα may have some biological actions in the cytosol, as well as in the nucleus. The Nrf2 (nuclear factor erythroid 2-related factor 2)-Keap1 (Kelch-like ECH-associated protein 1) system is known to play roles in the cell adaptation to oxidative and electrophilic stress.[28–30] Nrf2 is a nuclear transcription factor that regulates expression of several defensive genes, including detoxifying enzymes, and antioxidant genes.[28–30] In the absence of stress, cytosol Nrf2 is "trapped" by Keap1, ubiquitinated by the Cul3/Rbx1-dependent E3 ubiquitin ligase, and then subsequently degraded by the 26S proteasome.[31] Recent studies revealed that ProTα binds to Keap 1 and releases Nrf2 from the Nrf2-Keap1 complex ("switch on").[32] Interestingly, ProTα also mediates nuclear import of the Keap1/Cul3/Rbx1 complex to degrade nuclear Nrf2 ("switch off").[33] Thus, it is speculated that ProTα regulates the cell-defensive roles of Nrf2 by switch on/off mechanisms (Fig. 1C).

Inhibition of apoptosome formation

When the cell is under mitochondrial stress, such as in the case with a growth factor deprivation, cytochrome c (cyt. c)—a member of soluble mitochondrial intermembrane proteins (SIMPs)—is released from mitochondria (Fig. 2A). As a result, the apoptosome, composed of cyt. c and Apaf-1, is formed and followed by a cascade of caspase activation and apoptotic DNA fragmentation.[34,35] Recent studies revealed that ProTα inhibits apoptosome formation by interaction with Apaf-1.[5,36] During apoptosis, on the other hand, ProTα loses its C-terminus containing the NLS, due to the digestion by caspase-3, and is redistributed to the cytosol.[11,37,38] As both cytosolic full-length ProTα and truncated ProTα have a similar potential of apoptosome inhibition, it is interesting to speculate that ProTα has a potential as a natural inhibitor of apoptosis (Fig. 1D). This speculation is further supported by a report that ProTα inhibits apoptogenic compound–induced apoptosis by interacting with p8 (a nuclear protein-1 and candidate of metastasis-1).[39,40]

Extracellular functions

Identification of ProTα as a neuronal necrosis inhibitory factor

Based on the findings that cortical neurons at a low density rapidly die by necrosis under a serum-free or starving condition and that survival is density dependent,[10] we previously attempted to search for antinecrotic factors from the conditioned medium by using molecular weight cut-off ultrafiltration, ion-exchange filtration, SDS-PAGE separation, and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). A subsequent search of the nonredundant NCBI protein database for matching peptide mass fingerprints revealed 17 peptides consistent with the conclusion that the only active substance was acetylated ProTα.[9] After various approaches, we discovered an efficient way to obtain significant amounts of active materials that were unique to rat ProTα. Moreover, tandem MS analysis confirmed that the N-terminal of purified ProTα was an acetylated serine, in agreement with a previous report.[9]

Non-classical release mechanisms

ProTα was discovered in conditioned medium from serum-free or starving stress conditions of cultured cortical neurons. To examine the molecular basis of stress-induced ProTα release, we used C6 glioma (astrocytoma) cells because of their robustness against ischemia stress. On the analogy of the nonclassical release of FGF-1,[41,42] which lacks signal peptide sequence, ischemic stress caused a limiting extracellular release of ProTα (which also lacks signal peptide sequence). Our study revealed that the mechanisms underlying the nonclassical ProTα release from C6 glioma cells are mediated by the loss of ATP and Ca^{2+} influx through N-type voltage-dependent Ca^{2+} channel activity.[11] In this mechanism (Fig. 3A), the first step is the release of ProTα from the nucleus (passive diffusion) due to ATP loss, followed by a Ca^{2+}-dependent interaction with Ca^{2+} binding protein S100A13, a cargo protein. The release of ProTα from the nucleus upon an ischemic

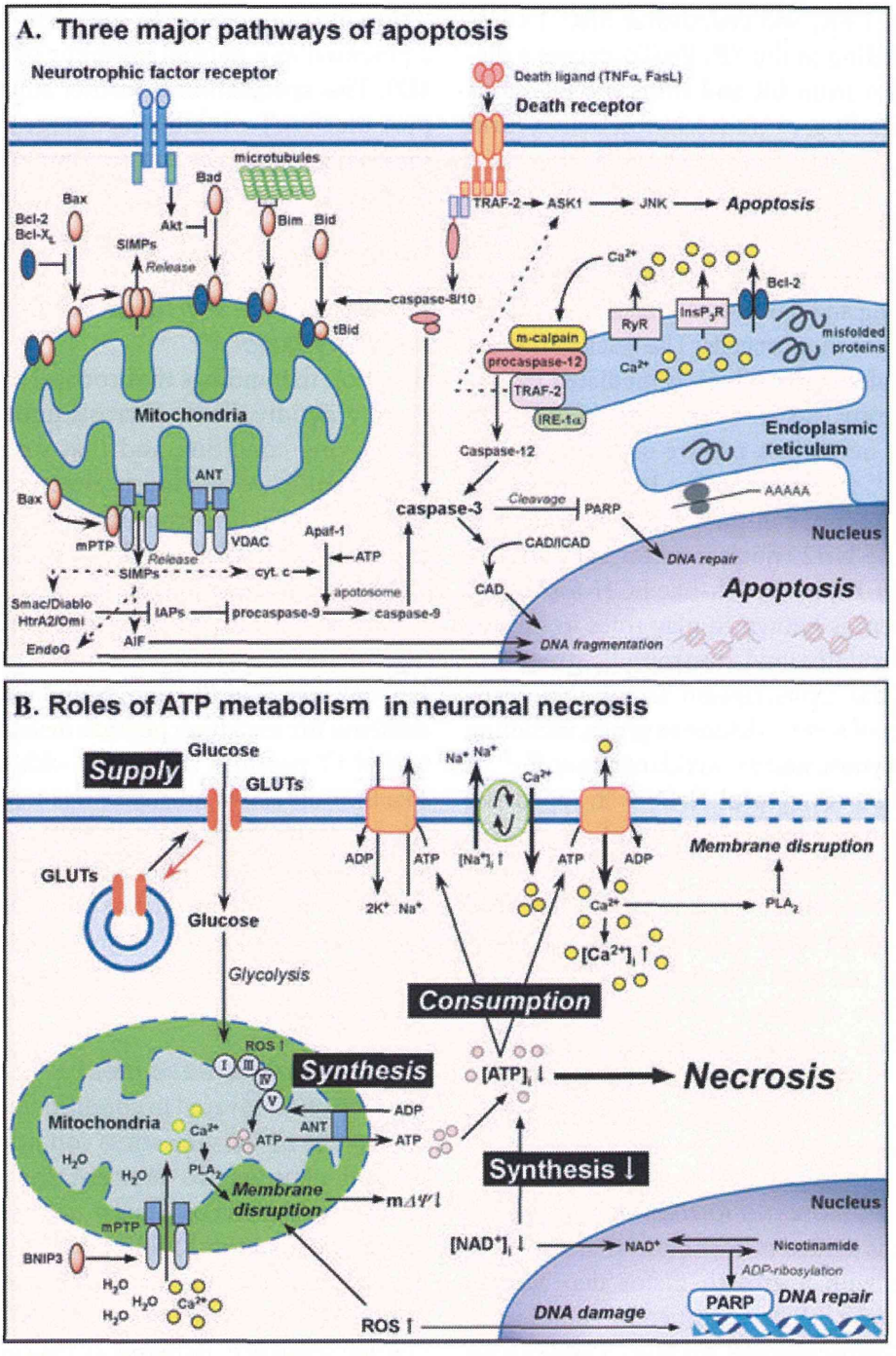

Figure 2. Schematic illustration of apoptosis and necrosis. (A) Three major pathways of apoptosis. Mitochondrial pathways are closely related to the expression of members of the Bcl-2 family of proteins. Proapoptotic Bak and Bax open mitochondrial permeability transition pores (mPTPs) to release soluble intermembrane proteins (SIMPs), including cytochrome c (cyt.c), apoptosis-inducing factor (AIF), Smac/DIABLO, EndoG, and HtrA2/Omi. Among these, cyt.c plays a major role in inducing apoptosis through activation of caspase-3 and caspase-activated DNase (CAD). Bcl-2 and Bcl-XL are major antiapoptotic proteins that inhibit the functions of these proapoptotic proteins. The other two pathways through death receptors (FAS and TNF-α receptors) or endoplasmic reticulum stress also use caspase-3 activation as the common execution pathway. (B) Roles of ATP metabolism in neuronal necrosis. Glucose transporters (GLUTs) are involved in the supply of cellular glucose (depicted as supply), a substrate for ATP production through glycolysis and oxidative phosphorylation (depicted as synthesis) in mitochondria. Some species of GLUT are constitutively localized, while others are translocated to the membrane upon cell stimulation by extracellular signals. Abundant cellular ATP molecules maintain intracellular ionic behavior (depicted as consumption). Poly (ADP-ribose) polymerase (PARP) restores the DNA damage caused by cellular stress, by using abundant NAD^+ molecules. NAD^+ reduction induces the decline of NADH-dependent ATP synthesis. A rapid decrease in the cellular ATP levels leads to necrosis.

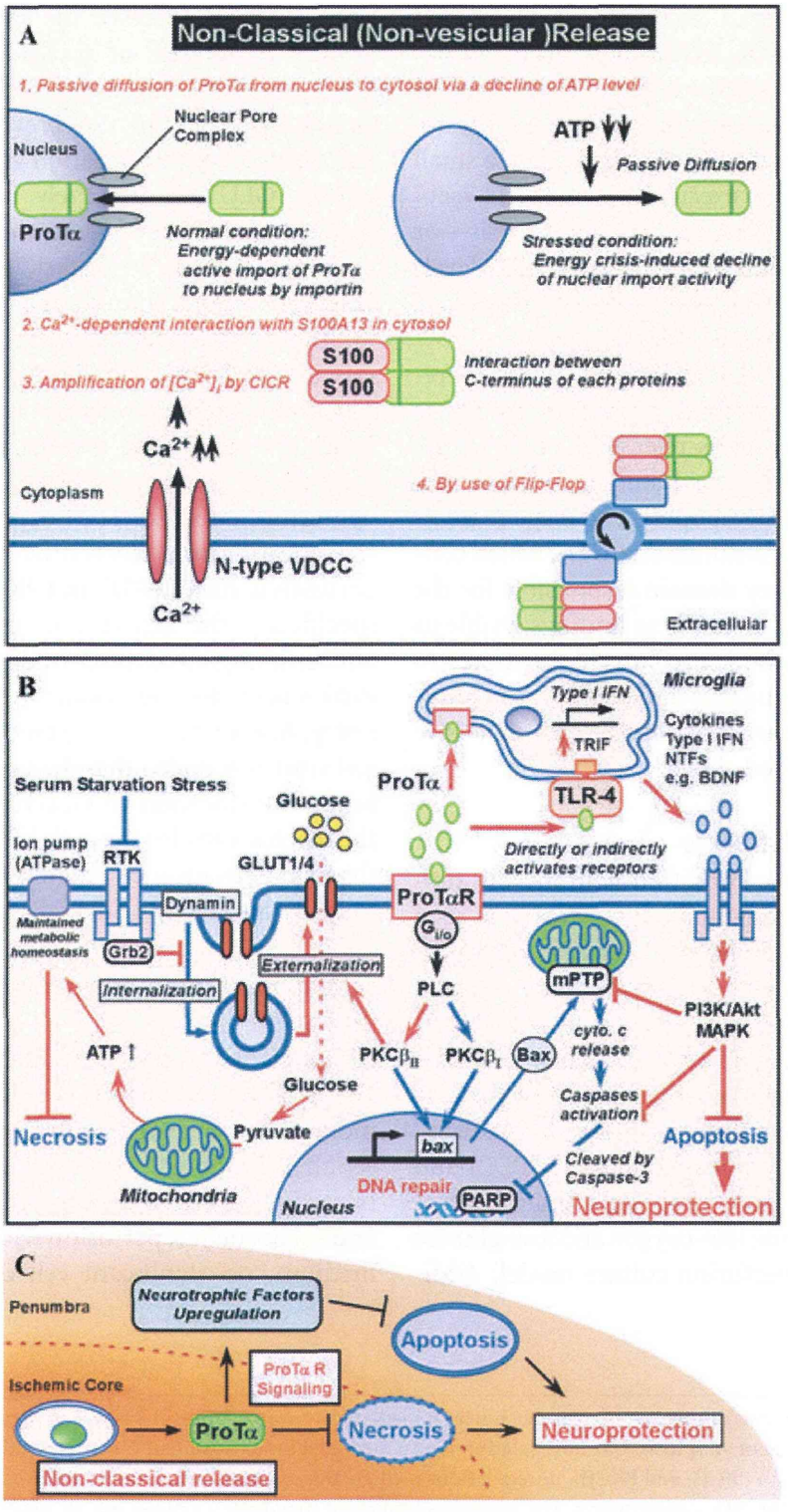

Figure 3. Schematic illustration for the extracellular release of ProTα and its neuroprotective roles in ischemic condition. (A) Necrotic stress–induced nonclassical release of ProTα. Necrosis-dependent energy crisis induces decline of nuclear import activity, subsequently ProTα, a small protein is passively diffused from the nucleus to the cytosol. Interaction between ProTα and extacellular cargo protein S100A13 is a Ca^{2+}-dependent manner through N-type voltage-dependent Ca^{2+} channel (VDCC) activity. Intracellular $[Ca^{2+}]_i$ is amplified by the mechanism of Ca^{2+}-induced Ca^{2+} release (CICR). Extracellular release of ProTα-S100A13 complex is driven by use of a flip-flop mechanism. (B) Mechanism of ProTα-induced cell death mode switch and neuroprotection. Serum-free or starvation stress leads to endocytosis of the glucose transporters GLUT1/4, which in turn causes bioenergetic catastrophe-mediated necrosis through a rapid loss of glucose supply. Addition of ProTα to ischemia-treated neurons causes translocation of GLUT1/4 to the membrane to allow sufficient glucose supply through activation of $G_{i/o}$, PLC and PKCβ_{II}. ProTα-induced apoptosis occurs

condition, causing rapid decrease in cellular ATP levels, is explained by the following possible mechanism. To retain ProTα in the nucleus against passive diffusion, the repeated recycling of importin α between cytosol and nucleus is required. Ran, a small G protein, is known to execute this importin recycling process.[43,44] Therefore, the energy crisis—or cellular ATP loss—leads to a decrease in GTP levels and disables importin recycling due to the decreased level of Ran in an active GTP-binding form.

It should be noted that there was no ProTα release when the cells were treated with apoptogenic reagents, though ProTα is released to the cytosol from the nucleus.[11] Detailed studies revealed that caspase-3, activated by apoptogenic reagent treatments, cleaves the C-terminus of ProTα, which contains an NLS and a key domain responsible for the interaction with S100A13. These findings enable us to speculate that cytosolic ProTα is redistributed to the cytosol from the nucleus under apoptotic conditions and may have an antiapoptotic self-defensive function, as mentioned above.

Antinecrosis mechanisms

Under the serum-free condition without any supplements, neurons rapidly die by necrosis, as seen in the typical necrosis features, such as disrupted plasma membranes and swollen mitochondria (in TEM analysis), and rapid decreases in [^{3}H]-2-deoxyglucose uptake and related cellular ATP levels.[9] Pharmacological studies revealed that the survival activity of recombinant ProTα is mediated through activation of phospholipase C (PLC) and protein kinase C (PKC) β.[9] Quite similar changes were reproduced in the low-oxygen and low-glucose (LOG) ischemia-reperfusion culture model. Addition of ProTα reversed the rapid decrease in the cellular ATP levels of cortical neurons following LOG-ischemic stress and reperfusion with serum-containing medium.[9] We previously reported that the membrane translocation of the glucose transporters GLUT1/4 is largely inhibited in serum-free cultures of cortical neurons, which leads to necrotic cell death.[45,46] In fact, LOG-stress decreased GLUT1/4 membrane translocation, as evaluated by immunocytochemistry and Western blot analysis, and addition of ProTα reversed these changes. This cell biological change with ProTα-induced GLUT1/4 membrane translocation enabled us to successfully characterize the putative receptor signaling mechanisms.[9] The ProTα-induced GLUT1/4 translocation was blocked by the treatments with pertussis toxin and PLC and PKCβ inhibitors. More specifically, the action was abolished by $PKC\beta_{II}$ antisense oligodeoxynucleotide (AS-ODN), though ProTα activates PKC isoforms, α, β_I, and β_{II}, but not γ, δ, ε, or ς. Taken together, the pharmacological studies revealed that the ProTα-induced membrane translocation of GLUT1/4, which underlies the mechanisms for necrosis inhibition, is mediated through activation of putative $G_{i/o}$-coupled receptor, PLC and $PKC\beta_{II}$ (Fig. 3B).

Inhibition of rapid necrosis by caspase activation

Although the addition of ProTα delayed the cell death of cortical neurons in serum-free culture, most of the neurons completely died by apoptosis after 24 hours. However, when neurons were treated with ProTα under conditions of ischemia and subsequent reperfusion with serum-containing medium, no significant cell death was observed

later at 12 h after the start of serum-free stress. The machinery is mediated by upregulation of Bax, which in turn causes mitochondrial cyto c release and subsequent apoptosis. Bax upregulation is also mediated by activation of $G_{i/o}$, PLC, and PKC, similar to the case for necrosis. However, both $PKC\beta_I$ and $PKC\beta_{II}$ upregulations mediate this apoptotic mechanism. Since caspase-3–mediated PARP degradation minimizes the ATP consumption, the apoptosis induction may have a crucial role in inhibiting the rapid necrosis. In addition, since pyruvate, a substrate for ATP production in mitochondria, inhibits necrosis but does not cause apoptosis, the apoptosis machinery seems to be independent of the necrosis inhibition. Neurotrophins, such as BDNF or EPO and type I IFN, which are expected in the ischemic brain and retina, can inhibit the apoptosis machinery at a later stage. (C) *In vivo* neuroprotective role of ProTα in the ischemic brain and retina. ProTα is first released upon ischemic stress in the ischemic core. Released ProTα exerts a suppression of the necrosis of neighboring neurons, which play a role of the early stage of neuroprotection. Expression of neurotrophic factors, such as BDNF and EPO will then occur and block the apoptosis in the penumbra (late stage of neuroprotection). Although dead cell-derived cytotoxic molecules also cause late apoptosis in the penumbra, the initial blockade of necrosis may minimize the occurrence of late apoptosis. This view may be consistent with the rationale for therapies for acute ischemic stroke.

for at least 48 hours.[9] These findings indicate that serum factors prevented ProTα-induced apoptosis. Indeed, further addition of nerve growth factor (NGF), brain-derived growth factor (BDNF), basic fibroblast growth factor (bFGF), or interleukin (IL)-6—representative apoptosis inhibitors[47–51]—rescued the cell survival in serum-free culture for 48 h, while these factors alone had no effects on the survival.[9] Similar effects were observed with BIP-V5, which blocks the translocation of Bax to mitochondria.[52] BIP-V5 also selectively inhibited the ProTα-induced apoptosis.

However, mixed addition of ProTα and *N*-benzyloxycarbonyl-Val-Ala-Asp (OMe)-fluoromethylketone (zVAD-fmk), a pan-type caspase inhibitor, caused necrosis at the later stage.[9,10] This unexpected results may be explained by the view that ProTα-induced continuous cyto.c release causes a loss of ATP and necrosis induction. The reason why ProTα alone causes apoptosis without causing necrosis may be explained by the fact that caspase-3 cleaves poly-(ADP-ribose) polymerase, PARP, which heavily uses NAD, and in turn leads to a decreased ATP synthesis (Fig. 2B). In other words, apoptosis-induction in the early stage after ischemia may play a defensive role in inhibiting rapid cell death by necrosis (Fig. 3C).

In vivo neuroprotection

Systemic and local injections of ProTα markedly inhibits the histological and functional damage induced by cerebral and retinal ischemia.[53,54] Although ProTα inhibits apoptosis as well as necrosis in *in vivo* studies, the discrepancy from *in vitro* studies may be explained by the fact that anti-apoptotic factors may inhibited the apoptotic machineries induced by ProTα. This speculation was successfully tested by the *in vivo* administration of antibodies against either brain-derived neurotrophic factor (BDNF) or erythropoietin (EPO), which restores apoptosis in cerebral and retinal ischemia models. Although ProTα upregulates BDNF levels in the retina only in the presence of ischemia, the underlying mechanisms remain elusive. Most recently, it was reported that ProTα causes immune responses through Toll-like receptor 4 (TLR4).[7,8] In this study, exogenous full-length ProTα, and endogenous ProTα released by $CD8^+$ T cells, may act as a signaling ligand for TLR4 and trigger the TRIF-mediated IFN-β induction and MyD88-mediated induction of proinflammatory cytokines, such as TNF-α, to suppress HIV-1 after the entry into macrophages.[7] As there are reports that stroke-induced brain damage is inhibited by a preconditioning treatment with LPS (an activator of TLR4),[55,56] this mechanism might cause the *in vivo* induction of antiapoptotic factors. There are several reports that TLRs cause induction of type-I IFN, which can be neroprotective.[56,57] TLR-4–mediated TRIF-IRF3 signaling may underlie major mechanisms for neuroprotection against stroke.[56,58] It is of interest that heterotrimeric G protein $G_{i/o}$ is important for the activation of MAPK and Akt downstream of TLR4, as well as for the full activation of IFN signaling downstream of TRIF-dependent signaling via TLR4.[59–61] The involvement of $G_{i/o}$ would be also consistent with the ProTα signaling, in terms of the antinecrosis pathway (see Fig. 3B).[9] In our preliminary studies, however, the preconditioning treatment of ProTα only partially inhibited retinal ischemia damage, while posttreatment with ProTα 24 h after the ischemia completely inhibited damage. Therefore, it is also speculated that additional receptor systems for ProTα play a more important for the retinal protection system.

Conclusions

ProTα and thymosin α1 possess a number of extracellular cytokine–like functions, including stimulating up-regulation of HA-DR, IL-2 receptor, dendritic cell maturation, chemotaxis, and possible antiviral, anticancer, and antifungal activities.[62–65] As we do not detect any similar neuroprotective actions with thymosin α1 for necrosis inhibition and neuroprotective actions, it is evident that the machineries underlying beneficial activities of both biologically active molecules are different. The most important take-home messages here is that ProTα plays key roles in the survival activity of intact cells, inhibits necrosis under the condition of neuronal necrosis, and inhibits apoptosis under the condition of apoptosis. The hypothesis is that ProTα acts as a "robustness" or cell death mode switch molecule from uncontrollable necrosis to neurotrophin-reversible apoptosis, and may provide a promising novel strategy for preventing serious damage in stroke (Fig. 3C). However, clarification of mechanisms underlying the intrinsic robust activity of ProTα must wait for the identification of ProTα-binding proteins, including

the $G_{i/o}$-coupled receptor for cell death mode switch. Further studies to examine how these candidate molecules play such multiple functions in cell death regulation are the next exciting step.

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Conflicts of interest

The authors declare no conflicts of interest.

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Flexible, layered biofuel cells

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ABSTRACT

Similar to conventional electrolyte batteries, biofuel cells often need to be stacked in order to boost their single cell voltage (< 1 V) up to a practical level. Here, we report a laminated stack of biofuel cells that is composed of bioanode fabrics for fructose oxidation, hydrogel sheets containing electrolyte and fuel (fructose), and O_2-diffusion biocathode fabrics. The anode and cathode fabrics were prepared by modifying fructose dehydrogenase and bilirubin oxidase, respectively, on carbon nanotubes-decorated carbon fiber fabrics. The total thickness of the single set of anode/gel/cathode sheets is just 1.1 mm. The laminated triple-layer stack produces an open-circuit voltage of 2.09 V, which is a 2.8-fold increase over that of a single set cell (0.74 V). The present layered cell (5 mm × 5 mm) produces a maximum power of 0.64 mW at 1.21 V, a level that is sufficient to drive light-emitting diodes.

1. Introduction

Enzyme-based biofuel cells (BFCs) that generate electricity through enzymatic oxidation of biological fuels like sugars and alcohols have attracted attention as ubiquitous, safe power sources (Heller, 2004; Barton et al., 2004; Cooney et al., 2008; Willner et al., 2009). In this decade, the output current of enzymatic BFCs have been dramatically improved from μA to mA level (Sakai et al., 2009; Gao et al., 2010; Zebda et al., 2011; Miyake et al., 2011a). In contrast, the cell voltage is determined by the coupling of redox reactions at anode and cathode, and is typically limited around 1.0 V, a value that is insufficient for many practical applications; for example, a single light-emitting diode (LED) requires a voltage larger than 1.6 V. Therefore, in a similar manner to electrolyte batteries, BFCs are often stacked for boosting the output voltage (Ferrigno et al., 2002; Sakai et al., 2009; Gellett et al., 2010; Holzinger et al., in press). When stacking with series-connections, each BFC should be isolated by proper packaging to prevent short-circuits via ion-conductive fuel solutions, and these packages are then interconnected electrically with metal lead. Such requirements, however, are often troublesome from the standpoint of exploiting the BFC's simplicity and disposability.

In this manuscript, we describe a layered biofuel cell constructed by laminating enzyme-modified carbon fabric (CF) strips and hydrogel film containing electrolyte and fuel as shown in Fig. 1. The hydrogel sheets ensure ion-conduction between anode/cathode fabrics, and also serve as the fuel tank that could eliminate the necessity of packaging. A BFC sheet using a conventional agarose (Haneda et al., in press) was thick and weak due to the fragile nature of agarose. In the present work, we employ a heavy-duty "double network (DN) hydrogel", resulting in a very flexible, thinner BFC (~1 mm thickness). The pre-modification of CF with carbon nanotubes (CNTs) was effective to improve the performances of both bioanode and biocathode. The laminated stack of the improved bioelectrodes was practical for LED lighting.

2. Experimental section

2.1. Preparation of carbon fabric anodes

A 5 mm × 5 mm strip (0.3 mm thickness) of carbon fabric (CF) (TCC-3250, donated from Toho Tenax Co.) was first modified with multiwalled carbon nanotubes (CNTs) (Baytubes, donated from Bayer Material Science Co.) to increase the specific surface area (Supplementary Fig. 1). The CNTs were pretreated by heating at 400 °C for 11 h and by immersing in mixed acid (H_2SO_4+HNO_3 in a 1:3 ratio) for 5 h. The treated CNT were dispersed in water containing 0.5% Triton X-100 surfactant. A 40 μl aliquot of the 10 mg ml^{-1} CNT dispersion was dropped on a CF strip and dried in air. After degassing the CNT-modified strip by immersion in a stirred McIlvaine buffer solution for more than 1 h under vacuum

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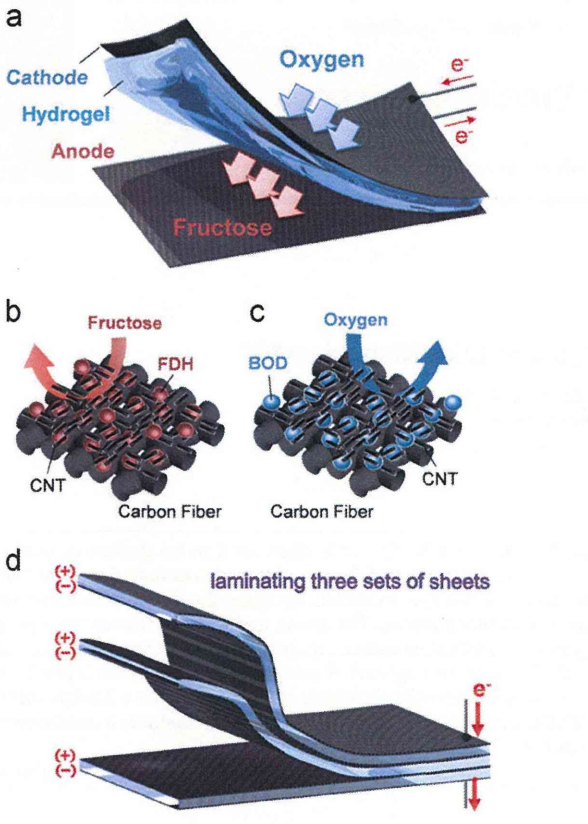

Fig. 1. (a) Schematic illustration of a biofuel cell sheet constructed by laminating enzyme-modified nanoengineered carbon fabric strips with a hydrogel film that retains electrolyte solutions and fructose fuel. (b) Schemes of fructose oxidation at the enzymatic anode. (c) Schemes of oxygen reduction at the enzymatic gas-diffusion cathode. (d) Schematic illustration of multi-lamination for boosting power.

(0.09 MPa), the CNT-modified strip shows hydrophilic property. Then, the CNT-modified CF strip was immersed in a stirred solution of D-fructose dehydrogenase (FDH) (EC1.1.99.11, 169.9 U mg^{-1}, ca. 140 kDa, from Gluconobacter, purchased from Toyobo Enzyme Co.) for FDH immobilization (Tominaga et al., 2009; Tsujimura et al., 2010; Miyake et al., 2011b). It has been reported that FDH works as an electrocatalyst for two-electron oxidation of fructose (Tominaga et al., 2007; Murata et al., 2009). The flavin-containing subunit of FDH accepts electrons from fructose, and transfers these electrons to the heme c-containing subunit that can electrically communicate with electrode. A geometric area of 0.564 cm^2 was utilized for calculation of the current density in cyclic voltammetry (CV).

2.2. Preparation of gas-diffusion carbon fabric cathodes

The preparation of the cathode basically followed the procedures used for our previous carbon particle (ketjenblack)-based BOD cathode (Miyake et al., 2011b; Haneda et al., in press). BOD is one of the multi-copper oxidases that can directly catalyze the four-electron reduction of O_2 to H_2O even without electron transfer mediators (Tsujimura et al., 2007; Wen et al., 2011). The type 1 Cu site of BOD accepts electrons from fabric electrode, and transfers these electrons to the type 2–3 cluster of BOD for O_2 reduction. A 40 μl aliquot of a 10 mg ml^{-1} CNT solution was put on a CF strip and dried in air, followed by thoroughly washing out the surfactant by soaking in an ethanol solution for more than 1 h with stirring. The surface of the CNT-modified CF electrode was further modified with a 0.1 ml solution of 5 mg ml^{-1} bilirubin oxidase (BOD, EC 1.3.3.5, 2.5 U/mg, from Myrothecium) in vacuum oven (AVO-205N, purchased from AS ONE, 0.09 MPa, 35 C°). The strip was additionally coated with the CNT solution to make the surface hydrophobic. A surface area of 0.25 cm^2 was utilized for calculation of the current density in cyclic voltammetry (CV).

2.3. Preparation of the hydrogel films

The fructose-containing double-network (DN) hydrogel films were prepared by a three-step process(Gong, 2010; Wu and Gong, 2011): (1) single network hydrogel formation, (2) second network formation into the single network hydrogel and (3) loading of 500 mM fructose. We used stock solutions A, B and C. Solution A contains 2-acrylamido-2-methlypropane (AMPS, 1 M), N,N-methylenebisacrylamide (MBAA, 40 mM), 2-oxoglutaric acid (OA, 1 mM) and ammonium persulfate (APS, 19 mM). Solution B was a mixture of acrylamide (AAm, 4 M), OA (1 mM), NaCl (80 mM) and APS (19 mM). Solution C contains AAm (2 M), OA (1 mM) and APS (19 mM). At first, the solution A was poured into a silicone mold, and preliminarily crosslinked by UV exposure (265 nm, 8 W) for 5 h. The formed soft gel was then immersed in solution B for 14 h to prevent dramatic swelling and further irradiated with the UV lamp for 5 h to reinforce the gel in order to become a sheet. After washing with water for 24 h, the gel sheet was immersed in solution C for 14 h followed by UV irradiation (5 h) to form a DN network. Finally, the DN gel sheet was immersed in 500 mM fructose solution for 24 h.

2.4. Electrochemical measurements

The performance of the CF electrodes was analyzed by a three-electrode system (BSA, 730C electrochemical analyzer) in solution using a Ag/AgCl reference and a platinum counter electrode. The FDH-modified anodes were evaluated in stirred McIlvaine buffer (pH 5.0) containing 500 mM fructose, while the BOD-modified cathodes were used in air-saturated McIlvaine buffer (pH 5.0). The performance of a biofuel cell sheet constructed using the fructose-containing DN hydrogel film (0.5 mm thick) was evaluated from the cell voltage upon connecting with a variable external resistance between 180 Ω and 10 kΩ. The current and the power were derived from the cell voltage and the resistance. Unless otherwise indicated, the electrochemical measurements were carried out at room temperature, around 25 °C.

3. Results and discussion

3.1. Performance of FDH/CNT/CF bioanodes

Fig. 2a shows cyclic voltammograms of the FDH/CNT/CF electrodes (solid plots) at 10 mV s^{-1} in a stirred buffer solution containing 500 mM fructose. In comparison with the FDH/CF electrode prepared without CNTs (broken line plot), the increased specific surface area produced by CNT-modification obviously increased the current density by at least an order of magnitude. In fact, the measured double-layer capacitance of the CNT-modified electrodes has a 2 orders larger value (ca. 6.7 mF cm^{-2}) than that of the original CF (0.07 mF cm^{-2}). The oxidation current density depended on the concentration of the Triton X-100 surfactant used for the CNT dispersion (Haneda et al., in press). The CNT dispersion with 0.5% surfactant is capable of entirely penetrating into the CF strip (see Supplementary Fig. 1b). This uniform modification with CNT would be a reason for the enhanced anode performance. In addition, the electrode