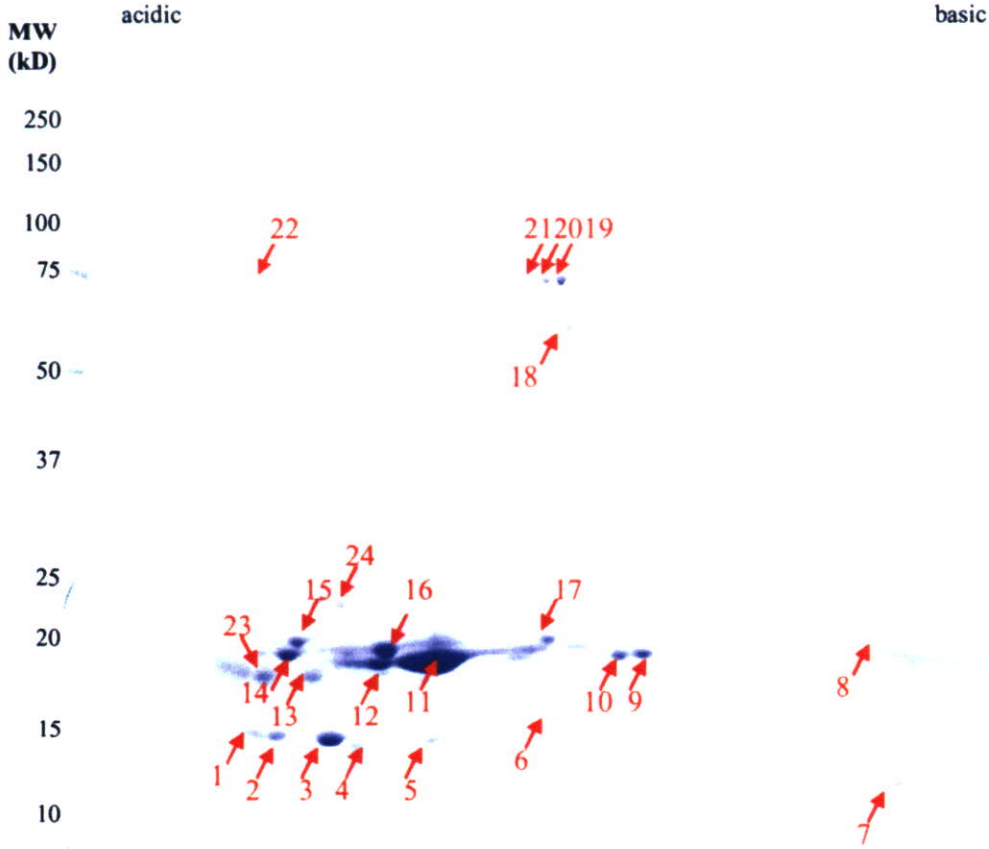
図 23　ラット尿中タンパク質の２次元電気泳動解析



（MALDI-TOF マス解析による同定結果）

spot No.	MS	MS/MS
1	nd	nd
2	nd	nd
3	nd	AY327506
4	nd	prostatic steroid-binding protein chain C3 precursor
5	nd	nd
6	nd	urinary protein 2 precursor
7	nd	nd
8	RATVPSP	RATVPSP
9	alpha-2u-globulin, cain A	alpha-2u-globulin, cain A (precursor)
10	alpha-2u-globulin, cain A	alpha-2u-globulin, cain A (precursor)
11	alpha-2u-globulin, cain A	alpha-2u-globulin, cain A (precursor)
12	alpha-2u-globulin, cain A	alpha-2u-globulin, cain A (precursor)
13	nd	urinary protein 2 precursor
14	alpha-2u-globulin (precursor)	alpha-2u-globulin (precursor)
15	alpha-2u-globulin (precursor)	alpha-2u-globulin (precursor)
16	nd	alpha-2u-globulin, cain A (precursor)
17	nd	alpha-2u-globulin, cain A (precursor)
18	Alpha-amylase	Alpha-amylase
19	Albumin	nd
20	Albumin	serum albumin precursor
21	Albumin	nd
22	Albumin	nd
23	nd	nd
24	alpha-2u-globulin, cain A	nd

図 24　抗体結合磁気ビーズによる高発現タンパク質の除去

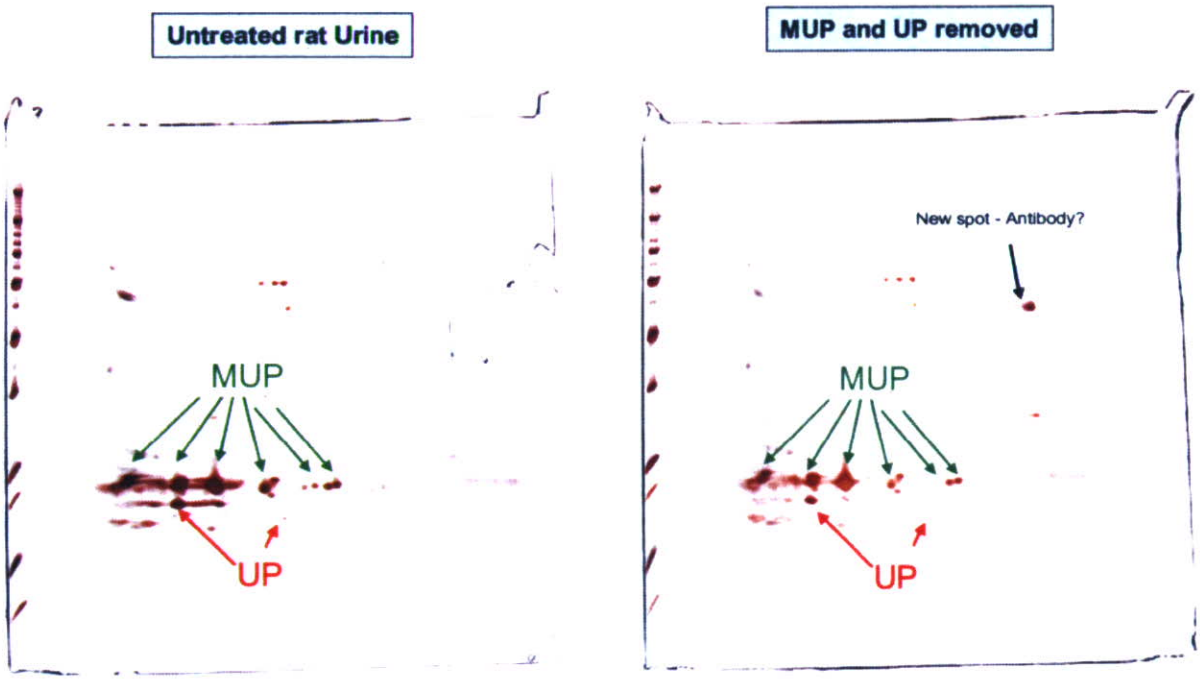
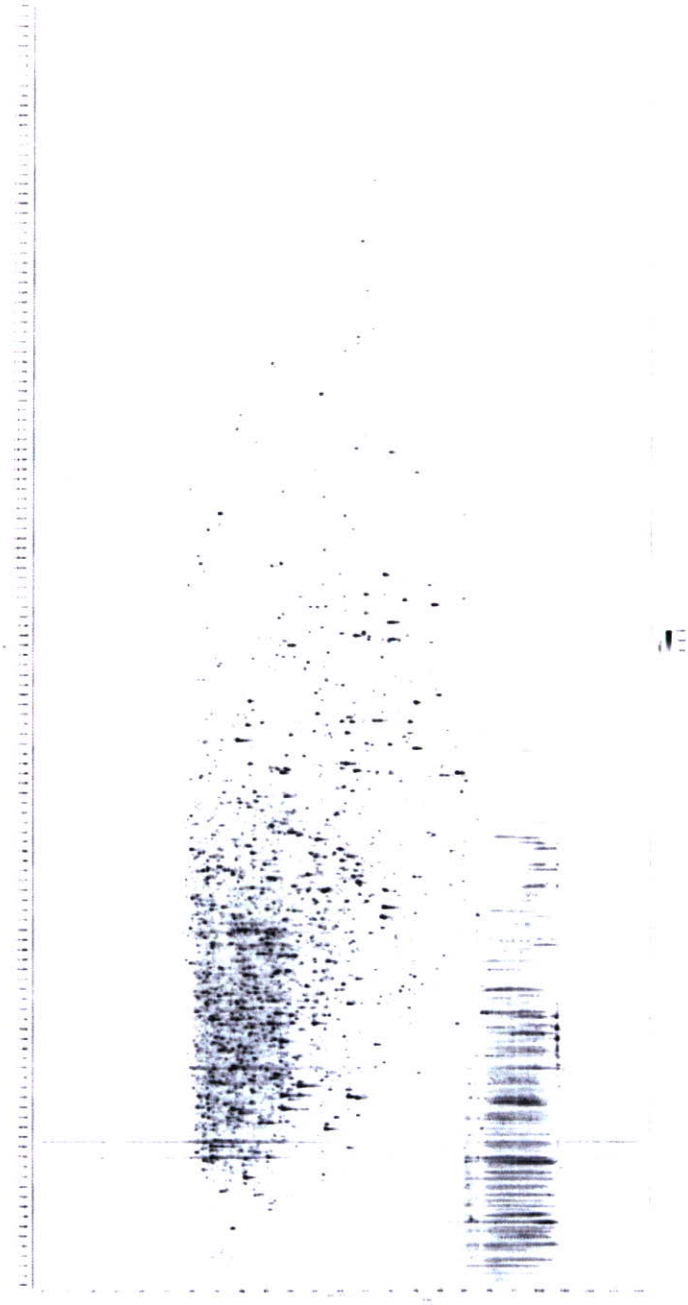


図 25　ヒト正常コントロール尿サンプルのプロテオーム解析



50 proteins was identified by standard LC/MSMS analysis

表1　ヒトプールド肝サイトゾル中でMASCOT検索により同定されたタンパク質

NAME	ID	Description
CPSM_HUMAN	(P31327)	Carbamoyl-phosphate synthase [ammonia], mitochondrial precursor (EC 6.3.4.16)
ADHB_HUMAN	(P00325)	Alcohol dehydrogenase beta chain (EC 1.1.1.1)
ADH4_HUMAN	(P08319)	Alcohol dehydrogenase class II pi chain (EC 1.1.1.1)
ALDOB_HUMAN	(P05062)	Fructose-bisphosphate aldolase B (EC 4.1.2.13) (Liver-type aldolase)
ADHA_HUMAN	(P07327)	Alcohol dehydrogenase alpha chain (EC 1.1.1.1)
ADHG_HUMAN	(P00326)	Alcohol dehydrogenase gamma chain (EC 1.1.1.1)
G3P2_HUMAN	(P04406)	Glyceraldehyde-3-phosphate dehydrogenase, liver (EC 1.2.1.12) (GAPDH)
FABPL_HUMAN	(P07148)	Fatty acid-binding protein, liver (L-FABP)
GSTA1_HUMAN	(P08263)	Glutathione S-transferase A1 (EC 2.5.1.18) (GTH1) (HA subunit 1) (GST-epsilon) (GSTA1-1)
ACTB_HUMAN	(P60709)	Actin, cytoplasmic 1 (Beta-actin)
ACTA_HUMAN	(P62736)	Actin, aortic smooth muscle (Alpha-actin-2)
HBB_HUMAN	(P68871)	Hemoglobin beta subunit (Hemoglobin beta chain) (Beta-globin)
GSTA2_HUMAN	(P09210)	Glutathione S-transferase A2 (EC 2.5.1.18) (GTH2) (HA subunit 2) (GST-gamma) (GSTA2-2)
HBA_HUMAN	(P69905)	Hemoglobin alpha subunit (Hemoglobin alpha chain) (Alpha-globin)
FTCD_HUMAN	(O95954)	Formimidoyltransferase-cyclodeaminase (Formiminotransferase-cyclodeaminase) (FTCD) (LCHC1)
DHE3_HUMAN	(P00367)	Glutamate dehydrogenase 1, mitochondrial precursor (EC 1.4.1.3) (GDH)
GSTA3_HUMAN	(Q16772)	Glutathione S-transferase A3-3 (EC 2.5.1.18) (GST class-alpha)
PRDX1_HUMAN	(Q06830)	Peroxiredoxin 1 (EC 1.11.1.15) (Thioredoxin peroxidase 2) (Thioredoxin-dependent peroxide
PRDX6_HUMAN	(P30041)	Peroxiredoxin 6 (EC 1.11.1.15) (Antioxidant protein 2) (1-Cys peroxiredoxin) (1-Cys PRX)
EF1A1_HUMAN	(P68104)	Elongation factor 1-alpha 1 (EF-1-alpha-1) (Elongation factor 1 A-1) (eEF1A-1)
ASSY_HUMAN	(P00966)	Argininosuccinate synthase (EC 6.3.4.5) (Citrulline--aspartate ligase)
DCXR_HUMAN	(Q7Z4W1)	L-xylulose reductase (EC 1.1.1.10) (XR) (Dicarbonyl/L-xylulose reductase)
ARGI1_HUMAN	(P05089)	Arginase-1 (EC 3.5.3.1) (Type I arginase) (Liver-type arginase)
ECHM_HUMAN	(P30084)	Enoyl-CoA hydratase, mitochondrial precursor (EC 4.2.1.17) (Short chain enoyl-CoA hydratase
FAS_HUMAN	(P49327)	Fatty acid synthase (EC 2.3.1.85) [Includes: [Acyl-carrier-protein] S-acetyltransferase
AL1A1_HUMAN	(P00352)	Retinal dehydrogenase 1 (EC 1.2.1.36) (RalDH1) (RALDH 1) (Aldehyde dehydrogenase family 1
ST2A1_HUMAN	(Q06520)	Alcohol sulfotransferase (EC 2.8.2.2) (Hydroxysteroid Sulfotransferase) (HST)
HMCS2_HUMAN	(P54868)	Hydroxymethylglutaryl-CoA synthase, mitochondrial precursor (EC 2.3.3.10)
IDHC_HUMAN	(O75874)	Isocitrate dehydrogenase [NADP] cytoplasmic (EC 1.1.1.42) (Oxalosuccinate decarboxylase)
PEBP_HUMAN	(P30086)	Phosphatidylethanolamine-binding protein (PEBP) (Prostatic binding protein) (HCNPpp)
SODC_HUMAN	(P00441)	Superoxide dismutase [Cu-Zn] (EC 1.15.1.1)
PPIA_HUMAN	(P62937)	Peptidyl-prolyl cis-trans isomerase A (EC 5.2.1.8) (PPIase) (Rotamase) (Cyclophilin A)
AK1C1_HUMAN	(Q04828)	Aldo-keto reductase family 1 member C1 (EC 1.1.1.-) (20-alpha-hydroxysteroid dehydrogenase
CH60_HUMAN	(P10809)	60 kDa heat shock protein, mitochondrial precursor (Hsp60) (60 kDa chaperonin) (CPN60)
ALDH2_HUMAN	(P05091)	Aldehyde dehydrogenase, mitochondrial precursor (EC 1.2.1.3) (ALDH class 2) (ALDH1)
SPYA_HUMAN	(P21549)	Serine--pyruvate aminotransferase (EC 2.6.1.51) (SPT) (Alanine--glyoxylate aminotransferase)
HBD_HUMAN	(P02042)	Hemoglobin delta subunit (Hemoglobin delta chain) (Delta-globin)
THIL_HUMAN	(P24752)	Acetyl-CoA acetyltransferase, mitochondrial precursor (EC 2.3.1.9)
LDHA_HUMAN	(P00338)	L-lactate dehydrogenase A chain (EC 1.1.1.27) (LDH-A) (LDH muscle subunit) (LDH-M)
ANXA6_HUMAN	(P08133)	Annexin A6 (Annexin VI) (Lipocortin VI) (P68) (P70) (Protein III) (Chromobindin-20)
GOGB1_HUMAN	(Q14789)	Golgi autoantigen, golgin subfamily B member 1 (Giantin) (Macrogolgin)
G3P1_HUMAN	(P00354)	Glyceraldehyde-3-phosphate dehydrogenase, muscle (EC 1.2.1.12) (GAPDH)
ANK2_HUMAN	(Q01484)	Ankyrin-2 (Brain ankyrin) (Ankyrin B) (Ankyrin, nonerythroid)
THIM_HUMAN	(P42765)	3-ketoacyl-CoA thiolase, mitochondrial (EC 2.3.1.16) (Beta-ketothiolase)
SYNE1_HUMAN	(Q8NF91)	Nesprin-1 (Nuclear envelope spectrin repeat protein 1) (Synaptic nuclear envelope protein 1) (Syne-1) (Myocyte nuclear envelope protein 1) (Myne-1) (Enaptin)
F16P1_HUMAN	(P09467)	Fructose-1,6-bisphosphatase 1 (EC 3.1.3.11) (D-fructose-1,6-bisphosphate 1-phosphohydrolase
IQGA2_HUMAN	(Q13576)	Ras GTPase-activating-like protein IQGAP2
HBG1_HUMAN	(P69891)	Hemoglobin gamma-1 chain (Hemoglobin gamma-A chain) (Hb F Agamma)
MLL3_HUMAN	(Q8NEZ4)	Myeloid/lymphoid or mixed-lineage leukemia protein 3 homolog
NEBU_HUMAN	(P20929)	Nebulin
ZF106_HUMAN	(Q9H2Y7)	Zinc finger protein 106 homolog (Zfp-106) (Zinc finger protein 474)
TENS_HUMAN	(Q9HBL0)	Tensin
UGPA1_HUMAN	(Q07131)	UTP--glucose-1-phosphate uridylyltransferase 1 (EC 2.7.7.9) (UDP-glucose pyrophosphorylase
ADH7_HUMAN	(P40394)	Alcohol dehydrogenase class IV mu/sigma chain (EC 1.1.1.1) (Retinol dehydrogenase)
FXL19_HUMAN	(Q6PCT2)	F-box/LRR-repeat protein 19 (F-box and leucine-rich repeat protein 19)
ACSL5_HUMAN	(Q9ULC5)	Long-chain-fatty-acid--CoA ligase 5 (EC 6.2.1.3) (Long-chain acyl-CoA synthetase 5)
PAR3L_HUMAN	(Q8TEW8)	Amyotrophic lateral sclerosis 2 chromosome region candidate gene protein 19
THIO_HUMAN	(P10599)	Thioredoxin (ATL-derived factor) (ADF) (Surface associated sulphydryl protein) (SASP)
ATP5H_HUMAN	(O75947)	ATP synthase D chain, mitochondrial (EC 3.6.3.14)
LYRIC_HUMAN	(Q86UE4)	LYRIC protein (Lysine-rich CEACAM1 co-isolated protein) (3D3/lyric)
DHSO_HUMAN	(Q00796)	Sorbitol dehydrogenase (EC 1.1.1.14) (L-iditol 2-dehydrogenase)
NCOA6_HUMAN	(Q14686)	Nuclear receptor coactivator 6 (Amplified in breast cancer-3 protein) (Activating signal cointegrator-2) (ASC-2) (Peroxisome proliferator-activated receptor-interacting protein)
SPTN5_HUMAN	(Q9NRC6)	Spectrin beta chain, brain 4 (Spectrin, non-erythroid beta chain 4) (Beta-V spectrin)
GNRP_HUMAN	(Q13972)	Guanine nucleotide-releasing protein (GNRP) (Ras-specific nucleotide exchange factor CDC25) (Ras-specific guanine nucleotide-releasing factor)
CLAP1_HUMAN	(Q7Z460)	CLIP-associating protein 1 (Cytoplasmic linker-associated protein 1) (Multiple asters homolog 1)
CJ012_HUMAN	(Q8N655)	Protein C10orf12
HYES_HUMAN	(P34913)	Epoxide hydrolase 2 (EC 3.3.2.3) (Soluble epoxide hydrolase) (SEH) (Epoxide hydratase) (Cy
M3K3_HUMAN	(Q99759)	Mitogen-activated protein kinase kinase kinase 3 (EC 2.7.1.37) (MAPK/ERK kinase kinase 3) (MEK kinase 3) (MEKK 3)
DHX37_HUMAN	(Q8IY37)	Probable ATP-dependent helicase DHX37 (DEAH-box protein 37)
TENX_HUMAN	(P22105)	Tenascin-X precursor (TN-X) (Hexabrachion-like protein)
DYH5_HUMAN	(Q8TE73)	Ciliary dynein heavy chain 5 (Axonemal beta dynein heavy chain 5) (HL1)
APC_HUMAN	(P25054)	Adenomatous polyposis coli protein (APC protein)
RTEL1_HUMAN	(Q9NZ71)	Regulator of telomere elongation helicase 1 (Helicase-like protein NHL)
LRFN5_HUMAN	(Q96NI6)	Leucine-rich repeat and fibronectin type-III domain-containing protein 5 precursor
ANR11_HUMAN	(Q6UB99)	Ankyrin repeat domain protein 11 (Ankyrin repeat-containing cofactor 1)
MYO1A_HUMAN	(Q9UBC5)	Myosin Ia (Brush border myosin I) (BBM-I) (BBMI) (Myosin I heavy chain) (MIHC)
PAPOA_HUMAN	(P51003)	Poly(A) polymerase alpha (EC 2.7.7.19) (PAP) (Polynucleotide adenylyltransferase alpha)
CF181_HUMAN	(Q6IEG0)	U11/U12 snRNP 48 kDa protein
CO7A1_HUMAN	(Q02388)	Collagen alpha 1(VII) chain precursor (Long-chain collagen) (LC collagen)
MYO5A_HUMAN	(Q9Y4I1)	Myosin-5A (Myosin Va) (Dilute myosin heavy chain, non-muscle) (Myosin heavy chain 12) (Myoxin)
CAR10_HUMAN	(Q9BWT7)	Caspase recruitment domain protein 10 (CARD-containing MAGUK protein 3) (Carma 3)
ZNF44_HUMAN	(P15621)	Zinc finger protein 44 (Zinc finger protein KOX7) (Gonadotropin inducible transcription repressor-2)
ZEP1_HUMAN	(P15822)	Zinc finger protein 40 (Human immunodeficiency virus type I enhancer-binding protein 1)
UN13B_HUMAN	(O14795)	Unc-13 homolog B (Munc13-2) (munc13)
GBF1_HUMAN	(Q92538)	Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1 (BFA-resistant
CHD8_HUMAN	(Q9HCK8)	Chromodomain-helicase-DNA-binding protein 8 (CHD-8) (Helicase with SNF2 domain 1) (Fragment)
AL1A2_HUMAN	(O94788)	Retinal dehydrogenase 2 (EC 1.2.1.36) (RalDH2) (RALDH 2) (RALDH(II))
ATM_HUMAN	(Q13315)	Serine-protein kinase ATM (EC 2.7.1.37) (Ataxia telangiectasia mutated) (A-T, mutated)
PCLO_HUMAN	(Q9Y6V0)	Piccolo protein (Aczonin)
CELR3_HUMAN	(Q9NYQ7)	Cadherin EGF LAG seven-pass G-type receptor 3 precursor (Flamingo homolog 1)
AKAP2_HUMAN	(Q9Y2D5)	A-kinase anchor protein 2 (Protein kinase A anchoring protein 2) (PRKA2) (AKAP-2)
CO4A3_HUMAN	(Q01955)	Collagen alpha 3(IV) chain precursor (Goodpasture antigen)
ATBF1_HUMAN	(Q15911)	Alpha-fetoprotein enhancer binding protein (AT motif-binding factor) (AT-binding transcrip
ABCAC_HUMAN	(Q86UK0)	ATP-binding cassette sub-family A member 12 (ATP-binding cassette transporter 12)
G3PT_HUMAN	(O14556)	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific (EC 1.2.1.12) (Spermatogenic cel
RASL1_HUMAN	(O95294)	RasGAP-activating-like protein 1
BRCA2_HUMAN	(P51587)	Breast cancer type 2 susceptibility protein (Fanconi anemia group D1 protein)
COPB2_HUMAN	(P35606)	Coatomer beta' subunit (Beta'-coat protein) (Beta'-COP) (p102)
HS90B_HUMAN	(P08238)	Heat shock protein HSP 90-beta (HSP 84) (HSP 90)
NIPBL_HUMAN	(Q6KC79)	Nipped-B-like protein (Delangin) (SCC2 homolog)
ATR_HUMAN	(Q13535)	Serine-protein kinase ATR (EC 2.7.1.37) (Ataxia telangiectasia and Rad3-related protein)
DYH9_HUMAN	(Q9NYC9)	Ciliary dynein heavy chain 9 (Axonemal beta dynein heavy chain 9)
BPAEA_HUMAN	(O94833)	Bullous pemphigoid antigen 1, isoforms 6/9/10 (Trabeculin-beta) (Bullous pemphigoid antigen) (BPA) (Hemidesmosomal plaque protein) (Dystonia musculorum protein) (Dystonin)
SUPT3_HUMAN	(O75486)	Transcription initiation protein SPT3 homolog (SPT3-like protein)
NYX_HUMAN	(Q9GZU5)	Nyctalopin precursor
HOOK2_HUMAN	(Q96ED9)	Hook homolog 2 (h-hook2) (hHK2)
PLK1_HUMAN	(P53350)	Serine/threonine-protein kinase PLK1 (EC 2.7.1.37) (Polo-like kinase 1) (PLK-1) (Serine/threonine-protein kinase 13) (STPK13)
KIF14_HUMAN	(Q15058)	Kinesin-like protein KIF14
TM16E_HUMAN	(Q75V66)	Transmembrane protein 16E (Gnathodiaphyseal dysplasia 1 protein)
CAC1B_HUMAN	(Q00975)	Voltage-dependent N-type calcium channel alpha-1B subunit
CO6A2_HUMAN	(P12110)	Collagen alpha 2(VI) chain precursor
POK3_HUMAN	(Q9WJR5)	HERV-K_19q12 provirus ancestral Pol protein (HERV-K(C19) Pol protein)
LAMA3_HUMAN	(Q16787)	Laminin alpha-3 chain precursor (Epiligrin 170 kDa subunit) (E170) (Nicein alpha subunit)
MPIP1_HUMAN	(P30304)	M-phase inducer phosphatase 1 (EC 3.1.3.48) (Dual specificity phosphatase Cdc25A)
DESP_HUMAN	(P15924)	Desmoplakin (DP) (250/210 kDa paraneoplastic pemphigus antigen)
ZN490_HUMAN	(Q9ULM2)	Zinc finger protein 490
I2C4_HUMAN	(Q9HCK5)	Eukaryotic translation initiation factor 2C 4 (eIF2C 4) (eIF-2C 4) (Argonaute-4)
DISC1_HUMAN	(Q9NRI5)	Disrupted in schizophrenia 1 protein
HS90A_HUMAN	(P07900)	Heat shock protein HSP 90-alpha (HSP 86)
LGR8_HUMAN	(Q8WXD0)	Relaxin receptor 2 (Leucine-rich repeat-containing G-protein coupled receptor 8)
FBX18_HUMAN	(Q8NFZ0)	F-box only protein 18 (EC 3.6.1.-) (F-box DNA helicase 1)
GEMI4_HUMAN	(P57678)	Component of gems 4 (Gemin-4) (p97)
ARP2_HUMAN	(P61160)	Actin-like protein 2 (Actin-related protein 2)
ZSWM5_HUMAN	(Q9P217)	Zinc finger SWIM domain containing protein 5
SMRC2_HUMAN	(Q8TAQ2)	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily C membe
VINC_HUMAN	(P18206)	Vinculin (Metavinculin)
SMCA4_HUMAN	(P51532)	Possible global transcription activator SNF2L4 (SNF2-beta) (BRG-1 protein) (Mitotic growth and transcription activator) (Brahma protein homolog 1) (SWI/SNF related matrix associated actin dependent regulator of chromatin subfamily A
BC11A_HUMAN	(Q9H165)	B-cell lymphoma/leukemia 11A (B-cell CLL/lymphoma 11A) (COUP-TF interacting protein 1) (Ecotropic viral integration site 9 protein) (EVI-9)
FBX46_HUMAN	(Q6PJ61)	F-box only protein 46 (F-box only protein 34-like)
JPH3_HUMAN	(Q8WXH2)	Junctophilin-3 (Junctophilin type 3) (JP-3)
RL10_HUMAN	(P27635)	60S ribosomal protein L10 (QM protein) (Tumor suppressor QM) (Laminin receptor homolog)
4ET_HUMAN	(Q9NRA8)	Eukaryotic translation initiation factor 4E transporter (eIF4E transporter) (4E-T)
STK31_HUMAN	(Q9BXU1)	Serine/threonine-protein kinase 31 (EC 2.7.1.37) (Serine/threonine-protein kinase NYD-SPK)
CEP2_HUMAN	(Q9BV73)	Centrosomal protein 2 (Centrosomal Nek2-associated protein 1) (C-NAP1) (Centrosome protein 250) (Centrosome associated protein CEP250)
CN145_HUMAN	(Q6ZU80)	Protein C14orf145
RP1L1_HUMAN	(Q8IWN7)	Retinitis pigmentosa 1-like 1 protein
KIF3C_HUMAN	(O14782)	Kinesin-like protein KIF3C
UBP8_HUMAN	(P40818)	Ubiquitin carboxyl-terminal hydrolase 8 (EC 3.1.2.15) (Ubiquitin thiolesterase 8) (Ubiquitin-specific processing protease 8) (Deubiquitinating enzyme 8) (hUBPy)
LAMA2_HUMAN	(P24043)	Laminin alpha-2 chain precursor (Laminin M chain) (Merosin heavy chain)
SMC3_HUMAN	(Q9UQE7)	Structural maintenance of chromosome 3 (Chondroitin sulfate proteoglycan 6) (Chromosome-associated polypeptide) (hCAP) (Bamacan) (Basement membrane-associated chondroitin
GOGA3_HUMAN	(Q08378)	Golgi autoantigen, golgin subfamily A member 3 (Golgin-160) (Golgi complex-associated protein of 170 kDa) (GCP170)
ACTZ_HUMAN	(P61163)	Alpha-centractin (Centractin) (Centrosome-associated actin homolog) (Actin-RPV) (ARP1)

表2　ラット尿中でMASCOT検索により同定されたタンパク質

NAME	ID	Description
ALBU_RAT	(P02770)	Serum albumin precursor
MUP_RAT	(P02761)	Major urinary protein precursor (MUP) (Alpha-2u-globulin) (Alpha(2)-euglobulin)
EGF_RAT	(P07522)	Pro-epidermal growth factor precursor (EGF) [Contains: Epidermal growth factor]
22P1_RAT	(P22282)	Cystatin-related protein 1 precursor (CRP-1) (Prostatic 22 kDa glycoprotein P22K16/P22K20)
UROM_RAT	(P27590)	Uromodulin precursor (Tamm-Horsfall urinary glycoprotein) (THP)
CPI3_RAT	(P05544)	Contrapsin-like protease inhibitor 3 precursor (CPI-23) (Serine protease inhibitor 1) (SPI
CPI1_RAT	(P05545)	Contrapsin-like protease inhibitor 1 precursor (CPI-21) (Kallikrein-binding protein) (KBP)
AMPN_RAT	(P15684)	Aminopeptidase N (EC 3.4.11.2) (rAPN) (Alanyl aminopeptidase) (Microsomal aminopeptidase)
AMBP_RAT	(Q64240)	AMBP protein precursor [Contains: Alpha-1-microglobulin; Inter-alpha-trypsin inhibitor lig
KLK7_RAT	(P35373)	Glandular kallikrein-7, submandibular/renal precursor (EC 3.4.21.35) (Tissue kallikrein)
TRFE_RAT	(P12346)	Serotransferrin precursor (Transferrin) (Siderophilin) (Beta-1-metal binding globulin)
A1AT_RAT	(P17475)	Alpha-1-antiproteinase precursor (Alpha-1-antitrypsin) (Alpha-1-proteinase inhibitor)
UP1_RAT	(P81827)	Urinary protein 1 precursor (RUP-1) (Liver regeneration-related protein LRRG05)
UP3_RAT	(P83121)	Urinary protein 3 precursor (RUP-3)
ACTB_RAT	(P60711)	Actin, cytoplasmic 1 (Beta-actin)
UP2_RAT	(P81828)	Urinary protein 2 precursor (RUP-2)
ACTC_RAT	(P68035)	Actin, alpha cardiac (Alpha-cardiac actin)
ACTS_RAT	(P68136)	Actin, alpha skeletal muscle (Alpha-actin-1)
AMYP_RAT	(P00689)	Pancreatic alpha-amylase precursor (EC 3.2.1.1) (PA) (1,4-alpha-D-glucan glucanohydrolase)
PSC3_RAT	(P02780)	Prostatic steroid-binding protein C3 chain precursor (Prostatein peptide C3)
KACA_RAT	(P01836)	Ig kappa chain C region, A allele
GGT1_RAT	(P07314)	Gamma-glutamyltranspeptidase 1 precursor (EC 2.3.2.2) (Gamma-glutamyltransferase 1)
HEMO_RAT	(P20059)	Hemopexin precursor
SODC_RAT	(P07632)	Superoxide dismutase [Cu-Zn] (EC 1.15.1.1)
SLC31_RAT	(Q64319)	Neutral and basic amino acid transport protein rBAT (B(0,+)-type amino acid transport prot
KLK9_RAT	(P07847)	Submandibular glandular kallikrein-9 precursor (EC 3.4.21.35) (Tissue kallikrein) (S3 kall
HBB2_RAT	(P11517)	Hemoglobin beta-2 subunit (Hemoglobin beta-2 chain) (Beta-2-globin) (Hemoglobin beta chain
KLK1_RAT	(P00758)	Nerve growth factor gamma chain precursor (EC 3.4.21.35) (Gamma-NGF) (Tissue kallikrein)
B2MG_RAT	(P07151)	Beta-2-microglobulin precursor
MEP1A_RAT	(Q64230)	Meprin A alpha-subunit precursor (EC 3.4.24.18) (Endopeptidase-2) (MEP-1) (Endopeptidase-2)
NOTC2_RAT	(Q9QW30)	Neurogenic locus notch homolog protein 2 precursor (Notch 2) [Contains: Notch 2 extracellu
PBAS_RAT	(P15399)	Probasin precursor (PB) (M-60)
SYCP1_RAT	(Q03410)	Synaptonemal complex protein 1 (SCP-1 protein)
SPA3M_RAT	(Q63556)	Serine protease inhibitor A3M precursor (Serine protease inhibitor 2.4) (SPI-2.4)
HBA_RAT	(P01946)	Hemoglobin alpha-1/2 subunit (Hemoglobin alpha-1/2 chain) (Alpha-1/2-globin)
ATOX1_RAT	(Q9WUC4)	Copper transport protein ATOX1 (Metal transport protein ATX1)
PSC2_RAT	(P02781)	Prostatic steroid-binding protein C2 chain precursor (Prostatein peptide C2)
MYH7_RAT	(P02564)	Myosin heavy chain, cardiac muscle beta isoform (MyHC-beta)
DNMT1_RAT	(Q9Z330)	DNA (cytosine-5)-methyltransferase 1 (EC 2.1.1.37) (Dnmt1) (DNA methyltransferase I)
KLK12_RAT	(P36376)	Glandular kallikrein-12, submandibular/renal precursor (EC 3.4.21.35) (Tissue kallikrein)
MYH10_RAT	(Q9JLT0)	Myosin-10 (Myosin heavy chain, nonmuscle IIb) (Nonmuscle myosin heavy chain IIb)
PLEC1_RAT	(P30427)	Plectin 1 (PLTN) (PCN)
MYH6_RAT	(P02563)	Myosin heavy chain, cardiac muscle alpha isoform (MyHC-alpha)
22P2_RAT	(P22283)	Cystatin-related protein 2 precursor (Prostatic 22 kDa glycoprotein P22K15)
PCLO_RAT	(Q9JKS6)	Piccolo protein (Multidomain presynaptic cytomatrix protein)
MOES_RAT	(O35763)	Moesin (Membrane-organizing extension spike protein)
GRK4_RAT	(P70507)	G protein-coupled receptor kinase 4 (EC 2.7.1.-) (G protein-coupled receptor kinase GRK4)
CAC1C_RAT	(P22002)	Voltage-dependent L-type calcium channel alpha-1C subunit (Voltage-gated calcium channel alpha subunit Cav1.2)
CAC1H_RAT	(Q9EQS0)	Voltage-dependent T-type calcium channel alpha-1H subunit
KLK3_RAT	(P15950)	Glandular kallikrein-3, submandibular (EC 3.4.21.35) (Tissue kallikrein) (S1 kallikrein)
XAB2_RAT	(Q99PK0)	XPA-binding protein 2 (Adapter protein ATM-55)
PLMN_RAT	(Q01177)	Plasminogen precursor (EC 3.4.21.7) [Contains: Plasmin heavy chain A; Activation peptide;
EZRI_RAT	(P31977)	Ezrin (p81) (Cytovillin) (Villin-2)
EI2BD_RAT	(Q63186)	Translation initiation factor eIF-2B delta subunit (eIF-2B GDP-GTP exchange factor)
PGFRA_RAT	(P20786)	Alpha platelet-derived growth factor receptor precursor (EC 2.7.1.112) (PDGF-R-alpha)
FAS_RAT	(P12785)	Fatty acid synthase (EC 2.3.1.85) [Includes: [Acyl-carrier-protein] S-acetyltransferase (E
THYG_RAT	(P06882)	Thyroglobulin precursor
CO4_RAT	(P08649)	Complement C4 precursor [Contains: Complement C4 beta chain; Complement C4 alpha chain; C4
ARNT_RAT	(P41739)	Aryl hydrocarbon receptor nuclear translocator (ARNT protein) (Dioxin receptor, nuclear translocator) (Hypoxia-inducible factor 1 beta)
MAP2_RAT	(P15146)	Microtubule-associated protein 2 (MAP 2) (MAP-2)
MYH11_RAT	(Q63862)	Myosin-11 (Myosin heavy chain, smooth muscle isoform) (SMMHC) (Fragments)
HD_RAT	(P51111)	Huntingtin (Huntington's disease protein homolog) (HD protein)
CSAD_RAT	(Q64611)	Cysteine sulfinic acid decarboxylase (EC 4.1.1.29) (Sulfinoalanine decarboxylase)
FOLH1_RAT	(P70627)	Glutamate carboxypeptidase II (EC 3.4.17.21) (Membrane glutamate carboxypeptidase)
RIP3_RAT	(Q9ERE8)	Rho-interacting protein 3 (p116RIP) (RIP3)
PLD2_RAT	(P70498)	Phospholipase D2 (EC 3.1.4.4) (PLD 2) (Choline phosphatase 2) (Phosphatidylcholine-hydroly
MAST1_RAT	(Q810W7)	Microtubule-associated serine/threonine-protein kinase 1 (EC 2.7.1.37) (Syntrophin-associated serine/threonine-protein kinase)
DPOG1_RAT	(Q9QYV8)	DNA polymerase gamma subunit 1 (EC 2.7.7.7) (Mitochondrial DNA polymerase catalytic subuni
P85A_RAT	(Q63787)	Phosphatidylinositol 3-kinase regulatory alpha subunit (PI3-kinase p85-alpha subunit) (PtdIns-3-kinase p85-alpha) (PI3K)
PAK3_RAT	(Q62829)	Serine/threonine-protein kinase PAK 3 (EC 2.7.1.37) (p21-activated kinase 3) (PAK-3) (Beta
CLH_RAT	(P11442)	Clathrin heavy chain
SHAN3_RAT	(Q9JLUM)	SH3 and multiple ankyrin repeat domains 3 (Shank3) (Proline-rich synapse associated protein 2) (ProSAP2) (SPANK-2)
PRC1_RAT	(Q71QF9)	Prickle-like protein 1 (REST/NRSF-interacting LIM domain protein 1)
GBRG2_RAT	(P18508)	Gamma-aminobutyric-acid receptor gamma-2 subunit precursor (GABA(A) receptor)
B3A2_RAT	(P23347)	Anion exchange protein 2 (Non-erythroid band 3-like protein) (AE2 anion exchanger) (Solute carrier family 4 member 2) (B3RP)
NOTC1_RAT	(Q07008)	Neurogenic locus notch homolog protein 1 precursor (Notch 1) [Contains: Notch 1 extracellu
VGFR1_RAT	(P53767)	Vascular endothelial growth factor receptor 1 precursor (EC 2.7.1.112) (VEGFR-1) (Tyrosine-protein kinase receptor FLT) (FLT-1)
YME1L_RAT	(Q925S8)	ATP-dependent metalloprotease YME1L1 (EC 3.4.24.-) (YME1-like protein 1) (ATP-dependent me
CYLN2_RAT	(O55158)	Cytoplasmic linker protein 2 (Cytoplasmic linker protein 115) (CLIP-115)
PRS7_RAT	(Q63347)	26S protease regulatory subunit 7 (MSS1 protein)
CAND1_RAT	(P97536)	Cullin-associated NEDD8-dissociated protein 1 (Cullin-associated and neddylation-dissociated protein 1) (p120 CAND1)
ABCC9_RAT	(Q63563)	Sulfonylurea receptor 2
MYST3_RAT	(Q5TKR9)	Histone acetyltransferase MYST3 (EC 2.3.1.48) (EC 2.3.1.-) (MYST protein 3)
SPTN2_RAT	(Q9QWN8)	Spectrin beta chain, brain 2 (Spectrin, non-erythroid beta chain 2) (Beta-II spectrin) (S
ABLM2_RAT	(Q6KC51)	Actin-binding LIM protein 2 (Actin-binding LIM protein family member 2) (abLIM2)
CASP1_RAT	(P43527)	Caspase-1 precursor (EC 3.4.22.36) (CASP-1) (Interleukin-1 beta convertase) (IL-1BC)
IL6RA_RAT	(P22273)	Interleukin-6 receptor alpha chain precursor (IL-6R-alpha) (IL-6R 1)
MINP1_RAT	(O35217)	Multiple inositol polyphosphate phosphatase 1 precursor (EC 3.1.3.62) (Inositol (1,3,4,5)-tetrakisphosphate 3-phosphatase)
INP5E_RAT	(Q9WVR1)	72 kDa inositol polyphosphate 5-phosphatase (EC 3.1.3.36) (Phosphatidylinositol-4,5-bispho
DVL1_RAT	(Q9WVB9)	Segment polarity protein dishevelled homolog DVL-1 (Dishevelled-1) (DSH homolog 1)
P55G_RAT	(Q63789)	Phosphatidylinositol 3-kinase regulatory gamma subunit (PI3-kinase p85-gamma subunit) (PtdIns-3-kinase p85-gamma) (p55PIK)
KCMA1_RAT	(Q62976)	Calcium-activated potassium channel alpha subunit 1 (Calcium-activated potassium channel,
TNR1A_RAT	(P22934)	Tumor necrosis factor receptor superfamily member 1A precursor (p60) (TNF-R1)
MYO1E_RAT	(Q63358)	Myosin Ie (Myosin heavy chain myr 3)
PLCG2_RAT	(P24135)	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma 2 (EC 3.1.4.11)
PSD1_RAT	(O88761)	26S proteasome non-ATPase regulatory subunit 1 (26S proteasome regulatory subunit RPN2)
ALDOC_RAT	(P09117)	Fructose-bisphosphate aldolase C (EC 4.1.2.13) (Brain-type aldolase)
MYH3_RAT	(P12847)	Myosin heavy chain, fast skeletal muscle, embryonic
PCSK5_RAT	(P41413)	Proprotein convertase subtilisin/kexin type 5 precursor (EC 3.4.21.-)
KCNH1_RAT	(Q63472)	Potassium voltage-gated channel subfamily H member 1
SRP54_RAT	(Q6AYB5)	Signal recognition particle 54 kDa protein (SRP54)
PTPRV_RAT	(Q64612)	Receptor-type tyrosine-protein phosphatase V precursor (EC 3.1.3.48)
CELR2_RAT	(Q9QYP2)	Cadherin EGF LAG seven-pass G-type receptor 2 (Multiple epidermal growth factor-like domains 3) (Fragment)
CAC1A_RAT	(P54282)	Voltage-dependent P/Q-type calcium channel alpha-1A subunit (Voltage-gated calcium channel
MBTP1_RAT	(Q9WTZ3)	Membrane-bound transcription factor site-1 protease precursor (EC 3.4.21.-)
KCNQ1_RAT	(Q9Z0N7)	Potassium voltage-gated channel subfamily KQT member 1 (Voltage-gated potassium channel subunit Kv7.1)
RORB_RAT	(P45446)	Nuclear receptor ROR-beta (Nuclear receptor RZR-beta)
SULF1_RAT	(Q8VI60)	Extracellular sulfatase Sulf-1 precursor (EC 3.1.6.-) (Sulfatase FP) (HSulfFP1)
MB21A_RAT	(O35821)	Myb-binding protein 1A (PAR-interacting protein) (PIP)

表３　既知標準混合サンプルのICAT法による定量予測

	Time	m/z-Light	m/z-Heavy	価数	親イオン[M+H]	Area-Light	Area-Heavy	H：L	Ave.	理論比	実測/理論
BSA											
	22.055-22.297	684.3770	687.3873	3	2051.1150	1892	1421	0.751			
	16.134-16.376	807.4157	811.9334	2	1613.8234	1589	1486	0.936	0.843	1.0	0.843
LDH-M											
	16.617-17.222	709.8737	714.3826	2	1418.7394	13497	7673	0.569		0.667	0.853
SOD											
	20.122-20.363	586.3432	590.8665	2	1171.6784	30469	20652	0.678			
	21.451-22.297	766.1079	769.1191	3	2296.3077	3570	1691	0.474	0.576	0.5	1.152
RNase A											
	14.322-14.926	543.2754	547.7853	2	1085.5428	5303	6704	1.264			
	24.109-24.832	798.7677	801.7729	3	2394.2871	12550	18428	1.468			
	14.322-14.926	837.9311	842.4474	2	1674.8542	5699	8335	1.463			
	24.349-24.712	1197.6519	1202.1606	2	2394.2958	19162	27464	1.433	1.407	1.5	0.938

表４　cICAT法による個別サイトゾルタンパク量の比較（プールドサイトゾルに対し）

HG23 (40/41)		HK27 (49/54)		HG30 (53/56)		HG43 (55/56)		（比決定/同定）
Accession #	Ratio	Accession #	Ratio	Accession #	Ratio	Accession #	Avg H/L	
ADH4_HUMAN (P08319)	1.2111	AATM_HUMAN (P00505)	0.8594	AATM_HUMAN (P00505)	0.9525			
ADHA_HUMAN (P07327)	1.3931	ADH4_HUMAN (P08319)	0.934	ADH4_HUMAN (P08319)	1.0718	ADH4_HUMAN (P08319)	0.5559	
ADHB_HUMAN (P00325)	1.4522	ADHA_HUMAN (P07327)	1.1474	ADHA_HUMAN (P07327)	1.2279	ADHA_HUMAN (P07327)	1.4898	
ADHQ_HUMAN (P00326)	1.4426	ADHB_HUMAN (P00325)	1.1494	ADHB_HUMAN (P00325)	1.2739	ADHB_HUMAN (P00325)	1.5482	
		ADHQ_HUMAN (P00326)	1.1585	ADHQ_HUMAN (P00326)	1.2348	ADHQ_HUMAN (P00326)	1.5434	
				ADHX_HUMAN (P11766)				
				ADO_HUMAN (Q06278)	0.9848	ADO_HUMAN (Q06278)	1.0638	
AKC1_HUMAN (Q04828)	1.1888	AKC1_HUMAN (Q04828)	1.4352	AKC1_HUMAN (Q04828)	1.2054	AKC1_HUMAN (Q04828)	1.4061	
AKC2_HUMAN (P52895)	1.1888	AKC2_HUMAN (P52895)	1.4352	AKC2_HUMAN (P52895)	1.2054	AKC2_HUMAN (P52895)	1.4061	
				AKC4_HUMAN (P17516)	1.0883	AKC4_HUMAN (P17516)	1.0101	
ALBU_HUMAN (P02768)	1.8222	ALBU_HUMAN (P02768)	2.5498	ALBU_HUMAN (P02768)	1.2229	ALBU_HUMAN (P02768)	2.4181	
ALFB_HUMAN (P05062)	1.303	ALFB_HUMAN (P05062)	1.6318	ALFB_HUMAN (P05062)	1.5547	ALFB_HUMAN (P05062)	1.054	
				AMPL_HUMAN (P28838)	1.0188			
ARGI_HUMAN (P05089)	0.7392	ARGI_HUMAN (P05089)	0.9815	ARGI_HUMAN (P05089)		ARGI_HUMAN (P05089)	0.8039	
		ASSY_HUMAN (P00966)	1.1922	ASSY_HUMAN (P00966)	1.4727	ASSY_HUMAN (P00966)	1.1136	
BHMT_HUMAN (Q93088)	1.0255	BHMT_HUMAN (Q93088)	0.9035	BHMT_HUMAN (Q93088)	1.134	BHMT_HUMAN (Q93088)	0.9016	
		BUP1_HUMAN (Q9UBR1)	1.2448					
				CATA_HUMAN (P04040)	1.5469	CATA_HUMAN (P04040)	1.2424	
				CH60_HUMAN (P10809)	0.8029			
						CATB_HUMAN (P07858)	1.2609	
CPSM_HUMAN (P31327)	1.1447	CPSM_HUMAN (P31327)	1.2454	CPSM_HUMAN (P31327)	1.2468	CPSM_HUMAN (P31327)	1.4751	
		DEF1_HUMAN (P59665)	0.8348					
DHA1_HUMAN (P00352)	1.0551	DHA1_HUMAN (P00352)	1.2413	DHA1_HUMAN (P00352)	1.1567	DHA1_HUMAN (P00352)	1.1799	
DHAM_HUMAN (P05091)	1.1082	DHAM_HUMAN (P05091)	1.0569	DHAM_HUMAN (P05091)	1.5259	DHAM_HUMAN (P05091)	1.3544	
DHCA_HUMAN (P16152)	1.0551	DHCA_HUMAN (P16152)	1.1787	DHCA_HUMAN (P16152)	1.3265	DHCA_HUMAN (P16152)	1.2183	
DHE3_HUMAN (P00367)	1.5682					DHE3_HUMAN (P00367)	1.2133	
DHSO_HUMAN (Q00796)	1.5494							
DOPD_HUMAN (P30046)	0.5875	DOPD_HUMAN (P30046)	1.0614	DOPD_HUMAN (P30046)	0.7498			
						DHE4_HUMAN (P49448)	1.1604	
				ECH1_HUMAN (Q13011)	1.0437			
				ECHM_HUMAN (P30084)	1.4051	ECHM_HUMAN (P30084)	1.0101	
ENOA_HUMAN (P06733)	1.101			ENOA_HUMAN (P06733)	1.2878	ENOA_HUMAN (P06733)	1.4443	
ENOB_HUMAN (P13929)	1.0362			ENOB_HUMAN (P13929)	1.1582			
ENOG_HUMAN (P09104)	1.0362			ENOG_HUMAN (P09104)	1.1582			
				F16P_HUMAN (P09467)	1.1634	F16P_HUMAN (P09467)	0.8796	
FAAA_HUMAN (P16930)	0.9861	FAAA_HUMAN (P16930)	0.9671	FAAA_HUMAN (P16930)	1.199			
FAS_HUMAN (P49327)	1.2834	FAS_HUMAN (P49327)	2.3302	FAS_HUMAN (P49327)	2.7567			
FTCD_HUMAN (O95954)	1.3394	FTCD_HUMAN (O95954)	1.4868			FTCD_HUMAN (O95954)	0.8726	
G3P1_HUMAN (P00354)	1.1018	G3P1_HUMAN (P00354)	1.2617	G3P1_HUMAN (P00354)	1.3352	G3P1_HUMAN (P00354)	1.1307	
G3P2_HUMAN (P04406)	1.0109	G3P2_HUMAN (P04406)	1.1408	G3P2_HUMAN (P04406)	1.2569	G3P2_HUMAN (P04406)	1.011	
				GAST_HUMAN (P01375)				
GHPR_HUMAN (Q9UBQ7)	1.2733	GHPR_HUMAN (Q9UBQ7)	1.4892	GHPR_HUMAN (Q9UBQ7)	1.3408	GHPR_HUMAN (Q9UBQ7)	1.5782	
						GLMT_HUMAN (O14746)	1.8924	
						GLRX_HUMAN (P35754)	0.8096	
GLYC_HUMAN (P34896)	0.9373	GLYC_HUMAN (P34896)	0.7653	GLYC_HUMAN (P34896)	1.0333	GLYC_HUMAN (P34896)	1.1558	
GTT1_HUMAN (P30711)				GTT1_HUMAN (P30711)		GTT1_HUMAN (P30711)	1.4208	
		HAO1_HUMAN (Q9UJM8)				HAO1_HUMAN (Q9UJM8)	1.6067	
HBB_HUMAN (P02023)	2.1978	HBB_HUMAN (P02023)	3.4439	HBB_HUMAN (P02023)	1.8415	HBB_HUMAN (P02023)	1.611	
		HBD_HUMAN (P02042)		HBD_HUMAN (P02042)	2.0921			
				HEM2_HUMAN (P13716)	1.2531			
		HMCM_HUMAN (P54868)	1.3887			HMCM_HUMAN (P54868)	1.0913	
HNT1_HUMAN (P49773)	0.7414							
				HS71_HUMAN (P08107)	2.1376	HS71_HUMAN (P08107)	1.5178	
				HS76_HUMAN (P17066)	2.1376	HS76_HUMAN (P17066)	1.5178	
						IDHC_HUMAN (O75874)	1.0817	
IF41_HUMAN (P60842)	1.1561					IF41_HUMAN (P60842)	1.5259	
IF42_HUMAN (Q14240)	1.1561					IF42_HUMAN (Q14240)	1.5259	
		KAG_HUMAN (P01834)	3.2598			KAG_HUMAN (P01834)	1.4945	
LDHA_HUMAN (P00338)	0.9771	LDHA_HUMAN (P00338)	0.7848	LDHA_HUMAN (P00338)	1.2233	LDHA_HUMAN (P00338)	1.197	
LDHB_HUMAN (P07195)	0.9771	LDHB_HUMAN (P07195)	0.8138	LDHB_HUMAN (P07195)	1.0166	LDHB_HUMAN (P07195)	1.0767	
LDHC_HUMAN (P07864)	0.9771	LDHC_HUMAN (P07864)	0.8138	LDHC_HUMAN (P07864)	1.0166	LDHC_HUMAN (P07864)	1.0767	
		LGUL_HUMAN (Q04760)	1.0446			LGUL_HUMAN (Q04760)	0.993	
		MDHM_HUMAN (P40926)	0.9712					
				METL_HUMAN (Q00266)	1.7116			
		MIF_HUMAN (P14174)	1.26	MIF_HUMAN (P14174)	1.1918	MIF_HUMAN (P14174)	0.8533	
				MPB1_HUMAN (P22712)	1.4313	MPB1_HUMAN (P22712)	1.5787	

いずれにおいてもH：L比が出ているタンパク質
HG30以外においてH：L比が出ているタンパク質
HG30以外で同定されているタンパク質

表5　マウス個体におけるシグナル変化の共通性の検索

			Olive oil						NNM						Quinoline					
Spot No	m/z	Charge	mouse-1	RT	mouse-2	RT	mouse-3	RT	mouse-1	RT	mouse-2	RT	mouse-3	RT	mouse-1	RT	mouse-2	RT	mouse-3	RT
1	568	2+	UP	25	UP	24	UP	28	DN	24	DN	24	AB	22	DN	27	DN	27	DN	26
2	554	2+	AB	24	AB	25	AB	29	UP	22	UP	24	UP	28	----	----	----	----	----	----
3	508	1+	UP	15	UP	18	PR	20	DN	15	DN	18	DN	20	DN	20	DN	22	DN	20
4	494	2+	UP	30	UP	30	UP	35	AB	27	AB	30	AB	34	----	----	----	----	----	----
5	468.7515	2+	UP	30	UP	32	UP	35	DN	30	DN	30	AB	27	DN	32	DN	34	DN	33
6	437	2+	UP	22	UP	25	UP	25	AB	18	AB	22	AB	27	DN	25	PR	26	DN	26
7	791	2+	AB	15	AB	18	AB	22	UP	15	UP	15	UP	15	----	----	----	----	----	----
8	789	1+	AB	22	AB	22	AB	22	UP	20	UP	24	UP	22	----	----	----	----	----	----
9	780	2+	UP	27	UP	27	UP	32	AB	25	AB	25	AB	30	AB	30	DN	25	AB	30
10	738	1+	AB	40	DN	45	AB	44	UP	44	UP	46	UP	48	----	----	----	----	----	----
11	699	2+	AB	22	AB	22	AB	22	UP	20	UP	25	UP	25	----	----	----	----	----	----
12	689	2+	DN	45	DN	46	DN	45	UP	44	UP	43	UP	49	----	----	----	----	----	----
13	661	2+	UP	25	UP	22	UP	30	AB	25	AB	25	AB	28	----	----	----	----	----	----
14	660	2+	PR	49	PR	38	DN	43	AB	36	AB	37	AB	40	----	----	----	----	----	----
15	613.7	2+	AB	70	AB	70	AB	70	UP	70	UP	70	UP	70	----	----	----	----	----	----
16	960	2+	UP	33	UP	36	DN	40	DN	33	DN	36	AB	40	----	----	----	----	----	----
17	942	2+	UP	55	UP	58	DN	58	AB	55	DN	53	AB	60	----	----	----	----	----	----
18	939	1+	AB	43	AB	40	AB	45	UP	44	UP	44	UP	46	----	----	----	----	----	----
19	923	2+	UP	50	UP	52	UP	57	DN	50	DN	51	DN	55	----	----	----	----	----	----
20	895	2+	UP	26	UP	27	UP	30	AB	25	AB	27	AB	29	DN	30	DN	30	AB	30
21	821	2+	PR	28	UP	28	UP	33	DN	25	DN	28	DN	28	----	----	----	----	----	----
22	818	2+	AB	25	AB	25	AB	25	PR	25	DN	27	DN	27	----	----	----	----	----	----
23	1081	2+	AB	20	AB	20	AB	20	UP	20	UP	24	UP	26	UP	24	UP	22	UP	20
24	1052	2+	UP	27	UP	27	UP	27	DN	25	DN	28	DN	25	----	----	----	----	----	----

DN; down regulated　UP; upregulated　PR; no change　----; not checked

RT-Retention time

表6　シグナル強度に差が見られたICATペアとその普遍性

				APAP 800 mg/kg									Control							
ID	m/z-L	m/z-H	Mass Diff	11	Int	RT	14	Int	RT	12	Int	RT	13	Int	RT	2	Int	RT	3	Int	RT	5	Int	RT	1	Int	RT	4	Int	RT
12- 1	1253.71	1255.74	2.03	np			LL	w	35.7-36	HH	w	35-36	np			LL	w	33.9-34.1	nps			LL	w	36-36.5	HH	w	36-36.5	HH	w	37.4-38
12- 2	1186.99	1191.52	4.53	nc	w	47.4-47.7	H	w	44.5-45	H	w	44.3-44.8	HH	w	45.7-46.1	H	w	42.3-43.4	nc	w	44.6-44.9	H	w	45.7-46.2	nc	m	44.4-45	H	m	46.3-46.7
12- 3	Ratio is not High																													
12- 4	1075.42	1081.11	5.69																											
12- 5	684.96	687.97	3.01	L	s	41.8-42.8	LL	s	38-39.8	H	s	39-40.2	H	s	40.4-41.2	LL	s	37.4-38.5	L	s	38.8-39.6	LL	s	39.7-40.6	H	s	38.8-41	HH	s	41-42
12- 6	1003.00	1004.80	1.80	HH	s	37.9-38.9	LL	s	35.6-36.4	HH	s	35.3-36	HH	s	36.8-37.1	LL	s	33.9-34.55	LL	w	35.3-36	LL	s	35.8-36.8	HH	S	35.8-36.6	HH	s	37.2-38.1
12- 7	886.82	891.33	4.51	np			LL	w	72.2-72.9	L	s	72.7-73.2	L	s	73-73.6	LL	w	70.9-71.5	np			L	w	72.5-73.1	L	w	73-73.6	L	w	73-73.5
12- 8	841.39	845.91	4.52	LL	s	41.6-42.4	LL	s	38.8-39.5	LL	s	38.8-41	LL	s	40-41.2	LL	s	37.2-38	LL	w	38.8-39.3	LL	m	39.6-40.15	LL	s	39.25-40	LL	s	40.7-41.8
12- 9	843.98	847.00	3.02	nc	s	23.3-24.5	HH	w	26-26.3	HH	m	22.5-23	HH	s	22.7-23.1	nc	s	19.4-20.2	np			nc	s	20.6-21.2	nc	m	21.9-22.4	H	w	26.9-27.3
12- 10	725.89	729.90	3.01	LL	w	61-61.5	LL	s	59-60.5	HH	s	59.5-60	HH	s	60.4-60.8	H			np			L			L			HH		
12- 11	Not consistent																													
12- 12	659.78	664.30	4.52	nc						HH			HH			L									nc			HH		
12- 13	530-531	Not consistent																												
12- 14	497.53	500.54	3.01	LL	Not consistent		L			LL																				
12- 15	491-492	Not consistent																												
12- 16	Not consistent																													
12- 17	448.70	453.22	4.52	Not consistent																										
12- 18	Not real peptide peak																													
12- 19	Not real peptide peak																													
12- 20	598.78	603.30	4.52	np			L	s	44.8-45.5	HH	s	44.7-45.4	HH	s	41.5-42	H	s	43.2-44.2	H	w	45-46.2	nc	s	46.3-47.1	L	s	46.5-47	H	s	46.7-47.2
13- 1	383.85	386.86	3.01	L	s	24-25	LL	s	9.3-9.8	HH	s	23-23.8	HH	s	23.3-24.2	H	s	19.8-20.5	H	s	21.1-22.3	nc	s	21.8-22.5	L	s	22.8-23.7	LL	s	27.326.4
13- 2	Not real pep																													
13- 3	nc																													
13- 4	Not consistent																													
13- 5	456.70	461.22	4.52	Not consistent			LL			LL			LL			H			nps			nps			LL			LL		
13- 6	456.89	459.90	3.01	Not consistent			LL						LL																	
13- 7	Not consistent																													
13- 8	Not consistent																													
13- 9	Not consistent																													
13- 10	509.90	512.91	3.01	L	s	38.5-38.8	LL	s	38-39.8	HH	s	39.1-39.8	HH	s	40.3-41	H	s	37.5-39.2	L	w	38.8-39.5	nc	s	39.8-40.5	L	s	39.5-40.4	HH	s	41-41.8
13- 11	523.89	526.90	3.01	nc			LL			HH			HH			nc			HH			nc			nc			HH		
13- 12	Not consistent																													

*Int
peak intensity of higher one
s 100+
m 50-100
w <50

Heavy peak is
HH Very high
H High
nc no change
L Low
LL Very Low

np Not observed
nps Not observed because of no spray
nA Not seen in Analyst

*Sample-label
Light
Heavy

表 7　候補ペプチドの同定結果

peptide ID	m/z-L	Identified	*Possible candidates*
12-5	684.96	Rat Serum Albumin	
12-10	725.99	*not identified*	
12-20	598.78	Major Urinary Protein Precursor	
13-1	393.85		*Retinoic acid receptor RXR-beta*
			Receptor-interacting serine/threonine-protein kinase 5
13-10	509.9		*E3 ubiquitin-protein ligase rififylin*

図表

Ⅶ　細胞レベルでのメタボロミクス技術の開発

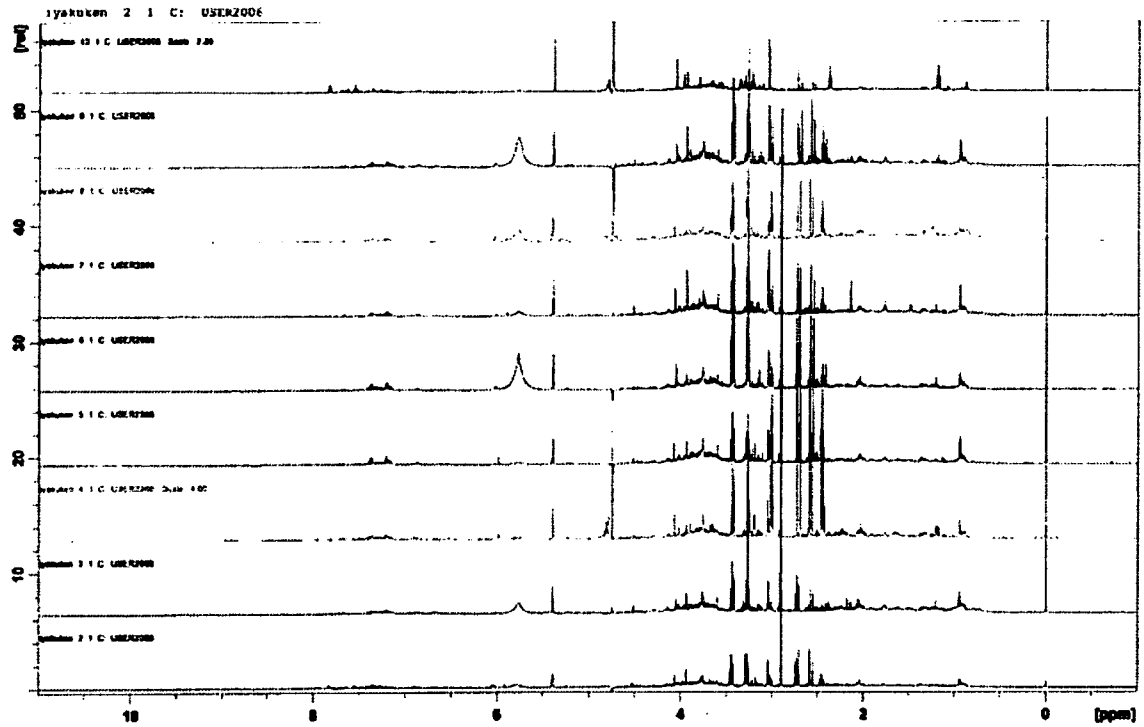


図１　病態モデルマウス尿試料のＮＭＲ

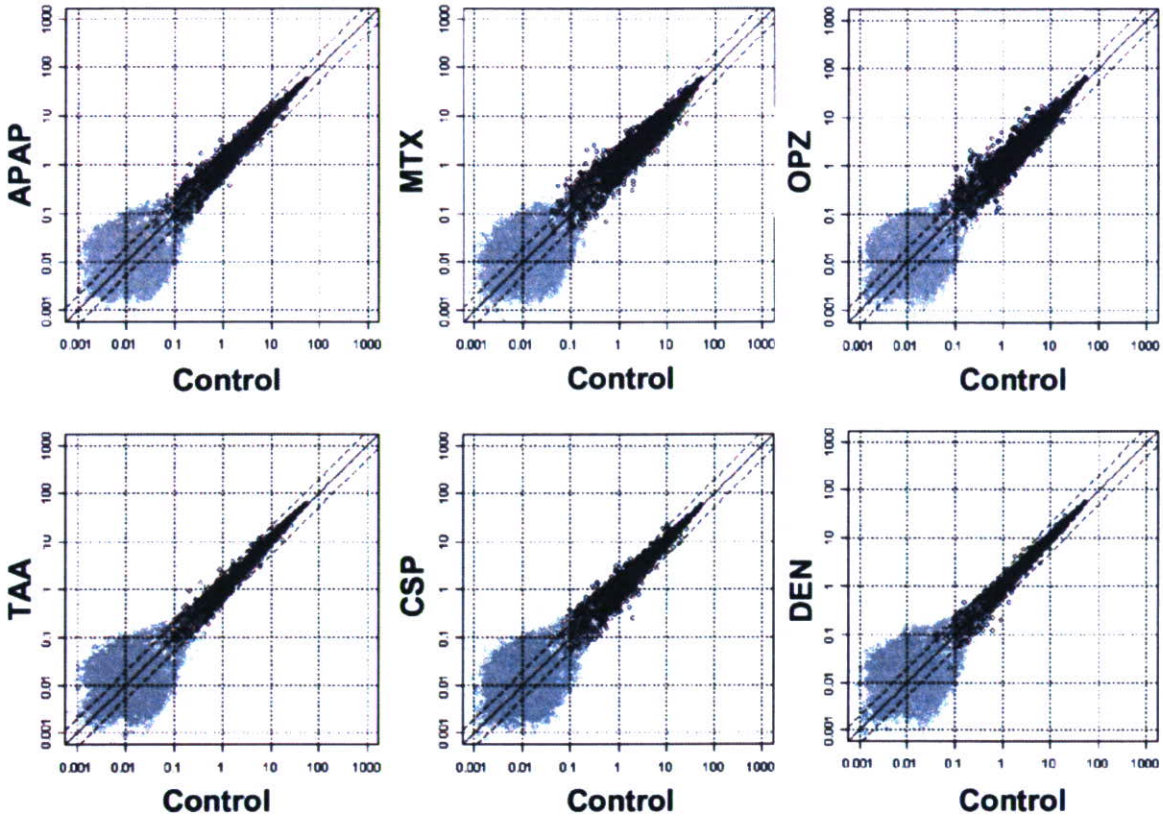


図２　遺伝子発現解析結果（薬剤処理と Control のスキャッタープロット）

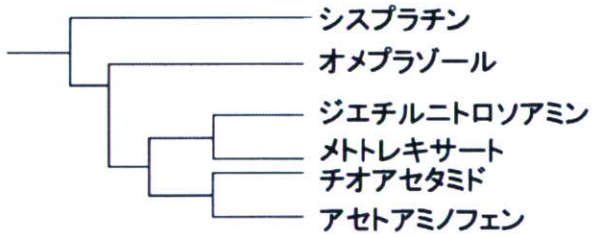


図３　薬剤処理による変動遺伝子を用いたクラスター解析

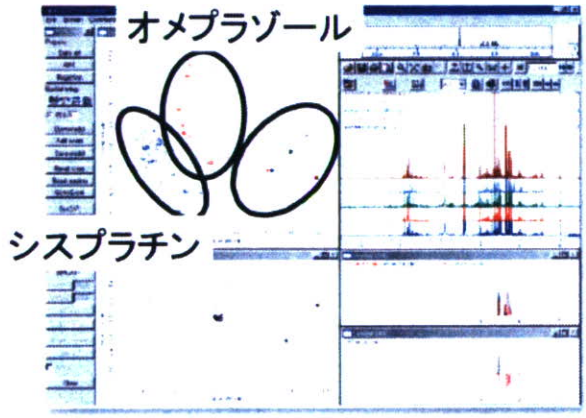


図４　薬剤処理細胞破砕液のＰＣＡ解析結果

別紙４

研究成果の刊行に関する一覧表

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Hydroxyl radical generation *via* photoreduction of a simple pyridine *N*-oxide by an NADH analogue†

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Photoreduction of pyridine *N*-oxide, which has a key structure of antitumor agents for hypoxic solid tumors, by 1-benzyl-1,4-dihydronicotinamide in deaerated aprotic media resulted in generation of hydroxyl radical, leading to the oxidation of salicylic acid to 2,3- and 2,5-dihydroxybenzoic acids, and catechol.

Recently, considerable effort has been made to develop effective drugs against solid tumors, which exist under hypoxic (oxygen-poor) conditions in inefficient vascular systems.[1,2] Tirapazamine (3-amino-1,2,4-benzotriazine 1,4-di-*N*-oxide), which has a heterocyclic *N*-oxide structure, is a clinically promising antitumor agent against hypoxic cells.[3] The DNA damage induced by tirapazamine is proposed to result from the generation of hydroxyl radical (•OH) or the direct oxidation of the deoxyribose backbone of DNA after one-electron reduction of the *N*-oxide to form an activated intermediate.[4-6] However, the actual DNA-damaging species has yet to be clarified. On the other hand, photosensitizers available for photodynamic therapy are advantageous to localize the toxicity to a selected site (tumor cells), thus avoiding toxicity to normal cells.[7] However, since most photosensitizers require O_2 to produce reactive oxygen species, they are not effective toward anaerobic solid tumors. Thus, photoactivated compounds, which generate reactive oxygen species under anaerobic conditions, are certainly required for the development of drugs effective against solid tumors without affecting normal cells.

We report herein •OH generation from a simple unsubstituted pyridine *N*-oxide (PyO), which has a largely negative reduction potential, *via* one-electron reduction by photoexcited 1-benzyl-1,4-dihydronicotinamide (BNAH) used as a model compound of dihydronicotinamide adenine dinucleotide (NADH) in DMF under anaerobic conditions. The effects of the substituent at the C-4 position of pyridine *N*-oxides on the mechanism of photoinduced electron transfer from BNAH to pyridine *N*-oxides as well as the reactivity of the corresponding radical anions are clarified based on the spectral and electrochemical data together with the calculated molecular structures by the density functional method, providing a valuable insight into the development of antitumor agents for hypoxic cells.

Salicylic acid (SA) was employed to detect •OH generated in the photoreaction of pyridine *N*-oxides with BNAH in deaerated DMF. SA reacts with •OH to form 2,3-dihydroxybenzoic acid (2,3-DHBA) and 2,5-dihydroxybenzoic acid (2,5-DHBA) as major products and catechol as a minor product.[8-13] These oxidized products of SA are stable and are readily isolated and quantified by a reverse-phase HPLC equipped with an electrochemical detector (HPLC-ECD). SA does not react with $O_2^{\bullet-}$ at an appreciable rate as compared to •OH. Although SA also reacts with singlet oxygen ($^1O_2$), only 2,5-DHBA is formed exclusively, instead of the mixture of 2,3-DHBA, 2,5-DHBA, and catechol.[14]

Aprotic solvents, such as DMF and acetonitrile (MeCN) were used because of the poor solubility of pyridine *N*-oxides toward water, although the reactivity in aqueous media is important for an *in vivo* situation. When a deaerated DMF solution of PyO (5.0×10^{-3} mol dm^{-3}) and BNAH (5.0×10^{-3} mol dm^{-3}) was irradiated with UV light ($\lambda > 290$ nm) in the presence of SA (3.2×10^{-2} mol dm^{-3}), 2,3-DHBA, 2,5-DHBA, and catechol were detected by the HPLC-EC analysis as shown in Fig. 1a. The ratio of the yields of 2,5-DHBA, 2,3-DHBA, and catechol are 48:35:16. Irradiation of PyO or BNAH alone in the presence of SA resulted in no formation of oxidized products of SA (Fig. 1c and d). Under dark conditions, neither DHBA product nor catechol was formed even in the presence of all the components, *i.e.*, PyO, BNAH, and SA (Fig. 1e). These results demonstrate that •OH is generated in the photoreaction of PyO with BNAH in deaerated DMF as shown in Scheme 1. In fact, addition of

† Electronic supplementary information (ESI) available: Transient absorption spectra of the photoreaction between BNAH and PyO (S1), EPR spectrum of $NO_2PyO^{\bullet-}$ (S2), spectral change in the photoreaction between BNAH and NO_2PyO (S3), and DFT minimized structures (S4). See http://dx.doi.org/10.1039/b509447j

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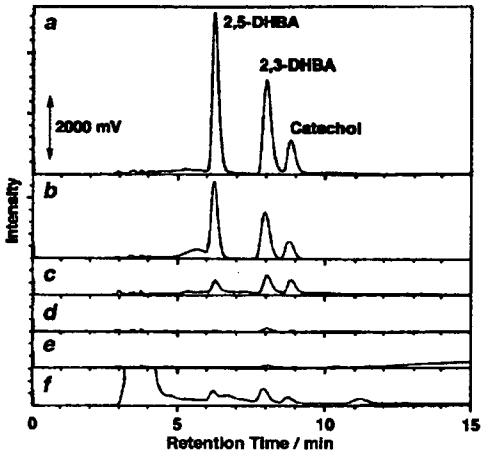
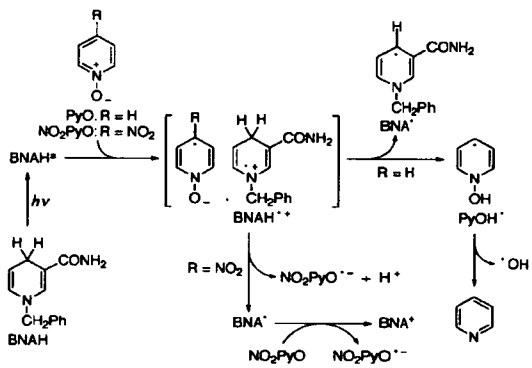


Fig. 1 HPLC-ECD chromatograms of products formed during irradiation (1 h) of (a) PyO (5.0×10^{-3} mol dm^{-3}), BNAH (5.0×10^{-3} mol dm^{-3}), and SA (3.2×10^{-2} mol dm^{-3}); (b) PyO (5.0×10^{-3} mol dm^{-3}), BNAH (5.0×10^{-3} mol dm^{-3}), SA (3.2×10^{-2} mol dm^{-3}), and ethanol (5.7×10^{-1} mol dm^{-3}); (c) PyO (5.0×10^{-3} mol dm^{-3}) and SA (3.2×10^{-2} mol dm^{-3}); (d) BNAH (5.0×10^{-3} mol dm^{-3}) and SA (3.2×10^{-2} mol dm^{-3}); (e) PyO (5.0×10^{-3} mol dm^{-3}), BNAH (5.0×10^{-3} mol dm^{-3}), and SA (3.2×10^{-2} mol dm^{-3}) without irradiation; (f) NO_2PyO (5.0×10^{-3} mol dm^{-3}), BNAH (5.0×10^{-3} mol dm^{-3}), and SA (3.2×10^{-2} mol dm^{-3}) in deaerated DMF at 298 K.



Scheme 1

ethanol, which is a •OH scavenger, resulted in a decrease in the yield of three oxidized SA products (Fig. 1b).

Photoirradiation of BNAH is known to give the singlet excited state, BNAH*.[15] The fluorescence of BNAH* is quenched efficiently by the addition of PyO in deaerated MeCN. The quenching rate constant is determined as 1.4×10^{10} mol^{-1} dm^3 s^{-1}, which is close to the diffusion limited value in MeCN. Nanosecond laser excitation of a deaerated MeCN solution of PyO and BNAH results in the formation of PyO•$^-$ (λ_{max} = 540 nm) and BNAH•$^+$ (λ_{max} = 380 nm)[16] (see Electronic Supplementary Information, Fig. S1†).

The reaction mechanism of electron-transfer reduction of PyO by BNAH is shown in Scheme 1. Photoinduced electron transfer from BNAH* to PyO takes place to produce a radical ion pair of BNAH•$^+$ and PyO•$^-$, since the oxidation potential of BNAH* (E^0_{ox} = − 2.65 V *vs.* SCE) determined in DMF[17] is more negative than the reported reduction potential of PyO (E^0_{red} = − 2.30 V) in DMF.[18] Proton transfer from BNAH•$^+$, thus produced, to PyO•$^-$ gives PyOH• and BNA•. The resulting PyOH• then undergoes the N–O bond cleavage to produce •OH and pyridine. Judging from the more positive oxidation potential of BNA• (E^0_{ox} = − 1.05 V)[17] in DMF than the reduction potential of PyO (E^0_{red} = − 2.30 V),[18] no electron transfer from BNA• to PyO would occur and instead a coupling between two molecules of BNA• may occur to give a dimer $(BNA)_2$.

When PyO is replaced by 4-nitropyridine *N*-oxide (NO_2PyO), neither DHBA products nor catechol was produced after irradiation of a deaerated DMF solution containing NO_2PyO, BNAH, and SA (Fig. 1f).

This result demonstrates that the radical anion of NO_2PyO ($NO_2PyO^{\bullet-}$) generated in photoinduced electron transfer from BNAH to NO_2PyO is relatively stable and does not undergo the N–O bond cleavage. In fact, an EPR spectrum of $NO_2PyO^{\bullet-}$, which has a *g* value of 2.0054, was observed after irradiation of a deaerated MeCN solution of NO_2PyO and BNAH (Fig. S2a†). The hyperfine coupling constants (hfc) of the observed EPR spectrum of $NO_2PyO^{\bullet-}$ were determined by comparison of the observed spectrum with the computer-simulated spectrum (Fig. S2b†) and they were assigned based on the reported hfc values for $NO_2PyO^{\bullet-}$ in DMF (Fig. S2†).[18]

The UV-vis spectral titration (Fig. S3†) indicates that BNAH acts as a two-electron donor to reduce 2 equiv. of NO_2PyO to $NO_2PyO^{\bullet-}$. Since the E^0_{ox} value of BNAH* (−2.65 V) is more negative than the E^0_{red} value of NO_2PyO (−0.77 V),[18] photoinduced electron transfer from BNAH* to NO_2PyO occurs to give a radical ion pair of $NO_2PyO^{\bullet-}$ and BNAH•$^+$. BNAH•$^+$ undergoes deprotonation to give BNA•. The delocalization of an electron on the $NO_2PyO^{\bullet-}$ molecule due to the electron-withdrawing NO_2 group may preclude the protonation of $NO_2PyO^{\bullet-}$. In fact, no hyperfine structure due to the N–OH proton was observed in the EPR spectrum of $NO_2PyO^{\bullet-}$. The subsequent electron transfer from BNA• to NO_2PyO may also occur rapidly, judging from the E^0_{ox} value of BNA• (−1.05 V), which is lower than the E^0_{red} value of NO_2PyO (−0.77 V).[18] Thus, once photoinduced electron transfer from BNAH to NO_2PyO occurs, 2 equiv of $NO_2PyO^{\bullet-}$ are produced.

DFT calculations using B3LYP/6-31G* basis set for PyOH• and NO_2PyOH• were carried out to investigate the difference in the reactivity of radical species of pyridine *N*-oxides depending on a substituent at the C-4 position (Fig. S4†). The calculated N–O bond length in PyOH• (1.50 Å) is significantly longer than that in NO_2PyOH• (1.40 Å). This suggests that the N–O bond cleavage in PyOH• may occur much easier than that in NO_2PyOH•. Furthermore, PyOH• is significantly bent as indicated by the out-of-plane N–O bond bending angle (α) of 152°, whereas NO_2PyOH• is relatively flat (α = 169°). Similar results have been reported for the N–O bond fragmentation in *N*-methoxy-substituted aromatic compounds.[19] The N–O bond cleavage requires mixing of π^* and σ^* orbitals, which is achieved by bending the N–O bond out of the plane of the aromatic ring.

In conclusion, photoreduction of a simple pyridine *N*-oxide by BNAH in deaerated aprotic medium resulted in generation of •OH, which can oxidize SA to 2,3-DHBA, 2,5-DHBA, and catechol. The electron-withdrawing group such as NO_2 on the aromatic ring can significantly stabilize the radical anion of pyridine *N*-oxide, precluding the subsequent •OH release. We are currently investigating the detailed effects of various substituents on the photoreactivities of pyridine *N*-oxides in the presence of various reducing agents.

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