Annual Autumn Meeting, Düsseldorf, Biol.Chem. Hoppe-Seyler Vol. 374, 742 (1993),V21

R. Kiehl

Evidence for mitochondrial FoF1-ATPase being a K^+-pump

Our experimente demonstrate that mitochondria contain an energy driven K^+/H+-antiporter. The energy is derived from substrate oxidation by the respiratory chain. This antiporter is Mg^{2+}-sensitive stimulated by N'-[N''-n-nonyl-4-sulfamoylphenyl)-maleimide$_o$, $Cd^{2+}{}_o$, dicyclohexyl-carbodiimimide$_o$ and $Ca^{2+}{}_o$. Quinine prevents the Mg^{2+}-sensitivity. Ruthenium red prevents $Cd^{2+}{}_o$- $Ca^{2+}{}_o$-sensitivity.

A mechanism for mitochondrial ATP synthesis on the 30 kDa Pi/H+ -symport system with oxidized glutathione as catalyst will be presented. The effect of the uncoupler DNP and arsenate in this mechanism will be discussed. Coupling between ATP synthase and ATPase is suggested. The mechanism represents the first description of a proton driven build up of high energy intermediates (activated disulfides, sulfenyl phosphate) and thereby performed phosphoryl transfer or transport activities. The sulfur analog of phosphate, namely thiophosphate, most likely proves sulfenylphosphate perticipation by inhibition of these activities.

The experiments prove further, that mitochondria contain an oligomycin-sensitive ATP-driven K^+ pump, which is identical with the oligomycin-sensitive FoF1-ATPase. This K -pump is stimulated by-N`-(N``-n-nonyl-4-Sulfamoylphenyl)maleimide, picrylacetate, Cd^{2+}(Mg^{2+}, Ca^{2+}) and inhibited by dicyclohexylcarbodiimide. A lot of earlier investigations pointed already to former reault. Artificial systems 'proving' ATP synthesis may then for instance be compared with the sarcoplasmic reticulum Ca^{2+}-transport ATPase/ 'synthase'. A physiological synthesis of ATP on the Pi/H^+-symporter is therefore most probable or even proved.

K+/H+-antiporter, K +-pump and Pi/H+-symporter/ATP synthase are linked together in versatile energy driven K+/H+ -cycling and oscillations. This system is controlled by 02 and the free Mg^{2+} and Ca^{2+} concentrations in the cytosol of the cells and most probably able to handle also 'abnormal' Ca^{2+} -concentrations. A high physiological K+ -gradient between cytosol (K^+ - high, ca.175 mM) and matrix (K = low, ca.1 mM) is established and maintained by the system. Under normal physiological conditions are no protons in the bulk phase detectable, they are therefore either moving along the membranes via hydrogen bridges or the proton pumping systems are directly linked.

The results imply a direct connection (and requlatory function) between nervous system (brain) and mitochondria (body and brain): thermoregulation, substrate oxidation as well as 02-uptake/ -reduction (anorexia/dystrophia) with build up of arteriosclerosis, heart attack, cancer on the one hand and build up of edema during anoxia/ hypoxia or shock, dilusion with coma (death) on the other hand are examples.

o - outside mitochondria

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13.Vortragstagung der GDCh, Fachgruppe Biochemie, Darmstadt, 16.-18.März 1994, A.p2.6.

IGE-REGULATION BY DITHIOL/ DISULFIDE INTERCHANGE: IN „VIVO“ STUDY ON BLOOD OF ATOPIC ECZEMA PATIENTS SUPERIOR TO CELL CULTURE SYSTEMS

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A dithiol/ diaulfide interchange mechanism is involved in IgE-synthesis. The associated redox state is sensitive to Hg2+, Diamide, gIFN and Il-4. I1-4 reacts antagonistically to gIFN in blood.samples and cell cultures, although to opposite directions. Elements involved in the signal transduction pathway from gIFN or Il-4 to IgE are their receptors, (a) cytosolic G protein(s), NADPH oxidase, a yet unknown electron transfer factor (etf), redox factor (ref), nuclear transcription factors and endonuclease. This electron transfer chain, NADPH to IgE, is coupled to ongoing mitochondrial energy formation. The involved reduced ref, stimulating DNA-binding, carries an essential sulfhydryl group, whereas the oxidized state, responsible for catalytic endonuclease activity, carries a catalytic diaulfide: in analogy to the mitochondrial transport and ATP synthesis, where activated disulfides were performing phosphoryl transfer or transport activities. The involvement of Mg2+- or Ca2+-sensitive serine residues in the signal transduction to IgE synthesis is suggested by APMSF/ EDTA-titrations. An important role in modulating IgE concentrations play kinases, esp. protein kinase C. Toxic mercury or reactive oxygen species seem to be not responsible for the changes in patients IgE-levels but for activation of metallo proteases and associated glucocorticoid sensitive inflammations. Metallo proteases degrade gIFN. Especially the course of leucocytes collagenase activity is opposite to gIFN concentration. Environmental pollutants, including formic aldehyde, sulfite/SO2, isocyanates and anhydrides, reacting irreversibly with the involved essential dithiol/disulfide redox state or CO, by binding to the NADPH oxidase, are shifting the redox state to the reduced form with enhanced probability for IgE synthasis. In addition, weakening of the immune system by dermal and intestinal dysbiosis (C.albicans), food (carbohydrate) as well an psychogenic stress (norepinephrine) leads in enhanced development of IgE antibodies. The first expression of, for instance, food- or inhalative allergen specific IgE may purely be incidental and relate to autoimmune diseases. The described
pathogenese of atopic eczema should be compared with development of cancer or AIDS.

Gesellschaft für Biol.Chemie, Herbsttagung, Würzburg, Sept 19-21, 1994, Biol.Chem.H.-S.375, S61 R. Kiehl

Sulfur associated redox reactions, involvement in signal transduction and phosphate transfer-relevance for pathogenesis of diseases

Some million years ago world's sulfur athmosphere changed into an oxygen atmosphere. During this time,however,cells maintained their sulfur for live functions. Evolutionary pressure could not alter this fact and oxygen remained poisonous until today.- I will present some important examplas for men and discuse the pathogenesis of diseases: 1.Activation of human PMN leucocyte proprocollagenases involve an intramolecular cysteine/disulfide rearrangement generating an accessible catalytically active zinc at the enzyme`s reactive site(1).The matrix metalloproteinases are involved in inflammatory processes, atopic eczema, cancer incl (2,3).-

2.Transport of anions and synthesis of ATP with the catalytic involvement of sulfenyl phosphate.The importance of ATP for live functions needs not to be stressed (4). 3.The pathogenesis of atopic eczema (IgE-regulation) and concomitantly cancer(proliferation) involve a dithiol/disulfide interchange mechanism (redox reaction) highly sensitive towards various treatments (Hg +,Diamide, gIFN, I1-4 etc.) (3). The essential intact electron transfer chain, NADPH to DNA(IgE), carries a catalytic disulfide (ox.ref) responsible for endonuclease activity. The involved thymidylate- and adenylosuccinate synthase(3) require sulfenic acid residues for function. The results discussed relate to development of AIDS and the most likely mechanism for its pathogenesis will be presented. -

1.J.Bläser et al.(1991) Eur.J.Biochem.202,1223-1230; S.Triebel et al.(1992) FEBS 298,280-284.
2.V.Knäuper et al.(1990) FEBS 263,355-357; V.Knäuper et al.(1991)FEBS 290,99-102.
3.R.Kiehl(1994)13.Vortragstag.Fachgr.Bioch.GDCh,Darmstadt,Abstr.P2.6andAnalyt.Conf. München, Abstr.p.195. R.Kiehl, H.Tschesche(1994) J.Immunology,subm.
4.R.Kiehl(1993) Biol.Chem. H.-S.374,742;and subm.

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14.Vortragstagung der GDCh, Fachgruppe Biochemie, Kaiserslautern, 15.-17.März1995,Abst 18:

Chemistry: Aid for identification of biochem. systems and solution of biochem. reaction mechanisms. Fall: ATP synthesis and Transport in mitochondria.

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The thiol reagent N`-[N``-n-nonyl-4-sulfamoylphenyl]-maleimide (NSPM) reacts meanly with adenine nucleotide binding sites because of its similarity with the adenine moiety of the corresponding nucleotides. We could show that NSPM competes in some nucleotide binding sites with phosphate binding thereby abolishing the phosphate- and uncoupler (2,4-dinitrophenol/ DNP or 2azido-4-nitro-phenol/ NPA) binding and transport (l] (table, fig.). - The sulfenyl reagent n-nonylthiouracil (NTU) reacts rapidly and specifically with sulfenyl groups in lipophilic environment [2] (table, fig.). The incubation of well coupled mitochondria with [^{35}S] NTU results finally in the isolation of [^{35}S] thiosulfenic acid of glutathione. At calculated 100 % inhibiting concentrations for State 4 - State 3 transition or DNP uncoupling by NTU is almost the whole glutathione pool involved. Phosphate modulates the bound and free glutathione concentrations. The effects of various sulfenyl- and thiol trapping compounds (incl. NSPM, Cd2+, Diamide, NTU, NPA, SPO_3^-) and the high energy compound picrylacetate (PA) in a postulated relais-mechanism suggest glutathione as endogenous regulatory factor for mitochondrial Pi/H+-symport [1,2]. - A mechanism for mitochondrial ATP synthesis on the Pi/H+- symport system with oxidized glutathione as catalyst has been presented [1]. The effects of the uncoupler DNP and arsenate in this mechanism were discussed. This mechanism is the first description of a proton driven build up of high energy intermediates (activated disulfides, sulfenyl phosphate) and thereby performed phosphoryl transfer or transport activities [1-4]. Thiophosphate presumably is functioning as „suicide" inhibitor for these activities and proves then sulfenylphosphate participation [1]. - Mitochondria contain an oligomicin sensitive ATP-driven K^+-pump and this pump is identical with the oligomicin sensitive FoF1-ATPase. The K^+-pump is stimulated by NSPM, PA, Cd^{2+} (Mg^{2+}, Ca^{2+}) and inhibited by dicyclohexylcarbodiimide (DCCD). A physiological synthesis of ATP on the Pi/H^+-symport system is therefore most

probable or even proven. Coupling between ATP synthase and ATPase is suggested [1](fig). Mitochondria contain an energy driven K+/H + -antiport-system. The energy is derived from substrate oxidation by the respiratory chain. This antiporter is Mg^{2+}-sensitive stimulated bv $NSPM_o$, Cd_o^{2+}, $DCCD_o$ and Ca_o^{2+}. Quinine prevents the Mg^{2+}-sensitivity. Ruthenium red prevents Cd_o^{2+}-and Ca_o^{2+}-sensitivity (o = outside) [1]. - The conclusion out of the results are clear for bioenergetics, the connection to medicine (incl. pharmacology/ toxicology) is obvious and will be discussed [1].

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Table I. ^{14}C-DNP-accumulation in the presence of NSPM

Conditions	^{14}C-DNP, Pellet nmol/mg	Accumulation nmol/mg	%
95 µM 14C-DNP	13.30 ± 0.33 (3)	6.63	100
95 µM 14C-DNP, + 223 nmoles Triton X 100/mg [a)]	6.67 ± 0.10 (3)	0	0
95 µM 14C-DNP, + 20 nmoles NSPM/mg	8.75 ± 0.15 (3)	2.08	31.4
20 nmoles NSPM/mg, + 95 µM ^{14}C-DNP	11.60 t 0.10 (3)	4.93	74.4

a) amount of Triton resulting in uncoupling; inhibition of RCR at 90 nmoles/mg

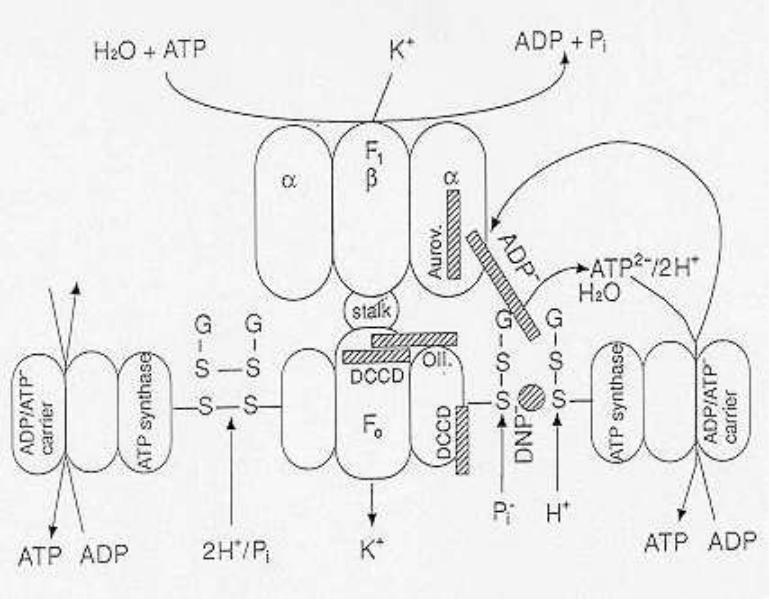
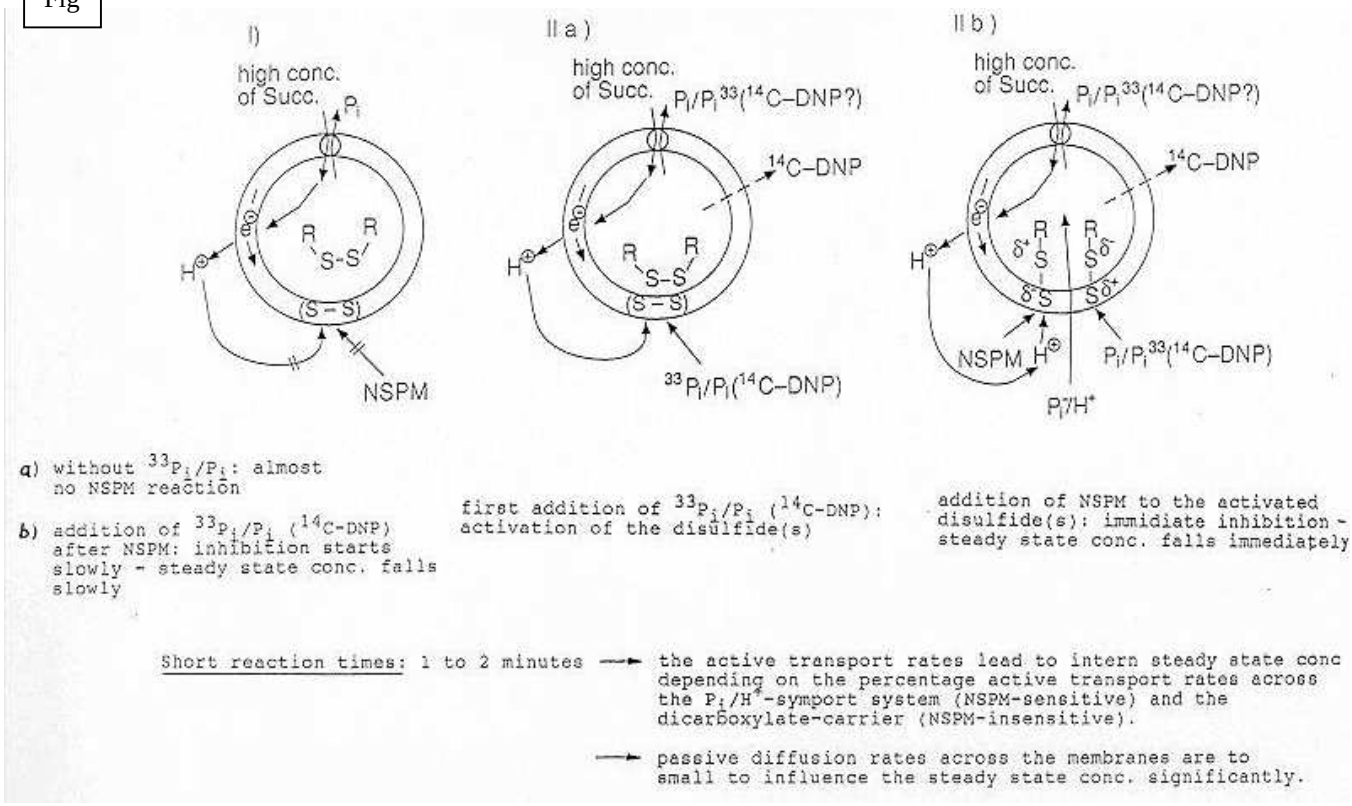


Fig. Coupling between ATP synthase, membrane bound ATPase (FoF1) and ATP/ADP-Translocator

Fig



4th Int.Congr.on Amino Acids, Vienna, 7-11th August 1995, Amino Acids 9(1), 20:

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Glutathione: Tlie essential factor for live functions

The inhibition of mitochondrial DNP- or phosphate transport by the thiol reagent NSPM (N' [N''-n-nonyl-4-sulfamoylphenyl]-maleimid) suggests the involvement of a regulatory factor in this transport activities [1,2]. This regulatory factor proved to be glutathione, meanly by its reactivity with the sulfenyl reagent NTU (n-Nonylthiouracil) and the modulation of free and bound -glutathione concentrations by phosphate [l,2]. A mechanism for mitochondrial ATP synthesis on the 30 kDa Pi/H^+-symport system with oxidized glutathione as catalyst has been presented. Sulfenylphosphate participation in this mechanism should be proven by the sulfur analog of phosphate namely thiophosphate, which presumably functions as suicide inhibitor for ATP synthesis [1. 2].

Mitochondrial K^+/H^+-antiport, K^+-pump and Pi/H^+-symport/ATP synthase are linked in versatile energy driven K^+/H^+-cycling and oscillations. The whole system is dependent on oxidized and reduced glutathione. Many diseases are due to perturbation of this system [l, 2]. The described system is also responsible for thermoregulation of our body. Acetylsalicylic acid presumably acts via nervous system (brain) on the center responsible for thermoregulation.

Antipyretic malaria therapeutics like chinin may change Ca^{2+}/Mg^{2+}-binding to mitochondria and by this way regulate the body temperature [1-3].

The functions of glutathione in the cells were extensively discussed and described [4]. The functions in mitochondria besides the involvement in ATP synthesis, are not as clear. Detoxification of mitochondria results in their "evolutionary development" with raised glutathione concentrations (relation to Moris hepatoma). A high turnover rate of chemicals (plasma-cell-mitochondria-cell-plasma) sharply rises glycolysis rates. which finally lead to enhanced proliferation of cells. Mitochondria are able to be inhibited by Ca^{2+} (via - for instance - complexation of glutathione) and this inhibition may lead to certain types of tumors (high rates of glycolytic formation of ATP).

Glutathione seems not to be directly involved in the redox potentials responsible for development of atopic eczema or AIDS and change in proliferation rates [5].

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5. Kiehl R (1994) Int ALK - Ciba Corning Joint Symposium. Benzheim

25.GDCh-Hauptversammlung, Münster, 10.-16.Sept 1995, Abstrakt-Buch p568, BC 7:

DIE ORG.CHEMIE EIN HILFSMITTEL ZUR IDENTIFIZIERUNG BIOCHEM. SYSTEME SOWIE AUFKLÄRUNG BIOCHEM. REAKTIONSMECHANISMEN: ATP SYNTHESE UND TRANSPORT IN MITOCHONDRIEN

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Das Thiolreagenz N`-[N`'-n-nonyl-4-sulfamoylphenyl)-maleimid (NSPM) reagiert hauptsächlich in Adeninnucleotid Bindungsseiten wegen seiner Ähnlichkeit mit dem Adeninteil der entsprechenden Nucleotide. Wir konnten zeigen, daß NSPM in der mitochondrialen Membran in einigen Nucleotid Bindungsseiten mit der Phosphat Bindung konkurriert und dabei die Phospat- und Entkoppler (2,4-Dinitrophenol/DNP oder 2-Azido-4-nitrophenol/NPA) Bindung sowie deren Transport verhindert [l]. - Das Sulfenylreagenz n-Nonyl-thio-uracil (NTU) reagiert schnell und spezifisch mit Sulfenylgruppen in lipophiler Umgebung [2]. Die Inkubation von sehr gut gekoppelten Mitochondrien mit [^{35}S] NTU resultiert im Endeffekt in der Isolierung der [^{35}S] Thiosulfensäure von Glutathion. Bei einer kalkulierten 100 % Inhibierung des State 4 state3 - Überganges oder der DNP-Entkopplung durch NTU ist fast der gesamte Glutathion-Pool involviert. Phosphat moduliert die gebundene sowie freie Glutathionkonzentration. Die Effekte von verschiedenen Sulfenyl- und Thiolgruppenreagentien (inkl. NSPM, Cd^{2+}, Diamid, NTU, NPA, SPO_3^-) sowie der 'Hochenergie'-Verbindung Picrylacetat (PA) in einem postulierten Relais-Mechanismus suggerieren Glutathion als endogenen Regulationsfaktor für mitochondrialen Pi/ H+-Symport [1,2]. - Ein Mechanismus für die mitochondriale ATP Synthese an dem Pi/H+-Symport-System mit oxidiertem Glutathion als Katalysator wurde von uns formuliert [1]. Die Effekte der Entkoppler DNP und Arsenat in diesem Mechanismus sind diskutiert worden. Dieser Mechanismus ist die erste Beschreibung einer Protonen getriebenen Bildung von Hochenergie Intermediaten (aktiviertem Disulfid, Sulfenylphosphat) und dabei durchgeführtem

Phosphattransfer oder Transportaktivitäten [1-4]. Thiophosphat fungiert wahrscheinlich als 'Suicide`Inhibitor für diese Aktivitäten und beweist damit Sulfenylphosphat Beteiligung [1]. - Mitochondrien enthalten eine Oligomycin sensitive ATP getriebene K+-Pumpe und diese Pumpe ist identisch mit der Olygomycin sensitiven FoF1-ATPase. Die K+-Pumpe ist stimulierbar durch NSPM, PA, (Mg^{2+}, Ca^{2+}) und sie wird inhibiert durch Dicyclohexyl-carbodiimid (DCCD). Eine physiologische Synthese von ATP an dem H^+-Symporter ist deshalb wahrscheinlich oder sogar bewiesen. Es scheint eine Kopplung zwischen ATPSynthase und ATPase vorhanden zu sein (1). - Mitochondrien besitzen ein Energie getriebenes K+/H+-Antiport-System. Die Energie wird geliefert durch Substrat-Oxidation in der Atmungskette. Der Antiport wird Mg2+-sensitiv stimuliert durch $NSPM_o$, $Cd2+_o$, $DCCD_o$ und Ca_o^{2+} . Quinin verhindert die Mg2+-Sensitivität. Ruthenium Rot verhindert Cd_o2+ - und Ca_o2+- Sensitivität (o = outside) [1]. Die Schlußfolgerungen aus den Resultaten sind für die Bioenergetik klar, die Verbindung zur Medizin (incl. Pharmakologie/ Toxikologie) ist eindeutig und wird diskutiert werden (1).

Literatur:l.Kiehl,R.(1994) J.of Mol.Med., No.1 to 4; 2.Kiehl, R. (1974) Diploma und (1977) Dissertation, Universität Heidelberg; 3.Bäuerlein, E. and Kiehl, R..(1976) FEBS Letters 61, 68-71; 4.Kiehl, R. and Bäuerlein, E. (1976) FEBS Letters 72; 24-28.

15.Vortragstagung der GDCh, Fachgruppe Biochemie,Giessen, 13.-15.März1996, Abstr.p.35, P3:

Is it possible to demonstrate the dynamic of enzymatic catalysis by (bio)chemical and (bio)physical methods? Considerations to a still unsolved problem on the energy-transduction across mitochondrial membranes.

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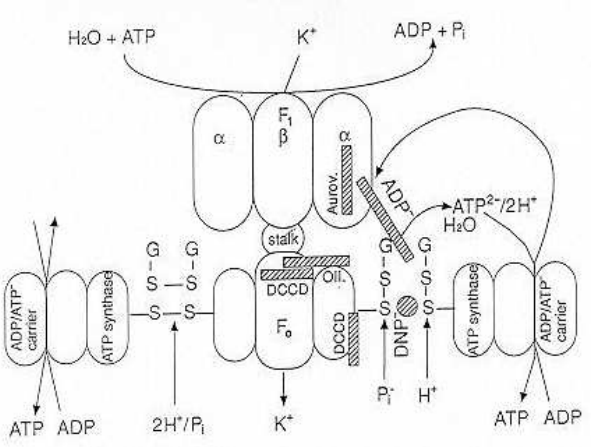
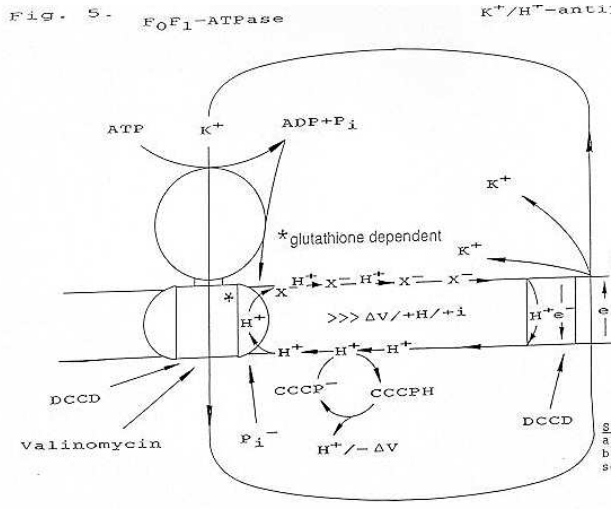
A) ATPsynthase/ATPase

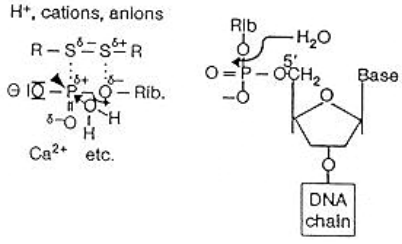


B)



Endonuclease

C



Statement: The dynamically living enzyme-systems (A-C) described by us [1-9] are with the static and dead x-ray diffraction not to analyse and molecular modeling on this systems is therefore not possible.
Presumably we are dealing with inner mitochondrial membrane surface reactions and we need thus methods to analyse such reactions. Ertl [10,11] is summarizing the

present available methods (LEED, FTIR, PEEM, MEM, REM, FEM, FIM and STM). Some of these methods are probably also reliable to solve our problems by interplay with the methods already used for biochemical purposes (e.g. freeze etching, freeze fracture).
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(Bunsen-Tagung, Jena, 1996, eingereicht, dann) 16.Vortragstagung der GDCh, Fachgruppe Biochemie, Kassel, 12.-14.März, Abstr.p.3:

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Kopplungsfaktor Glutathion

Einführung in die Elektrophysiologie und Thermodynamik von Mitochondrien

Th. Wieland und E. Bäuerlein schlugen 1968 für die Umwandlung von Oxidationsenergie in die "energiereiche" Diphosphat-Bindung, z.B. von ATP, als phosphorylierendes Agens ein gemischtes Sulfensäure-Phosphorsäure Anhydrid R- S-O-PO_3H vor [1]. Painter und Hunter zeigten 1970 in einem Modellsystem mit oxidiertem Glutathion als Katalysator die Bildung von hohen Ausbeuten an ATP [2]. 1976 konnte an der Mitochondrien-Membran die Beteiligung von Glutathion an einem (H+) aktivierten Disulfid unter katalytischer Sulfenylphosphat-Beteiligung gezeigt werden [3]. Die letzten 20 Jahre Forschung führten zwangsläufig zur Formulierung des mitochondrialen F0F1-Komplexes als gekoppelte K+/H_20-Pumpe mit H+/Pi--induzierbarer ATPsynthase sowie zum Atmungsketten-Substrat getriebenen K+/H+-Antiport-System. Verbunden sind diese Einzelsysteme über antizyclische K+/H_20 und H^+/Pi-Bewegungen (Schwelungen plus Kontrak- tionen = Oszillationen), kontrolliert durch und die freien Mg^{2+} und Ca^{2+}-Konzentrationen im Cytosol der Zellen [4]. Das Gesamtsystem ist verantwortlich für die Thermoregulation unseres Körpers.
Die zyklische Hydrolyse/Synthese von ATP und die damit gekoppelte Freisetzung/Bindung von Mg^{2+} in dem "steady state flow system" führt zur Bildung von Wärme (q) mit steigender Temperatur (ΔT). Diese Wärme wird durch die oszillierenden Mitochondrien sowie das pumpende Herz über den Gesamtkörper verteilt und durch die normalen Körperfunktionen verbraucht.

Störungen dieses Systems werden normalerweise durch niedere/höhere Atmungsraten kompensiert [5]. Eine vereinfachte Beschreibung zur Thermodynamik von Mitochondrien liefert die Theorie von Carnot [6]. Die hauptsächlich von Eisen und seinem Oxidationszustand abhängigen H+/e--Verschiebungen, Strom (i), führen zu hohen lokalen Spannungen (ΔV) über der Membran mit entsprechenden magnetischen Feldern (H). Die unter physiologischen Bedingungen arbeitende mitochondriale Membran fungiert hierbei gleichzeitig als Kondensator, Transmitter und als Supraleiter (37^{o}C!). Um einen besseren Eindruck von diesem System zu erhalten, sollte man zur Einführung die Theorie von Maxwell und Kirchhoff lesen. Weitere Studien an Faradays und Oerstedts Gleichungen sollten in die Kompliziertheit der mitochondrialen Elektrophysiologie und in die Zusammenhänge von Gedächtnis und Denken, sowie in die Grundlagen der chinesischen Akupunktur fuhren [6].

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5th Int.Congr.on Amino Acids, Chalkidiki, 25.-29th August 1997, Amino Acids 13, p50:

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Coupling factor glutathione: Introduction into the electrophysiology and thermodynamics of mitochondria

Th. Wieland and E. Bäuerlein formulated in 1968 a mixed anhydride of sulfenic and phosphoric acid, R-S-0PO3 H-, to explain the conversion of energy obtained by oxidation into the energy-rich binding between two phosphate groups, as for instance found in ATP. In 1970, Painter and Hunter showed high amounts of ATP-formation in a model system containing oxidized glutathione as essential catalyst. During my attempt to elucidate the mechanism of oxidative phosphorylation in mitochondria, i came to the conclusion that an (H^+) activated disulfide in the lipophilic membrane had to be involved [3]. I proposed a phosphate reaction with ATP on this activated disulfide in a synchronous reaction mechanism without the real build-up of a sulfenylphosphate intermediate. The research performed during the last 20 years led inevitably to the formulation of the mitochondrial FoF1-complex as coupled K^+/H2O-pump (similar to the nAChR) with H^+/Pi-inducible ATPsynthase as well as to the respiratory chain substrate driven K^+/H^+-antiport system. These systems are linked together in anticyclic energy driven K^+/H20, H^+/Pi-movements and oscillations (swelling plus contraction of the mitochondrial matrix space by osmotically active K^+-ions), controlled by 02 and the free Mg^{2+}- and C^{2+} -concentrations in the cytosol of the cells. The system is responsible for the thermoregulation of our body. - The

cyclic hydrolysis/synthesis of ATP and the concomitantly cyclic release/binding of Mg^{2+} in the "steady state flow system" releases heat and the temperature (ΔT) is permanently raised. The released heat is constantly distributed throughout the entire body by the oscillating mitochondria, as well as the pumping heart, and is used up by the normal body functions. Disturbances of this system are normally compensated for by lower/higher respiration rates. For clarification, one should study the theory of Carnot. The essentially by iron and its state of oxidation dependent H^+/e--displacements, current (i), lead to high local voltages (ΔV) over the membrane with corresponding magnetic fields (H). The under physiological conditions operating mitochondriotic membrane acts thereby simultaneously as capacitor, transmitter and supraconductor (37^oC!). *The entire system is dependent on oxidized and reduced glutathione.* To gain more insight into this system, one should read an introduction to the theory and equations of Maxwell and Kirchhoff. Further studies based on Faraday's and Oerstedt's equations should lead to the complexity of mitochondrial electrophysiology, its influence on momory and thinking, and the basics of the chinese acupuncture.

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