GDCh

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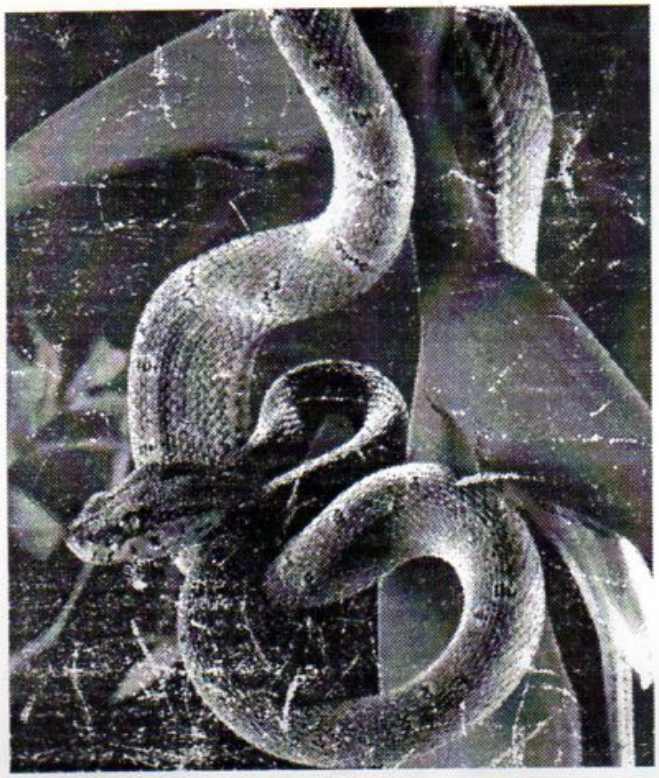
Deutscher Chemiker

Fachgruppe
Biochemie

Abstracts

14. Vortragstagung

Struktur, Funktion und Design: Vom Protein zum niedermolekularen Wirkstoff



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Chemistry: Aid for identification of biochem. systems and solution of biochem. reaction mechanisms. Fall: ATP synthesis and transport in mitochondria. Reinhold Kiehl, Laboratory and Research GmbH for Mol. Medicine/ Biology, 93437 Furth, Germany.

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 2-azido-4-nitro-phenol/ NPA) binding and transport [1] (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, Cd^{2+}, 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 P_i/H^+-symport [1,2]. - A mechanism for mitochondrial ATP synthesis on the P_i/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 F_0F_1-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 P_i/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 by $NSPM_o$, 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 for bioenergetics clear, the connection to medicine (incl. pharmacology/ toxicology) is obvious and will be discussed [1].

References: 1. Kiehl, R. (1994) J. of Mol. Med., No. 1 to 4; 2. Kiehl R. (1974) Diploma and (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; 24-28.

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

Conditions	^{14}C-DNP, Pellet nmol/mg	Accumulation nmol/mg	%
95 µM ^{14}C-DNP	13.20 ± 0.33 (3)	6.63	100
95 µM ^{14}C-DNP, + 223 nmoles Triton X100/mg[a]	6.67 ± 0.10 (3)	0	0
95 µM ^{14}C-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 ± 0.10 (3)	4.93	74.4

a) amount of Triton resulting in uncoupling

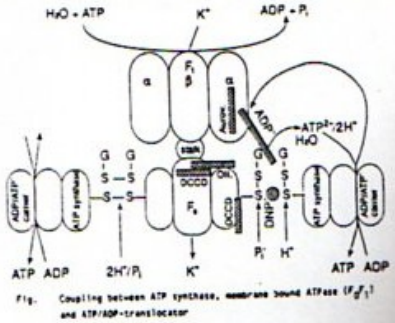

Fig. Coupling between ATP synthase, membrane bound ATPase (F_0F_1) and ATP/ADP-translocator