Scripps Clinic and Research Foundation

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Department of Biochemistry

July 24, 1979

Dr. Reinhold Kiehl
Institut für Physiologische Chemie
Rühr-Universität Bochum
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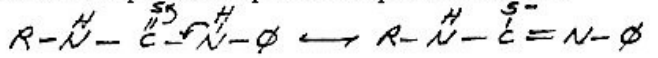
Universitätsstrasse 150
West Germany

Dear Reinhold,

A thought has occurred to me with regard to NPTU, which might be interesting for your paper. The thought is whether NPTU could act as a proton carrier in mitochondria. For example, the following reaction could produce a potential proton carrier:



with $=N-\phi$ structure stabilized by resonance through the benzene ring. The question is whether the $-SH$ function in NPTU can be detected at various pH values by NMR, and whether NPTU might increase the conductance of lipid bilayers. I don't know whether we have any NPTU here. Since Yves is away, you might let me know about this so that I can arrange to have the lipid bilayer conductance experiment done. You might already have the NMR data, or you might be able to do them in Bochum. At any rate, please write and let me know what you think.

Best regards,

Youssef Hatefi

YH/jlh

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August 9, 1979

Dr. Reinhold Kiehl
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Dear Reinhold,

A copy of your DCCD paper is enclosed for your perusal and comments. Should you agree with the paper as is, or have only minor comments, please cable or phone, so that we can submit the paper to Biochemistry without delay. Should you have extensive comments, then you would have to write to me. However, you might still cable or phone that you have received the manuscript and that your comments will follow. At any rate, please remember that I would not agree to overspeculation based on thin evidence. Also, please check the Methods and Materials and the Results sections as well as the Tables, figure legends and references very carefully for possible errors.

With very best regards to you, Walter, and to your family,

Very sincerely,

Youssef Hatefi

YH/jlh

Scripps Clinic and Research Foundation

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Department of Biochemistry

August 30, 1979

Dr. Reinhold Kiehl
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Dear Reinhold,

The DCCD paper has been revised in accordance with your suggestions and those of others. The manuscript will be sent off today to Biochemistry, and a copy is enclosed for your files. Any other minor modification can be made on the proofs should the paper be accepted for publication.

Regarding your comment to page 16, I checked this with several people here, and it seems that you had misunderstood the intent of the references to Graf & Sebald, and Sebald et al. The point made in this sentence is concerned only with the number of DCCD-binding polypeptides in Neurospora, yeast and beef-heart mitochondria. There is no mention of ATPase complex & ATP-Pi exchange activity. Nevertheless, I changed the wording of "These results agree with ..." to "These results recall ...". Also, I do not want to talk about 2/3, because this is a soft calculation rather than an accurate determination, and it introduces an unnecessary problem.

I have sent both NPTU and NPU to McLaughlin to compare their ability to increase lipid bilayer conductance as compared to a protonophoretic uncoupler of medium potency, such as DNP. We have agreed that I reveal the nature of the compounds to him after the data have been obtained. Incidentally, I did not realize until a few days ago that in the only assay run for NPU (i.e., ATP-Pi exchange activity of Complex V), this compound was just as inhibitory as its thiourea analog. It would be nice to have an NPA equilibrium-binding competition curve also for NPU. Furthermore, in your NMR experiments, it would be useful to know also whether NPU forms the C-OH containing isourea at pH 4 to 10.

With very best regards, also to Ilse and Walter,

Sincerely yours,

Youssef Hatefi

YH/jlh

Bo., 5.5.80

Dear Joe,

I am sending you the pictures for the NPTM paper. Two pictures have to be done again (Fig 2: first has to be Pat, and Fig. 10: with resolving for high and low affinity binding for NPA).

I did for some of the inhibition curves Hill-plots, since the curves show no normal inhibition behavior of the compound NPTM.

For ATP driven reactions (Fig. 4 and 5) - involvement of the ATPase - n is between 3 and 4, which is suggesting involvement of at least 4 subunits in the reaction - which goes parallel with blocking the ^{14}C-DCCD-binding in C$\underline{V}$ and numbers of DCCD-binding sites. The inhibition of the ATP-P_i-exchange shows normal behavior (linear up to 90% inhibition).

The proton release itself (NADH-jump) gives a n value of about 2, which is suggesting involvement of two subunits in the release! which could be a dimeric UBP. There is also high competition between NPA and NPTM (Fig 10 new - factor of about 5).

The succinate driven transhydrogenase is differently inhibited as the ATP-driven, there seems to be involvement of fewer subunits in catalysis ($n \sim 2.6$ and conc. for half-maximal inhibition is ~ 34 nmol/mg) → relation to proton release?

In the presence of Rotenone instead of KCN - the inhibition kinetic is changed at the beginning ($n: 1.4 \rightarrow 3.4$).

The effect on the C$\underline{V}$-ATPase (Fig. 12) is to resolve in two different effects - first inhibition and second stimulation.

The nucleophilic power of the different compounds are to correlate with the ATP-P_i-exchange inhibition as shown in Fig. 11. Studies on rat-liver mitos with the compounds are

showing similar results: NPTU, the oxygen analog, tert.butyl-n-nonyl-thiourea are uncoupling at about the same conc. with succinate as substrate - nonyl-tert.butylurea does not!

I am furthermore working with the different compounds, but for the paper this are enough data, I think (together with the things I wrote you already).

To the struct. requirement: a) There is no need for the phenyl - as I wrote priviously - but the nucleophilic power of the sulfur!

b) similarity to DCCD is important for competition

Suggestion: as before - reaction on electrophilic groups - actio. by $H^{\oplus}$ (i.e. -COOH).

Please tell me if you are still interested in the paper - if not could you send back the material and I would try writing it alone. If you are still interested - I hope so - please write it up as soon as you can. Tell me if you are satisfied with the pictures.

Since I am on the way for habilitation I need the papers fairly fast. Also waiting longer doesn't do any good - since there are lots of new data coming up.

Sorry, I don't want to push you - but you know that the job situation is not the best at the moment, and one needs to do a lot in order to get a tenured position.

Greatings to everyone.

Sincerely yours

Reinhold

enclosure: 4 drawings
3 pictures

DEPARTMENT OF BIOCHEMISTRY

Scripps Clinic and Research Foundation

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May 14, 1980

Dr. Reinhold Kiehl
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Dear Reinhold,

Thank you for your letter of May 5, 1980, and the accompanying data on NPTU. I would like to put the data together in a paper, but there are two problems. First of all, I'll be busy for the rest of this month with Study Section work, then follows ASBC meetings and Study Section meeting both in New Orleans, then I must come back to La Jolla and prepare for the move of the lab to the Torrey Pines building. After that, i.e., mid July, I have other priorities. At any rate, I could not write a paper without figure legends and the section on Methods for which you will have to prepare the draft. The second problem is that the discovery of yet another mildly potent uncoupler is not an important enough event. Therefore, I am afraid that your paper may not be accepted for publication unless there is a new and important feature of this particular uncoupler which will advance the understanding of the mechanism. This is why I was interested to know whether the S in NPTU and O in NPU could be protonated at near neutral pH.

At any rate, my suggestion is that I return to you all the figures you have sent plus the folder I still have on NPTU and NPU, and let you think about whether or not you want to write the paper with the available data. Also, if I may make one or two other suggestions, I think that the Hill plots should not be shown or discussed, because of the enormous complexity of the system. If you had obtained in most cases a uniform Hill number, then one could have discussed it, but as things are I think you should not emphasize this point. You can state in the Discussion section that the Hill numbers are variable and greater than unity, indicating mechanistic complexities, but not much more. The oxygraph traces are crowded with too much writing. For example, all the initial additions should be deleted and explained in the legend, and concentrations of additives

May 14, 1980

(e.g. ADP, DNP, etc.) also explained in the legend when they are the same throughout. I should be happy to read your draft and put in my 2 cents worth, but I must reserve judgement as to whether the available data will make a satisfactory paper.

With best wishes,

Sincerely yours,

Youssef Hatefi

YH:caf