ANALYTICAL BIOCHEMISTRY

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June 8, 1982

Dr. Reinhold Kiehl
Inst. f. Phys. Chem. MA 2/136
Ruhr-Univ. Bochum
Universitatsstr. 150
Bochum 4630-1
WEST GERMANY

Dear Dr. Kiehl:

Your manuscript entitled "Dodecylsulfate-polyacrylamide gel electrophoresis at acidic pH suitable for detection of labile protein bound functional groups" has been carefully reviewed. One of the reviewers, whose comments are enclosed, finds that the results presented are not a focused study which describes new methodology. Therefore, I regret to inform you that the manuscript is not suitable for publication in Analytical Biochemistry.

The copies of the manuscript which were submitted will be returned to you under separate cover.

Sincerely,

W. S. Allison
for the Editors

WSA:jm

Enc.

ANALYTICAL BIOCHEMISTRY

The enclosed manuscript has been submitted for publication in Analytical Biochemistry. Your evaluation of the manuscript would be greatly appreciated. Please return regular papers with your evaluation within two weeks of receipt. If you do not have the time to review this manuscript would you please ask one of your colleagues to do so. PLEASE SIGN AND RETURN THIS FORM, RETAINING THE YELLOW COPY FOR YOUR FILES.

YOUR APPRAISAL SHOULD INCLUDE A SPECIFIC RECOMMENDATION FOR ACCEPTANCE, REVISION, OR REJECTION, AND SPECIFIC REASONS FOR THIS RECOMMENDATION SHOULD BE INCLUDED.

Manuscript by: REINHOLD KIEHL

Ms. No.: E596-A43
Date: 5/11/82

Title: Dodecylsulfate-polyacrylamide gel electrophoresis at acidic pH suitable for detection of labile protein bound functional groups

Comments: (Please continue on additional sheet(s) if necessary, using ordinary paper.)

Unfortunately the principle focus of this manuscript does not appear to be the description and characterization of new methodology. Instead, the author presents a highly specific and poorly substantiated study of the effects of chemical modification on the electrophoretic mobility of F_1 subunits. In addition, parts of the manuscript lack clarity and there is frequent use of unconventional abbreviations. In the opinion of this reviewer the manuscript does not warrant publication in Analytical Biochemistry.

Specific examples to illustrate each of these general criticisms follow:

1. It would be of interest to see how the Weber and Osborn procedure compares with the author's in separating polypeptides without loss of covalently-bound, radioisotope-labeled, chemical reagents. What other procedures have been described to isolate modified proteins and peptides stable at acid pH (i.e., H.V. paper electrophoresis, chromatography, etc.)? How does the author's procedure compare to such alternatives?

2. It is suggested in discussing Fig. 3 that the ability of azide to promote cross-linking of a few percent of the subunits of F_1 may give a hint as to its action on the ATPase activity. How does the amount of cross-linking relate to the

AUTHOR'S COPY

amount of inhibition obtained at the concentration of azide used? The author reports that the subunit stoichiometry of chloroform released enzyme is $\alpha_4 \beta_3 \gamma_2 \delta_1 \varepsilon_{1\text{-}2}$ based on the relative staining of the subunits on gels. The data are not shown. If the author wishes to propose a new stoichiometry for F_1 subunits, a very thorough documentation would be necessary.

3. The introduction is not well written. Transitions between th^oughts are abrupt.

4. To my knowledge BF_1 is not a common abbreviation for mitochondrial F_1 even if obtained from beef heart. In addition, BF_1 is used by some laboratories to refer to bacterial F_1. I would also question whether W/O gels (Weber and Osborn gels) and K (for phosphorylase kinase) are appropriate abbreviations.

Dr. R. Kiehl

Dr. H Neurath
Editor, Biochemistry
University of Washington
Seattle, Washington 98195
USA

19.4.1983

Dear Dr. Neurath:

I would like to subumit the manuscript entitled

"Interaction of Picryl acetate with the mitochondrial F_1-ATPase"

for publication in Biochemistry.

As expert reviewers I would like to suggest:

Dr. R.L. Cross, Department of Biochemistry, Public Health Res. Inst., City of New York, Inc., 455 First Avenue, New York, NY 10016, USA.

Dr. A.E. Senior, Department of Biochemistry, University of Rochester Medical Center, Rochester, New York 14642.

Dr. E.C. Slater, Laboratory of Biochemistry, B.C.P. Jansen Institute, University of Amsterdam, Plantage Muidergracht 12, Amsterdam (The Netherlands).

Dr. P.V. Vignais, Laboratoire de Biochimie, Département de Recherche Fondamentale, Centre d'Etudes Nucléaires de Grenoble, et Faculté de Médecine de Grenoble, Grenoble, France.

Sincerely yours,

(Reinhold Kiehl, Ph.D.)

Biochemistry

HANS NEURATH, EDITOR
University of Washington
Seattle, Washington 98195
Phone (206) 543-1690

May 23, 1983

Dr. Reinhold Kiehl
Ruhr Universitat Bochum
Institut fur Physiologische Chemie
Universitatsstr. 150
4630 Bochum 1, West Germany

Dear Dr. Kiehl:

Thank you for sending us the manuscript entitled "Interaction of Picryl acetate with the mitochondrial F_1-ATPase". As usual, the manuscript was examined by two independent reviewers who are knowledgeable in this field. Their relevant comments are enclosed.

Both reviewers recommended that publication of the manuscript be declined and we must regretfully concur with their negative recommendation. We are sorry to convey this decision to you, but we should like to thank you for having given us an opportunity to consider your manuscript.

Sincerely yours,

William Parson

William W. Parson
Associate Editor

K3143K

WWP:ss
Enclosures

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BIOCHIMICA ET BIOPHYSICA ACTA

Please reply to:
Editorial Secretariat
Biochimica et Biophysica Acta
P.O. Box 1345
1000 BH Amsterdam
The Netherlands

Dr. Reinhold Kiehl,
Institut fur Physiologische Chemie,
Ruhr-Universitat Bochum,
D 4630 Bochum 1,
West Germany.

Ref. No. RPB 4271

Amsterdam, February 20, 1985

Dear Dr. Kiehl,

On behalf of the Managing Editors of Biochimica et Biophysica Acta, we would like to thank you for submitting the manuscript entitled "Interaction of Picrylacetate with the Mitochondrial F1-ATPase".

We are sorry to have to inform you that this paper is not acceptable for publication in BBA. This decision has been reached in the light of the enclosed comments made by the reviewers.

Yours sincerely,

Julie Egan
EDITORIAL SECRETARIAT

Encl.

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Amsterdam Handelsregister No. 158992

BIOCHIMICA ET BIOPHYSICA ACTA

Editorial Secretariat
P.O. Box 1345
Amsterdam, The Netherlands

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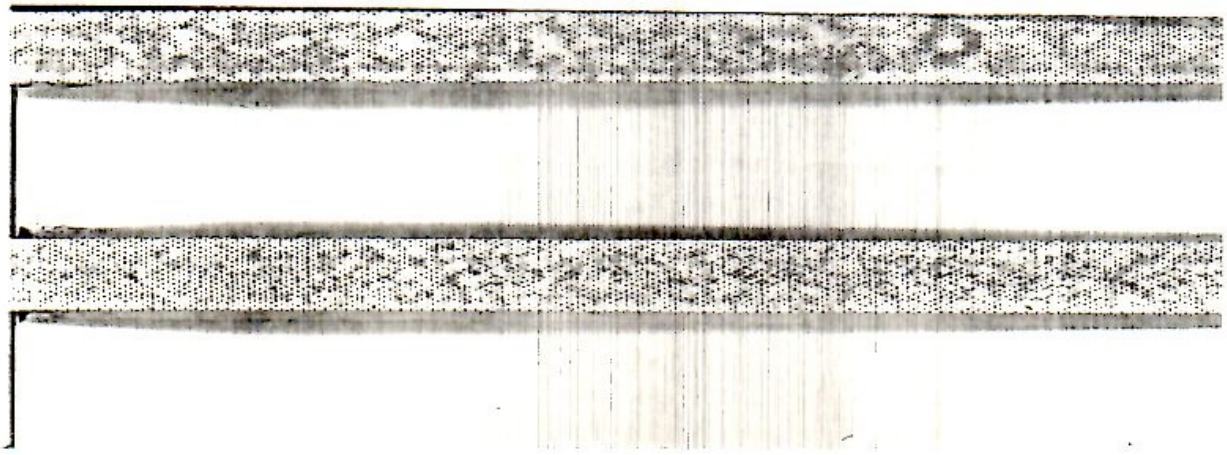
Article no: RP B 004271 Date of Receipt: 17 December, 1984
Title: Interaction of picrylacetate with the mitochondrial F1-ATPase
Corresponding Author: Kiehl R, Bochum

COMMENTS Reviewer I

The aim of this study is not very clear.
Besides, the legends of the Figures are not sufficient to understand how the experiments were conducted and the experimental conditions are never precisely described. It is therefore impossible to appreciate the validity of the results.
All the paper is rather confused and much too long especially Introduction and Discussion.
Therefore the manuscript cannot be accepted for publication in its present form.

Main Detailed remarks

1 - The authors do not mention whether their preparation is depleted in tightly-bound nucleotides and devoid of protein natural inhibitor.
2 - p. 6 The author mentions that only F_1 preparations with ATPase activity above 50-60 micromoles/mn/mg protein were used. It is then difficult to understand why in most experiments with phosphate release measurements, the enzyme is used under conditions strongly inhibiting its ATPase activity (14 to 20 micromoles per mn and mg protein) ; one can wonder whether this inhibitionis due to 0.3 M saccharose or to a denaturation of the enzyme. If it is a denaturation, the interpretations become questionnable.
3 - Fig. 1 It is not obvious from the legend, whether BSA or DTE are always added to the assays. The final volume is not given.
Fig. 2 The separation of alpha and beta is very bad. Therefore one cannot be sure of the conclusions. The degree of inhibition is not mentioned. What is the meaning of the stair levels if gels were cut at the arrows ?
Fig. 3 Precise the statistic significance between controls (1) and (2) since interpretations are based on the difference of activities. Besides, the legend does not specify either the pH or the F_1-ATPase preparations used for each incubation (the preparation of control (1) or (2) ?
Fig. 4 The concentration of F_1-ATPase is not given. The text refers to 50 % inhibition concentrations which are not mentioned in the legend. The legend is relatively precise for b and c but definitely insufficient for a, d, e.
Fig. 5 Concentration of F_1-ATPase ? and total volume ? What is meant by control (2)?
Fig. 6 Almost the same effects are obtained with both concentrations of NBD-Cl ; therefore how were these concentrations chosen and what was the degree of inhibition of ATPase activity ?
4 - p. 16 and scheme I : The author should not involve the role of monoionic Pi since the pH-dependence of Pi effects has not been studied.
5 - Minor : Almost all along the manuscript, the author speaks of phosphorus release instead of phosphate release.



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COMMENTS REVIEWER II

I have found it difficult to judge the scientific value of this paper because so many aspects are explained in an imprecise manner. It should not be necessary for the reader to figure out/guess what the author means. I cannot give an exhaustive list here, but some examples of this imprecision are:

page 7, line 17 'phosphate assay mixture' ; presumably this means assay mixture for determining ATPase activity but literally it means the assay mixture used for determining phosphate.

legend to Fig. 1 'before start of reaction with ATP' means that the protein was chemically reacted with ATP. Presumably the author means before determination of ATPase activity. The construction of this legend and its relation to the symbols in the figure could be much clearer.

legend to Fig. 3 the legend reads as if control (1) has two specific activities. Why not label the experiments a through f and give clear definitions in the legend?

legend to Fig. 4 is very unclear to this reviewer. (d) and (e) are not included explicitly in the legend. I am not certain that I understand what is meant by NBD-Cl, Picrate + NBD-Cl and NBD-Cl + PA when I read both the legend and the figure; I have to guess.

scheme 1 CT is not defined although in the text charge transfer is mentioned. So presumably this is what CT means, but the scheme ought to be comprehensible in itself.

The cumulative effect of such imprecisions throughout the paper has left this reviewer very uncertain as to the possible scientific value of this contribution. Nevertheless some scientific points are as follows:

1. The phosphorolysis scheme shown in Scheme 1 is highly speculative. Acetyl phosphate can survive in water for some time and so the authors could try to check their scheme with fairly simple experiments.

contd/

REVIEWER II (contd.)

2. Fig 2. Is it really possible to assign counts to the α and β subunits with such precision when the resolution of the α and β polypeptides is far from complete ?

3. According to Fig 3 less than 1 nmol ^{3}H acetyl is incorporated per nmol F_1 on a β chain. As there three β chains per F_1 one might have expected specific labelling to at least reach an incorporation of 1 nmol per nmol F_1. As NBD-Cl specifically modifies one tyrosine on one of three β chains then modification by NBD-Cl before treatment with PA should either have little effect on PA incorporation (if a specific PA reaction site is distinct from the NBD site) or a major protective effect if the same tyrosine reacts with both NBD or PA. From Fig. 6 it seems that there is [redacted] a small effect. But if there was a unique site of PA reaction one might expect that at least 1 nmol PA per nmol F_1 would be incorporated and then a [redacted] protective effect of prior modification by NBD-Cl would be comprehensible.

My overall conclusion is that the reaction of PA with F_1 has not been documented sufficiently extensively to warrant publication in BBA at this stage. A general non-specific reaction is not of much interest unless a valuable application of the procedure is in sight. However, the author, if he disagrees with this view, might like to consider resubmission of an improved version to either BBA or another journal. A clearer version might reveal aspects of the work that the present version has obscured.

MAX-PLANCK-INSTITUT
FÜR
ERNÄHRUNGSPHYSIOLOGIE

Professor Dr. A. Maelicke

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1206-320

16. Januar 1986

5. Januar

Kopplung
Sundaram, P.V.

Herrn
R. Kiehl
Universität Bielefeld
Postfach 4640
Fakultät für Chemie

4800 Bielefeld 1

Lieber Herr Kiehl,

vielen Dank für Ihren Brief vom 2. Januar. Ihre Frage ist nicht einfach zu beantworten. Zwar ist es recht einfach, "Kanäle" in Rekonstitutionsversuchen zu sehen, doch besagt das noch wenig. Glaubhaft ist erst, wenn folgende Kriterien gelten:

1. Die statistische Analyse der Kanalereignisse muß genügend Ereignisse enthalten und muß zu einem "vernünftigen" Ergebnis führen.
2. Die Meßprotokolle müssen wie bei natürlichen Membranen einzelne und mehrfache Ereignisse nebeneinander enthalten.
3. Die pharmakologische Spezifität, d.h. die etablierten Unterschiede zwischen Agonisten, Antagonisten, Lokalanästhetika usw. müssen vorhanden sein.

Setzt man diese Kriterien an, so sind Rekonstitutionen nur schwierig und unter sehr selektiven biochemischen und biophysikalischen Bedingungen zu erhalten. Man kann umgekehrt sagen, daß es offenbar sehr viele Artifakte gibt, die ohne die geeigneten Kontrollen zu leicht als echte Meßergebnisse erscheinen.

Was die genauen Bedingungen der Rekonstitution angeht, so gibt es z.Zt. noch keine Obereinstimmenden Ansichten zwischen den verschie-

denen Arbeitsgruppen, und die Zeit der Alchemie ist noch nicht überwunden. Zu diesem Punkt möchte ich deshalb keine bewertende Meinung abgeben, sondern schlage Ihnen vor, daß Sie einfach mit den entsprechenden Labors (Schindler, Boheim, Hanke) in Kontakt treten. Wir sind zwar selbst keine Rekonstruierer, stehen Ihnen aber jederzeit für Diskussionen und Information zur Verfügung. Kommen Sie doch einfach mal vorbei, wenn Sie in der Nähe sind.

Mit herzlichen Grüßen

Alfred Maelicke

Alfred Maelicke