ALFRED BENZON SYMPOSIUM I

THE ROLE OF NUCLEOTIDES FOR THE FUNCTION AND CONFORMATION OF ENZYMES

EDITED BY
HERMAN M. KALCKAR, HANS KLENOW
AGNETE MUNCH-PETERSEN,
MARTIN OTTESEN, JØRN HESS THAYSEN

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The Role of Nucleotides for the Function and Conformation of Enzymes

THE ALFRED BENZON FOUNDATION

29 Halmtorvet, 1700 Copenhagen V, Denmark

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WELCOME BY THE FOUNDATION

The Alfred Benzon Foundation was founded in 1952 by Dr. Bøje Benzon in commemoration of his father Alfred Benzon, who was an outstanding pharmacist in Copenhagen.

The aim of the Foundation is to support the medical, pharmaceutical, and basic biological sciences. The Foundation has done so for many years by giving financial support to young Danish scientists. In later years the Foundation has widened its scope of activities to include an annual Alfred Benzon lecture, on which occasion the Alfred Benzon prize is awarded to the speaker in recognition of his contributions to science. Three distinguished foreign scientists have until now been our honoured guests. Furthermore, it has been decided to inaugurate a series of symposia under the sponsorship of the Foundation. We were very fortunate indeed to have Professor Herman Kalckar and the Danish "trio" Professors Klenow, Munch-Petersen, and Ottesen to organize the scientific programme of the first Alfred Benzon Symposium. We are indebted to them for their initiative as well as for their enthusiastic collaboration. We want to thank especially Professor Kalckar because he has so graciously offered to chair the meeting, and add to this our heartfelt congratulations on his 60th anniversary, which he has celebrated this year.

The Foundation also wants to extend its thanks to the Royal Danish Academy of Sciences, which has put its premises at the disposal of the symposium. We are grateful to the president of the Academy, Professor Johannes Pedersen, and are very happy to be able to tell him that many of the participants in the symposium are here in a double right, as members of the symposium and as honorary members of the Academy.

Last but not least we are grateful to all of the members of the symposium who have so graciously accepted the invitation to come to Copenhagen. Many of you have travelled over very long distances, and all of you are very busy scientists to whom the difficulties in cutting out several days of your normal schedule must be very big. We are very honoured to have you here as our guests. We can only try to show our gratitude by making the frame of the symposium as effective and as pleasurable as possible.

Meteorologists of this city usually give us relatively poor weather forecasts, and we regret that their forecasts are normally correct. However, today the weather of Copenhagen is unusually good, and we do know from reliable sources that the weather forecast is good for all the days during which you are staying in Copenhagen, so that you will experience one of our rare Indian summers. Just as the sun has come out to give all of you a hearty welcome to Denmark, we do hope that you feel how welcome you are as the guests of the Foundation, and that you may derive some pleasure from the social activities that we have tried to arrange for your relaxation during the hard work of the following days.

Jørn Hess Thaysen

CHAIRMAN'S OPENING ADDRESS

In the fall of 1967 I visited my good friend Professor Poul Plesner, at the University of Odense. He was the first to present me with the plan for a biochemistry symposium for which I should serve as Chairman and eventually as Editor and which would be supported by a new Danish foundation. I believe that the first nucleus, a trio composed of Professors Hans Klenow, Agnete Munch-Petersen, and Martin Ottesen, was aware of Poul Plesner's artistic diplomacy and skillful persuasion in inducing me to accept the invitation to chair such a Danish-sponsored international symposium. Later Klenow wrote me and presented me with the great news that the Alfred Benzon Foundation had generously offered to sponsor a series of international symposia in biochemistry and molecular biology. The maiden symposium was scheduled to take place in September, 1968, in Copenhagen. Our thanks are foremost due to Dr. Bøje Benzon, Professor Jørn Hess Thaysen, M.D., and Director Niels Steinø of the Alfred Benzon Foundation for creating a most attractive background for such an international symposium. I was, of course, grateful and honored that the Alfred Benzon Foundation as well as the Danish quartet Klenow, Munch-Petersen, Ottesen, and Hess Thaysen specifically invited me to serve as Chairman and suggested that the topic of the first symposium be related to my own research approach. The help rendered by the "symposium quartet" and by Director Steinø of the Alfred Benzon Foundation was much greater than most of us are probably aware of.

It was a pleasure for us to assemble in the distinguished hall of the Royal Danish Academy and to have an opportunity to meet the President, Professor Johannes Pedersen, and the chairman of the Biology Division, Professor Einar Lundsgaard.

While this book was under preparation I was told of Professor

Einar Lundsgaard's death and it feels in every way natural for us to pay a special tribute to the memory of this great personality and the distinguished role which he played in the development of biology. His revolutionary discovery of the by-pass of glycolysis as an important phenomenon in the bioenergetics of higher organisms has been of fundamental importance in the development of physiology and biochemistry. With profound insight Lundsgaard disclosed the strategic role of phosphocreatine and phosphorylations in general in bioenergetics. His tool, a simple organic chemical at that time little known, iodoacetic acid, has become a key tool in the study of enzyme nucleotide functions ever since Lundsgaard first described its fateful action forty years ago.

It has been a great satisfaction for all of us that we were able to attract a number of distinguished scholars and exciting investigators from Europe and United States to come to Copenhagen and be active participants as well as authors for the first volume of the symposium. Among the exciting active participants permit me to single out three pioneers who made nucleotides fashionable more than a quarter of a century ago, Professors Carl Cori, Fritz Lipmann, and Hugo Theorell.

The topic selected, "The Role of Nucleotides for the Function and Conformation of Enzymes", is becoming one of increasing importance. It affects regulatory functions as shown in numerous examples presented at this symposium. It actually goes back to the so-called Bohr effect described 65 years ago by Christian Bohr and his coworkers K. A. Hasselbalch and August Krogh. This well-known phenomenon, the effect of varying tensions of carbon dioxide on the dissociation curve of oxyhemoglobin, scarcely needs further comment. It has been the subject of many elegant analyses recently, tying it up with feed-back inhibitions in enzymes, one of the main topics of this symposium.

The equally important aspect of conformation and of conformational changes of the peptide backbone goes back to Kaj Linderstrøm-Lang. Not only did he stress the importance of

secondary and tertiary structures (the names stem also from him) in native and denatured proteins; but besides these conceptional aspects, he was also the inventor of a new experimental approach. I refer to his fundamental studies of the hydrogen exchange of the polypeptides in native and denatured proteins and his demonstration of what he called "motility" of proteins. This is studied by means of exchange with water. Perhaps Copenhagen (although displaying anomolous sunshine during this meeting) with its normally prevailing rain inspired Lang to use water for probing the motility of the α-helix which, after all, was born in the dry, crystal-clear climate of Linus Pauling.

We decided to include in this symposium some aspects of protein biosynthesis (represented by three pioneers in the field, Professors Hoagland, Zamecnik, and Lipmann) because of the observations of nucleotide interaction in this complicated machinery, in which conformational changes presumably will turn out to play an important role as well.

It was not possible at this symposium to include a session on crystallography. It is hoped that an extension of the present topic with more emphasis on the approach of 3-dimensional crystallography will be possible in the near future under the same sponsors. In any event a beginning, and an exciting beginning, has been made, thanks to the participants as well as to the sponsors.

Herman M. Kalckar

The Role of Sugars in Nucleotide Binding by Yeast Hexokinase

Sidney P. Colowick with Frances C. Womack & Judith Nielsen

The main new topic to be discussed in this paper is the measurement of the binding of sugars and of nucleotides by various forms of yeast hexokinase using a rapid method which was recently developed in our laboratory (Colowick & Womack 1969). Although some of you may not be particularly interested in the results of our own binding measurements, the method itself may prove useful for other studies.

We will present today the evidence for the following sequence of events in the hexokinase reaction:

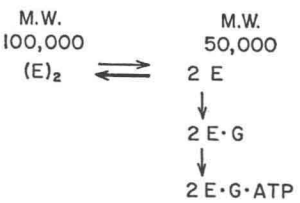

Diagram 1. Proposal illustrating (a) selective binding of glucose (G) to the dissociated form of hexokinase, and (b) the dependence of nucleotide (ATP) binding on prior formation of the enzyme-glucose complex.

In this sequence we deal with an enzyme which occurs naturally as a species of molecular weight 100,000, in equilibrium with a very low concentration of a dissociated form of molecular weight 50,000. Our evidence suggests that glucose combines preferentially with the 50,000 molecular weight species, and thereby promotes dissociation of the 100,000 molecular weight form. This dissociation of hexokinase by the addition of the substrate, glu-

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cose, has been known now for some time (Schachman 1960) and is referred to in our laboratory as the Schachman effect. In addition, we will present evidence that nucleotides are not bound detectably by hexokinase unless a sugar or sugar analogue is present, suggesting that an ordered reaction is involved in which the sugar adds first and then the nucleotide.

I. PROPERTIES OF NATIVE AND PROTEASE-MODIFIED HEXOKINASES

The present studies were made possible by earlier work carried out in our laboratory, which clarified the nature of hexokinase isozymes (Schulze *et al.* 1965, Schulze *et al.* 1966, Schulze & Colowick 1969). Prior to that work we had been using crystalline enzyme made by a method (Darrow & Colowick 1962) which, as it turned out, was permitting marked modification during the isolation because of the presence of a yeast protease. The presence of protease in our crystalline preparations was first brought to our attention by Dr. Howard Schachman (personal communication). Dr. Schulze was able to demonstrate that this proteolytic modification could be avoided either by use of DEAE cellulose chromatography, which resulted in retention of the protease on the column, or by treatment with phenylmethane sulfonyl fluoride, which inactivated the protease. She could then demonstrate the presence of two naturally occurring forms which we refer to now as P-I and P-II. These forms, which correspond to the forms A and B described in Barnard's laboratory (Lazarus *et al.* 1966), are distinct in their catalytic activity and in their amino-acid composition (Gazith *et al.* 1968), as well as in serological tests (F. C. Womack unpublished). They are also distinct in their appearance upon crystallization. One can see in Fig. 1 that P-I tends to crystallize much more slowly than P-II and thus to produce very much smaller crystals. Fig. 2 illustrates how forms P-I and P-II are obtained chromatographically on DEAE cellulose. The enzyme after adsorption onto DEAE cellulose is eluted by means