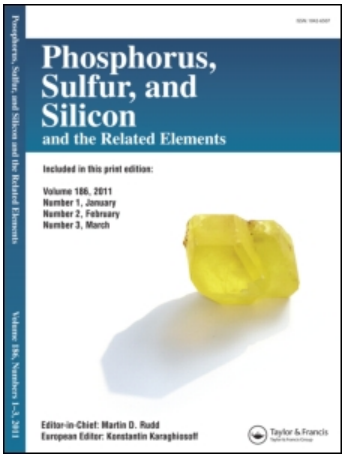

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Models of biotin coenzyme reactions activated through phosphorylation

C. Blonski[a]; G. Etemad-moghadam[b]; M. B. Gasc[a]; A. Klaebe[c]; J. J. Perie[a]
[a] ERA 264 U. E. R.-P. C. A., Université Paul Sabatier, [b] ERA 316 Université Paris Sud bt 410, ORSAY [c] ERA 926 118, route de Narbonne, TOULOUSE Cedex

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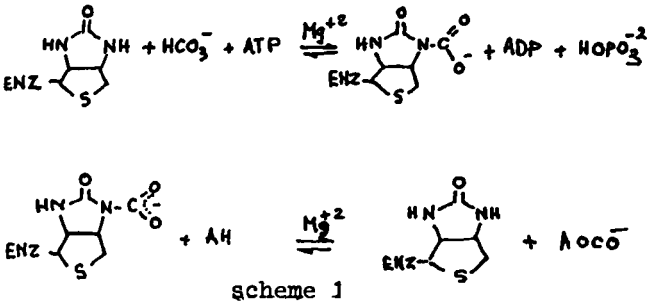
MODELS OF BIOTIN COENZYME REACTIONS ACTIVATED THROUGH PHOSPHORYLATION

C. BLONSKI[(a)], G. ETEMAD-MOGHADAM[(c)], M.B. GASC[(a)], A. KLAEBE[(b)], J.J. PERIE[(a)].
(a) ERA 264 U.E.R.-P.C.A., Université Paul Sabatier
(b) ERA 926 118, route de Narbonne 31062 TOULOUSE Cedex
(c) ERA 316 Université Paris Sud bât 410, 91405 ORSAY

Abstract The reactivity of models of enzymatic carboxylation reactions mediated by biotin coenzyme is presented ; a possible role of the requiered ATP molecule for these reactions was investigated.
Several models of biotin activated through phosphorylation - according to the O-phosphobiotin model - are described and their reactivity to wards carboxylating agents discussed. Models also exhibit phosphoryl transfer properties which may mimic known ATP synthesis from ADP, catalyzed by biotin carboxylases. Possible involvement of the same O-phosphobiotin structure is considered.

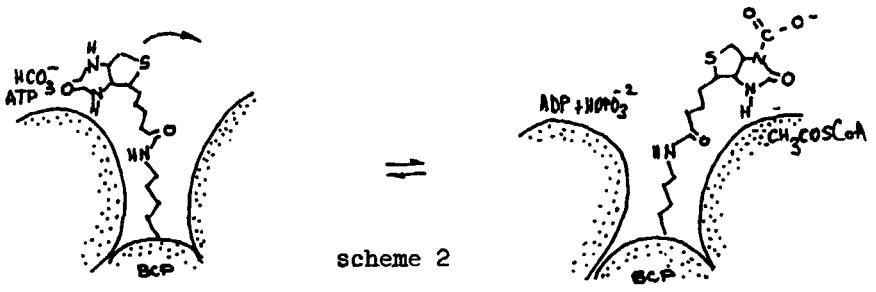
INTRODUCTION

This work is related to the mechanism of enzymatic carboxylations catalyzed by biotin carboxylases. It is known[1] that this reaction is a two-step process according to the scheme :



scheme 1

the two steps occuring on two different sub-units of the enzyme ; the biotin coenzyme binds the hydrogeno-carbonate group and then transfers it to the acceptor, according to a translocation process.



scheme 2

Concerning the reactivity, several points remain unclear, particularly the role played by the ATP molecule, **requiered** in the process. Two activation modes have been postulated : either phosphorylation of biotin leading to the so called "O-phosphobiotin model"[2] corresponding to scheme 3, in which a more nucleophilic imidonitrogen would be formed, or phosphorylation of the carbonate moiety : the mixed anhydride phosphocarbonate formed, being highly reactive toward the ureido nitrogen of biotin ; a such intermediate has been evidenced in the reaction of the carbamylphosphate synthetase[3].

scheme 3

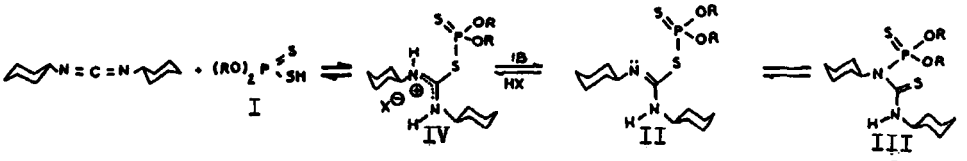
We consider in this work the O-phosphobiotin route, since although suggested in 1959, this pathway has never been investigated except an approach by Kluger[2], probably due to the lack of suitable models.

1- SYNTHESIS OF MODELS

The different routes used are :

- Addition of phosphoric and thiophosphoric esters on carbodiimides

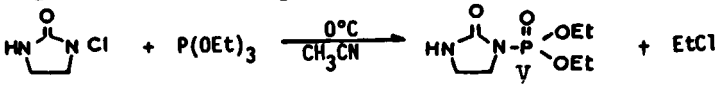
The reaction of the former leads to the classical pyrophosphate synthesis[4], whereas with the later, the intermediate can be trapped and settles IV as phosphobiotin model



Several features of this reaction have previously be published[5] ; also a kinetic study indicates that compounds I, II, III and IV interconvert through a set of reactions which are controlled by proton transfer and stereoelectronic effects[6].

- Reactions of phosphites with N-chloro-ureas

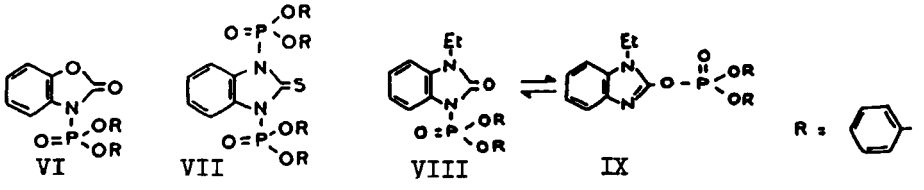
According to the following reaction



Which can be used with phosphites bearing an easily eliminating R group in the phosphonium intermediate.

- Phosphorylation of ureas and analogues with chlorophosphates and triochlorophosphates

Models obtained through this route are :

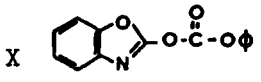


from which, reactivity of N-phospho and O (S)-phospho compounds can be investigated. Phosphorylation by chlorophosphates of unconjugated substrates (imidazolidinone or biotin) gives a different reaction (phosphoryl transfer) discussed in section 3.

2- CARBOXYLATION REACTIONS

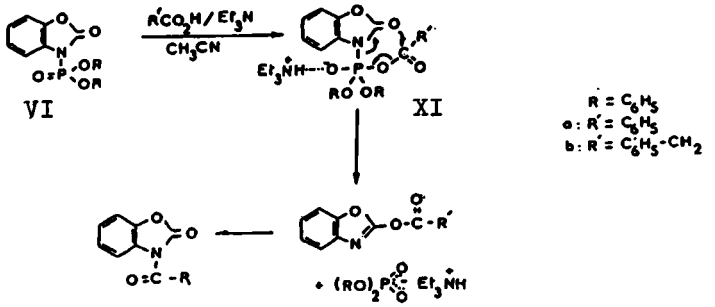
The chemical feasability of phosphobiotin route was laid down with these models using carbonates and carboxylates ; results are the following :

- with carbonates an highly unstable carbonate is formed, wich cannot be isolated nor its N-rearranged product (reaction followed by IR) ;
- with less reactive carboxylates N-acyl derivatives of VI and VII are obtained



according to a fast and quantitative reaction at room temperature. For substrates IV, V and IX no reaction occurs.

^{31}P NMR studies at low temperature for reactions of the N-phosphorylated substrates VI and VII suggest the following pathway :



Intermediate XI is observed on the NMR spectra and at the same chemical shift for the two substrates VI and VII. At completion, the diphenyl phosphate anion is the only phosphorylated species.

This reaction observed with N-phosphorylated substrates which has to be extended to biotin itself, opens a new possibility of pathway for the enzymatic reaction, a N-phosphobiotin route. It is noteworthy that the proposed scheme is in accordance with labelling experiments in the enzymatic reactions[1].

These reactions on models indicate clearly that activation requires phosphorylation. Phosphorylation may act in several ways making with the carboxylating agent a reactive species, acting as template and eliminating as a good phosphate leaving group.

3- PHOSPHORYL TRANSFER REACTIONS

As mentionned above, phosphorylation by the chlorophosphate route of the imidazolidinone ring leads, not to a N- or O-phosphorylated compound but to a pyrophosphate in high yield ; also in the synthesis of VIII and IX, diphenyl pyrophosphate is formed whereas no pyrophosphate was obtained in the reactions (section 2) leading to the only N-phospho compound. It comes out that in both situations the O-phosphorylated structures formed exhibit phosphoryl transfer properties ; the feature has to be related to an enzymatic synthesis of ATP from ADP and carbonyl phosphate through a reaction catalyzed by a biotin carboxylase[7] and where the same O-phosphorylated intermediate becomes very likely.

Pyrophosphate bond formation catalysed by an imidazolidinone which mimics the ATP synthesis, implies a nucleophilic attack on the O-phosphorylated species by a phosphate group ; we show that this phosphate group is formed by a Veilsmeier-Haack type reaction of the chlorophosphate with the solvent DMF.

Finally on the basis of model studies, these two reactions of biotin : carboxylation requiering an ATP molecule and ATP synthesis, might be rationnalized on the basis of reactions of the N and the O-phosphorylated forms of biotine, respectively.

REFERENCES

1. M.J. WIMMER, I.A. ROSE, Ann. Rev. Biochem., 47, 1031 (1978).
2.a/ J. RETEY, F. LYNEN, Biochem. J., 102, 381 (1967).
b/ R. KLUGER, P.D. ADAWADKAR, J. Amer. Chem.Soc., 98, 3741 (1976).
3. S.G. POWERS, A. MEISTER, J. of Biol. Chem., 253, 1258 (1978).
4. M. MIKOLAJCZYK, P.KIELBASINSKI, Tetrahedron, 37, 233 (1981).
5. C. BLONSKI, M.B. GASC, A. KLAEBE, J.J. PERIE, Tetrahedron letters 23, 2773 (1982).
6. C. BLONSKI, M.B. GASC, A.F. HEGARTY, A. KLAEBE, J.J. PERIE, J. amer. Chem. Soc., to be published.
7. S.E. POLAKIS, R.B. GUCHAIT, E.E. ZWERGEL, M.D. LANE, T.G. COOPER, J. of Biol Chem., 249, 6657 (1974).