Hypothesis

A mechanism for the transfer of the carboxyl-group from 1′-*N*-carboxybiotin to acceptor substrates by biotin-containing enzymes

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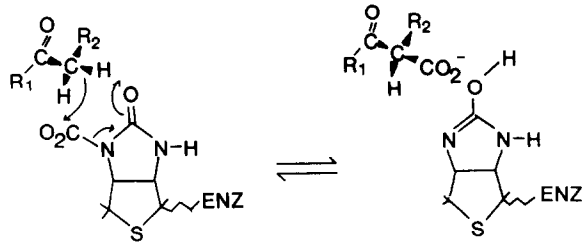
Previous proposals for the mechanism by which biotin-dependent enzymes catalyse the transfer of the carboxyl group from 1′-*N*-carboxybiotin to acceptor molecules do not appear to be consistent with all of the experimental observations now available. We propose a multi-step mechanism in which (a) substrate and then carboxybiotin bind at the second partial reaction site, (b) a base positioned adjacent to the 3′-*N* of the carboxybiotin abstracts a proton from the 3′-*N* and (c) the resulting enolate ion and the acceptor substrate undergo a concerted reaction resulting in carboxyl-group transfer.

Biotin *Carboxylase* *Carboxyl-group transfer* *Pyruvate carboxylase* *Propionyl-CoA carboxylase* *Reaction mechanism*

1. INTRODUCTION

The biotin-dependent carboxylases each catalyze their respective carboxylation reactions in two steps. In the first partial reaction a stable covalent intermediate, 1′-*N*-carboxybiotin, is formed [1]. In the second partial reaction, at a separate site on the enzyme [2,3], a carboxyl group is transferred from 1′-*N*-carboxybiotin to the acceptor substrate. The experimental evidence to date is consistent with the common assumption that the enzymes all use the same catalytic mechanism in the second partial reaction. In each case the carboxylation partial reaction occurs with retention of configuration about the α-carbon of the substrate [4–6] and the α-proton of the substrate does not exchange with the medium without the substrate becoming carboxylated [7–9]. Based on these observations the concerted reaction mechanism shown in scheme 1 was proposed [4].



Scheme 1. The concerted reaction mechanism. R_1 = CO_2^-; R_2 = H (pyruvate), or R_1 = CoA; R_2 = CH_3 (propionyl-CoA).

The fact that there is no exchange of protons between the substrate and solvent in the absence of carboxylation does not rule out the possibility of a pre-transition state proton abstraction step since it is conceivable that the proton abstracted from the substrate may not be able to exchange with solvent protons. There is evidence that proton abstraction from propionyl-CoA precedes carboxylation by both propionyl-CoA carboxylase and transcarbox-

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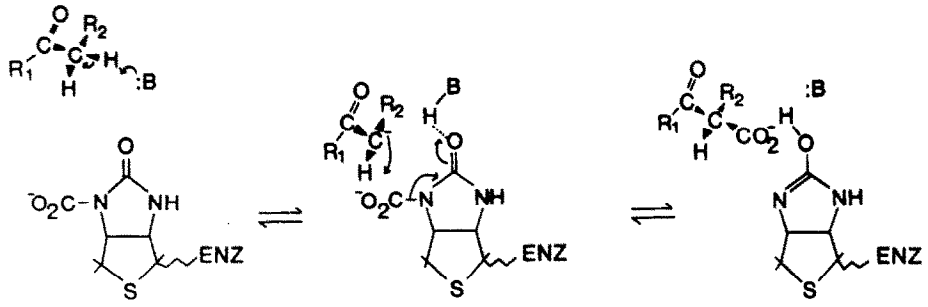


Scheme 2. The reaction mechanism involving a carbanion intermediate. $R_1 = CO_2^-$; $R_2 = H$ (pyruvate), or $R_1 = CoA$; $R_2 = CH_3$ or CH_2F (propionyl-CoA, β-fluoropropionyl-CoA).

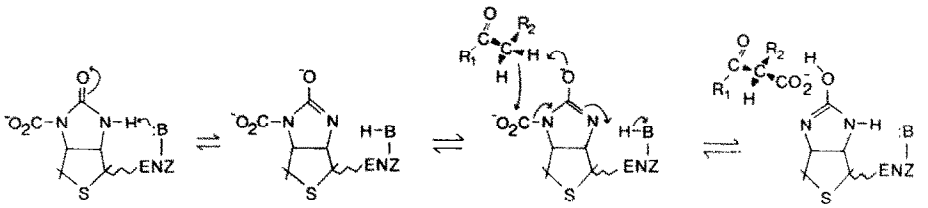
ylase [10,11]. Both enzymes catalyze the elimination of HF from β-fluoropropionyl-CoA resulting in the formation of acrylyl-CoA, but no detectable carboxylation products of β-fluoropropionyl-CoA. Therefore a two-step mechanism has been proposed (scheme 2) in which a proton is first abstracted from β-fluoropropionyl-CoA by a base on the enzyme, leaving the CoA derivative as a carbanion. The protonated base then forms a hydrogen bond with the ureido oxygen of carboxybiotin, thereby increasing the electrophilicity of the *N*-carboxyl group making it more susceptible to nucleophilic attack by the carbanion form of the acceptor substrate.

Consistent with both mechanisms is the observation that there is a small but significant amount of hydrogen transferred between the substrates of the two partial reactions catalyzed by transcarboxylase [9]. This indicates that in transcarboxylase there is a proton carrier which oscillates between the two partial reaction sites. The proton carrier was suggested to be the carbonyl oxygen of the ureido group of biotin, even though an enol proton would rapidly equilibrate with the medium. In both of the reaction mechanisms discussed so far the proton removed from the substrate becomes attached to the 2′-oxygen of the enol form of biotin. However, recent observations cast doubt on the feasibility of both mechanisms.

2. DISCUSSION OF PREVIOUSLY PROPOSED MECHANISMS

The problem with the mechanism shown in scheme 1 is the unreactive nature of carboxybiotin [12–16]. *N*-carboxyimidazolidone, a model compound for carboxybiotin, loses its carboxyl group by an intramolecular cyclic decarboxylation reaction and has no tendency to transfer it to a nucleophile [12]. Crystal structure analyses of biotin [17] and *N*-methoxycarbonyl biotin methyl ester [16] indicate that the carboxylation of biotin decreases its ability to withdraw a proton from a substrate. Furthermore, of the 3 compounds that participate in the transcarboxylase reaction (oxaloacetate, carboxybiotin and methylmalonyl-CoA), carboxybiotin is the most stable [18]. The proposal [8] that transannular interaction between the carbonyl group and the sulphur atom of biotin might contribute to the polarization of the carbonyl group and thus assist the removal of a proton from the substrate molecule is untenable [19]. Obviously, the enzymic process involves more than just aligning the acceptor substrate with the carboxybiotin.

Our objection to the mechanism shown in scheme 2 arises from experiments using pyruvate carboxylase. In this enzyme, the normally stable carboxybiotin intermediate undergoes hydrolysis in the presence of low concentrations ($<K_m$) of pyruvate [20] or analogues of pyruvate such as oxamate, glyoxylate and hydroxypyruvate [21]. The binding of pyruvate to the enzyme triggers the translocation of carboxybiotin to the second partial reaction site [20]. If pyruvate dissociates and the pyruvate binding site is not occupied when the carboxybiotin arrives (a situation which is quite feasible at non-saturating levels of pyruvate [22,23]), then the enzyme catalyzes transfer of the carboxyl group to water to form HCO_3^-. The key point against the carbanion mechanism shown in scheme 2 is that the pyruvate analogues oxamate



Scheme 3. The proposed two-step mechanism. $R_1 = CO_2^-$; $R_2 = H$ (pyruvate), or $R_1 = CoA$; $R_2 = CH_3$ (propionyl-CoA).

and glyoxylate cannot form carbanions, but do promote the hydrolysis of carboxybiotin [21]. Presumably these analogues trigger the translocation of carboxybiotin to the second partial reaction site, where groups on the enzyme catalyze the hydrolysis.

3. THE PROPOSED MECHANISM

To account for all of the observations cited above, we propose that the carboxylation reactions catalyzed by biotin-dependent enzymes occur by an enolate-ion relay mechanism as shown in scheme 3. In this mechanism, the binding of the acceptor substrate at the second partial reaction site induces a conformational change in the enzyme resulting in the translocation of the carboxybiotin from the vicinity of the first partial reaction site to the second partial reaction site. In the second partial reaction site, a base positioned adjacent to the 3′-*N* of the carboxybiotin abstracts a proton from the 3′-*N*, producing an enolate-anion of carboxybiotin. A cyclic reaction similar to the concerted mechanism (scheme 1) then occurs, except that it is the enolate-anion of carboxybiotin that reacts rather than the ureido form. Once the carboxylated product is formed, it dissociates from the enzyme and the enol-biotin returns to the first partial reaction site, in most cases tautomerizing to the ureido form on the way.

The proposed mechanism is consistent with the observations of retention of configuration, lack of exchange of protons between substrate and water when the enzyme is not carboxylated, and transfer of tritium between the substrates of transcarboxylase. The abortive hydrolysis of carboxybiotin by pyruvate carboxylase at low concentrations of pyruvate [20] could occur if the pyruvate molecule, having induced the carboxybiotin to shift to the second partial reaction site, dissociates from the enzyme. The dissociation of pyruvate would allow access of a molecule of water to the enolate anion of carboxybiotin and a cyclic reaction would occur with water as the acceptor molecule instead of pyruvate. Pyruvate analogues such as oxamate and glyoxylate would induce the decarboxylation of carboxybiotin in the same way.

The proposed mechanism also accounts for the observation that propionyl-CoA carboxylase and transcarboxylase catalyze the elimination of the fluoride ion from β-fluoropropionyl-CoA with concomitant decarboxylation of carboxybiotin [10,11]. In this mechanism, the binding of β-fluoropropionyl-CoA to the enzyme induces carboxybiotin to move to the second partial reaction site where the enolate-anion of carboxybiotin is formed. The enolate-anion then abstracts a proton from the C-2 of the substrates in the normal reaction, but instead of an immediate attack by the C-2 on the 1′-*N*-carboxyl group of carboxybiotin, fluoride is eliminated from the β-fluoropropionyl-CoA and CO_2 is released from carboxybiotin (in [11] several reasons why fluoride elimination occurs in preference to carboxylation were suggested). The net result is the formation of HF and acrylyl-CoA from β-fluoropropionyl-CoA and enol-biotin and CO_2 from the enolate-anion.

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