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# Carboxybiotin translocation mechanisms suggested by diffraction studies of biotin and its vitamers

(enzyme mechanism/x-ray diffraction/swinging arm model/$CO_2$ migration/role of sulfur)

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ABSTRACT **Biotin is a coenzyme that fixes $CO_2$ for transfer in a family of carboxylase, decarboxylase, and transcarboxylase enzymes. Their enzyme reactions involve two basic steps during which a carboxybiotinyl intermediate forms at one site and translocates to a second (distinct) site for $CO_2$ transfer. Our diffraction studies of biotin and its vitamers suggest that translocation involves rotation about one, or at most two, bonds in biotin's valeryl chain. The rotations are energetically economical *gauche* ⇄ *trans* rotations about the two valeryl bonds nearest the biotin bicyclic ring. They move a carbon atom of a $CO_2$ moiety bound at N-1′ approximately 7 Å, a distance in accord with spectroscopic measurements of one of the biotin enzymes. From our studies we infer that sulfur in biotin imparts to the valeryl chain a conformational variability necessary for bond rotation and, hence, translocation between catalytic sites.**

Biotin (structure Ia) participates as coenzyme in a variety of carboxylase, transcarboxylase, and decarboxylase enzyme systems in which its role is to fix $CO_2$ for eventual transfer. Biotin-mediated reactions involve at least two steps, during which an $N^{1'}$-carboxybiotinyl intermediate (structure Ib) is formed (1). Kinetic and protein subunit experiments suggest that the half-reactions in which biotin participates take place at physically distinct catalytic sites. For example, the transcarboxylase reaction

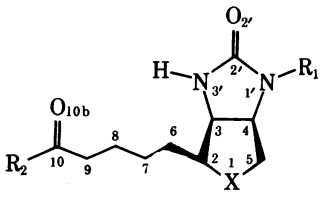
*S*-Methylmalonyl-CoA + pyruvate
⇄ propionyl-CoA + oxaloacetate

catalyzed by methylmalonyl-CoA:pyruvate carboxyltransferase (EC 2.1.3.1) from *Propionibacterium shermanii* displays nonclassical "two-site" bi (uni uni) ping pong kinetics (2). The enzyme can be dissociated into three nonidentical protein subunits, the 12S, 5S, and 1.3S proteins. Only the last of these contains biotin, covalently bound to the ϵ-amino group of the Lys-87 residue. Reconstitution experiments (3) suggest the following reaction mechanisms in line with the kinetic data

*S*-Methylmalonyl-CoA + 1.3S–biotin
$\xrightleftharpoons{12S}$ propionyl-CoA + 1.3S–biotin ~ $CO_2$

1.2S–biotin ~ $CO_2$ + pyruvate
$\xrightleftharpoons{5S}$ 1.3S–biotin + oxaloacetate.

In order to participate in both half-reactions, the carboxybiotinyl prosthetic group must translocate between catalytic sites on the 5S and 12S subunits. Evidence that the sites on transcarboxylase are approximately 7 Å apart comes from a combination of nuclear magnetic and electron spin resonance studies of the enzyme (4–6).




| | X | $R_1$ | $R_2$ |
|---|---|---|---|
| a. Biotin | S | H | OH |
| b. "Carboxybiotin" intermediate | S | $CO_2^{\ominus}$ | —NH—$(CH_2)_4$—CH(NH—)(O—C—) |
| c. Dethiobiotin | H,H | H | OH |
| d. Carbobiotin | $CH_2$ | H | OH |
| e. Oxybiotin | O | H | OH |
| f. Selenobiotin | Se | H | OH |
| g. Biotin sulfoxide | SO | H | OH |
| h. Biotin sulfone | $SO_2$ | H | OH |
| i. Biotin methyl ester | S | H | $OCH_3$ |
| j. Azabiotin | NH | H | OH |

STRUCTURE I.

Our previous diffraction study (7) of biotin revealed an unusual twisted conformation of its valeryl chain; our study of dethiobiotin (structure Ic) (8) suggested that the sulfur of biotin might play a role in maintaining the conformation. Here we describe diffraction studies of six biotin vitamers: the heterobiotins, carbobiotin (structure Id) (9), oxybiotin (structure Ie) (10), and selenobiotin (structure If) (11); the oxidation products, biotin-*d*-sulfoxide (structure Ig) and biotin sulfone (structure Ih); and the methyl ester of biotin (structure Ii). We also discuss their relevance to the mechanics of the translocation process.

## EXPERIMENTAL DETAILS

Except for crystals of biotin methyl ester, which were grown from acetone/cyclohexane, crystals of the vitamers were grown from aqueous or aqueous ethanol solutions. X-ray diffraction data were measured on automated counter diffractometers by use of copper radiation. Intensity data were integrated by $\theta$–$2\theta$ scans, and were corrected for Lorentz and polarization effects but not for absorption or extinction. Because the linear absorption coefficient for selenobiotin is rather large (48.7 $cm^{-1}$),

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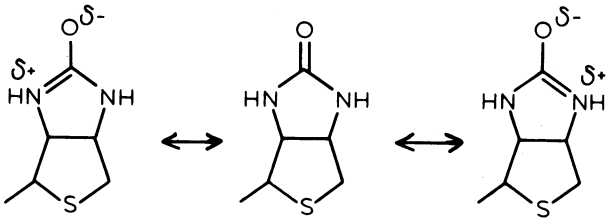
the data for it were measured in a minimum pathlength mode. Selenobiotin is isomorphous with biotin, and its structure was directly refined from the coordinates of biotin. The other structures were determined by routine applications of direct methods or Patterson techniques and were refined by least-squares. The function minimized was $\Sigma w_i(|F_o| - |F_c|)_i^2$, where $w = 1/\sigma_F^2$, $\sigma_F = (|F_0|/2) \times [(\sigma_I/I)^2 + C^2]^{1/2}$. $F_o$ and $F_c$ are, respectively, the observed and calculated structure factors; $I$ is the measured intensity; $\sigma_I$ represents statistical counting uncertainties; and $C$ represents instrumental uncertainties. Values of $C$ were 0.02–0.05, and only reflections with $|F_o| > 2\sigma_F$ were used in the refinement. A summary of crystal data and refinement results is given in Table 1. Positional parameters for the six vitamers are collected in Table 2.

## RESULTS

The conformations of the six vitamers, along with biotin, dethiobiotin, and azabiotin (structure Ij) (12), are shown in Fig. 1. Selected parameters of the bicyclic ring geometries are collected in Table 3.

In all of the vitamers, the ureido C2′-O2′ bonds are longer, and C2′-N bonds are shorter, than the corresponding bonds in barbiturates (13) and approach those in urea (14). The bond lengths are in accord with a resonance delocalization and polarization:



The empirical formula, $d_{C=O} = 3.36$ Å $- 1.57 \times d_{\langle C\text{-}N\rangle}$, describes the relationship well in the region bounded by the observed structures (Fig. 2). The degree of polarization might depend on the heteroatomic species in the sulfur position. Thus, the points of the graph associated with oxy, sulfo, and seleno substitution form a regularly spaced progression from a more delocalized ureido function for oxybiotin to a less delocalized function for selenobiotin.

Our earlier analyses (7, 8) ascribed part of the delocalization in biotin and dethiobiotin to an enhancement of the polarization of the ureido carbonyl bonds by strong hydrogen bonds (O···O ≈ 2.55 Å) involving the O2′ oxygen atoms. Our new results underscore the importance of resonance delocalization and polarization as inherent characteristics of the biotinyl ureido group, but shed little light on the influence hydrogen bonding might have on the electronic polarization. The strong hydrogen bond observed in biotin is observed also in oxybiotin and selenobiotin (Fig. 3), but the latter two define the extremes of the observed polarizations (Fig. 2).

The bicyclic rings are all *endo* shaped, with the heteroatoms tucked in towards the ureido rings. Out-of-plane distances for the heteroatoms (Table 3) range from 0.58 to 0.98 Å. Departures from ideal bicyclic mirror symmetry, measured by the asymmetry parameters (7) in Table 3, range from very small ($\Phi_B = 1°$) for carbobiotin to very large ($\Phi_B \geq 15°$) for azabiotin and oxybiotin. The distortion, brought about by a twist around the C3-C4 bond, moves N3′ and C5 towards, and N1′, C2, and C6 away from, the viewer (Fig. 1); thus, C6 becomes more nearly equatorial and the crowding of C6 and N3′ is lessened. Dethiobiotin is twisted about C3-C4 in the sense opposite the other vitamers, suggesting that the bicyclic ring geometries of the vitamers are affected by both the valeryl chain and the heteroatom.

The conformations of the valeryl chains and of the chain–ring junctions vary. The junction geometries are of the *gauche-trans* type, C7 either *+gauche* to C3 and *trans* to the heteroatom or *trans* to C3 and *−gauche* to the heteroatom. The former situation describes all of the vitamers with a heteroatom smaller than sulfur, plus biotin sulfone and the bis(*p*-bromoanilide) of $N^{1'}$-carboxybiotin (15); the latter, the remaining vitamers with sulfur or selenium. Beyond the junction, the chains take up one of the three minimum energy conformations available about the C6-C7 bond: *+gauche* in biotin and selenobiotin, *−gauche* in biotin-*d*-sulfoxide, and *trans* in the other vitamers. Farther from the bicyclic moieties, all of the chains adopt the all-*trans*, planar conformation. The carboxyl group junctions also vary. In most cases the protonated oxygen O10a continues the all-*trans* arrangement of the chains, but in biotin, selenobiotin, and biotin methyl ester, the O10a atoms are *−gauche* to C8. In biotin-*d*-sulfoxide the carbonyl oxygen O10b is approximately *trans*, and the hydroxyl oxygen O10a is approximately *cis*, to C8.

Our finding of the sulfoxide oxygen equatorial in biotin-*d*-sulfoxide confirms the assignment of the *d*-oxygen configuration (*S* stereochemistry at sulfur) that had been deduced from nuclear magnetic resonance studies (16).

Table 1. Crystal data for the six biotin vitamers

| | Carbobiotin | (±)Oxybiotin | Selenobiotin | Biotin sulfone | Biotin-*d*-sulfoxide | Biotin methyl ester |
|---|---|---|---|---|---|---|
| *a* (Å) | 7.931(2) | 11.101(2) | 5.340(1) | 15.906(3) | 10.461(2) | 7.638(1) |
| *b* (Å) | 26.987(5) | 4.813(1) | 10.343(2) | 7.581(2) | 7.585(1) | 35.491(10) |
| *c* (Å) | 5.466(1) | 22.728(4) | 21.100(5) | 5.067(2) | 7.664(1) | 4.775(1) |
| $\beta$ (°) | 90. | 113.79(1) | 90. | 93.91(1) | 101.12(1) | 90. |
| Space group | $P2_12_12_1$ | $P2_1/c$ | $P2_12_12_1$ | $P2_1$ | $P2_1$ | $P2_12_12_1$ |
| Formula | $C_{11}H_{18}O_3N_2$ | $C_{10}H_{16}O_4N_2$ | $C_{10}H_{16}O_3N_2Se$ | $C_{10}H_{16}O_5N_2S$ | $C_{10}H_{16}O_4N_2S$ | $C_{11}H_{18}O_3N_2S$ |
| $M_r$ | 226.3 | 228.3 | 291.2 | 276.3 | 260.3 | 258.3 |
| $\mu_{Cu}$ ($cm^{-1}$) | 7.8 | 9.0 | 48.7 | 24.8 | 24.3 | 21.6 |
| $\rho_{obs}$ ($g \cdot cm^{-3}$) | 1.29 | 1.37 | 1.65 | 1.50 | 1.46 | 1.33 |
| $\rho_{calc}$ | 1.29 | 1.36 | 1.66 | 1.51 | 1.45 | 1.32 |
| $Z$ | 4 | 4 | 4 | 2 | 2 | 4 |
| $N_{obs}$ | 1045 | 1174 | 1130 | 911 | 1303 | 1114 |
| $N_{var}$ | 217 | 209 | 145 | 223 | 153 | 213 |
| $R_o$ | 0.041 | 0.098 | 0.055 | 0.048 | 0.085 | 0.050 |
| $R_w$ | 0.060 | 0.106 | 0.056 | 0.054 | 0.096 | 0.060 |

Temperature, 295 K; wavelength, 1.5418 Å; $\mu_{cu}$ is the linear absorption coefficient for copper radiation; $\rho$ is the calculated crystal density for $Z$, the number of crystal chemical units per unit cell; $N_{obs}$ and $N_{var}$ are the number of observed data and the number of least-squares refined parameters, respectively; $R_o = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $R_w = (\Sigma w(F_o - F_c)^2/\Sigma wF_o^2)^{1/2}$ ($R_o$ and $R_w$ refer only to the observed, i.e., refined, data).

Table 2. Nonhydrogen positional parameters ($\times 10^4$) for the six biotin vitamers;* estimated SDs are given in parentheses for the least significant parameter digits

| X = | Carbobiotin C | (±)Oxybiotin O | Selenobiotin Se | Biotin sulfone S | Biotin-*d*-sulfoxide S | Biotin methyl ester S |
|---|---|---|---|---|---|---|
| X1 | 2604(5) | 2966(3) | −314(2) | 8993(1) | 1627(1) | 8216(2) |
| | 769(1) | 2920(9) | 3647(1) | 7722(3) | 2497(0) | 1382(0) |
| | −673(6) | 6264(2) | 4557(0) | 8225(3) | 554(1) | 3642(3) |
| C2 | 3285(4) | 4079(4) | −3566(14) | 7984(3) | 2552(4) | 6378(6) |
| | 1293(1) | 4610(10) | 4442(8) | 8046(8) | 783(6) | 1500(1) |
| | −1073(5) | 6348(2) | 4323(3) | 6447(11) | −361(5) | 1341(12) |
| C3 | 5111(4) | 3958(4) | −3587(15) | 7966(3) | 2480(4) | 6697(6) |
| | 1206(1) | 5221(9) | 4417(8) | 10053(9) | 1405(6) | 1906(1) |
| | −1933(6) | 5673(2) | 3603(3) | 6019(12) | −2283(5) | 0480(10) |
| C4 | 5139(5) | 2431(5) | −2201(16) | 8882(4) | 2528(5) | 8726(6) |
| | 671(1) | 5402(11) | 3188(8) | 10842(9) | 3452(6) | 1970(1) |
| | −3050(6) | 5289(3) | 3349(4) | 6043(13) | −2344(5) | 0302(11) |
| C5 | 3327(5) | 1886(5) | −1064(19) | 9523(4) | 2444(8) | 9676(6) |
| | 478(1) | 4304(15) | 2423(7) | 9367(9) | 4135(7) | 1621(1) |
| | −2777(6) | 5755(3) | 3887(4) | 6631(12) | −504(7) | 1265(13) |
| C6 | 3091(4) | 5332(4) | −4014(16) | 7240(4) | 1988(5) | 4636(6) |
| | 1638(1) | 3212(11) | 5807(7) | 7276(8) | −1074(7) | 1413(2) |
| | 1119(6) | 6812(2) | 4574(4) | 7826(12) | −196(6) | 2661(12) |
| C7 | 3825(4) | 6518(4) | −4348(17) | 6408(3) | 2057(5) | 4436(7) |
| | 2158(1) | 5119(11) | 5932(9) | 7616(11) | −1687(7) | 1000(2) |
| | 805(5) | 7010(2) | 5297(4) | 6345(11) | 1726(6) | 3490(15) |
| C8 | 3438(5) | 7686(4) | −1990(17) | 5655(4) | 3406(5) | 2650(7) |
| | 2477(1) | 4075(10) | 5827(8) | 7167(10) | −1908(7) | 895(2) |
| | 3027(6) | 7576(2) | 5684(4) | 7859(13) | 2816(6) | 4602(14) |
| C9 | 4254(4) | 8873(4) | −2431(17) | 4844(4) | 3371(4) | 2561(8) |
| | 2984(1) | 5915(11) | 6254(8) | 7882(13) | −2658(9) | 486(2) |
| | 2973(6) | 7778(3) | 6370(4) | 6479(13) | 4648(5) | 5436(16) |
| C10 | 3725(4) | 10029(4) | −124(18) | 4056(4) | 4612(4) | 766(8) |
| | 3314(1) | 5037(11) | 6026(7) | 7527(10) | −2507(9) | 349(2) |
| | 5027(5) | 8346(2) | 6769(3) | 7886(12) | 5999(5) | 6284(17) |
| O10a | 4285(3) | 11120(4) | 272(12) | 3423(3) | 5673(3) | 41(5) |
| | 3771(1) | 6315(8) | 4824(5) | 8538(8) | −2453(9) | 567(1) |
| | 4847(5) | 8457(2) | 6919(3) | 6923(11) | 5317(4) | 8205(10) |
| O10b | 2836(4) | 9990(4) | 1264(14) | 3998(3) | 4651(4) | 103(6) |
| | 3171(1) | 3152(12) | 6888(7) | 6530(10) | −2679(10) | 72(1) |
| | 6671(4) | 8689(2) | 6944(3) | 9647(12) | 7571(4) | 5417(11) |
| N1′ | 6344(4) | 2204(4) | −339(14) | 8891(3) | 1334(4) | 8991(5) |
| | 419(1) | 3590(9) | 3741(6) | 12156(7) | 3882(5) | 2294(1) |
| | −1489(5) | 4751(2) | 2920(3) | 8076(11) | −3653(5) | 2127(9) |
| C2′ | 7015(4) | 3276(4) | −409(19) | 8140(4) | 653(4) | 7536(7) |
| | 710(1) | 2130(11) | 5053(7) | 12300(8) | 2433(7) | 2392(1) |
| | 251(6) | 4825(2) | 2906(3) | 9204(13) | −4283(4) | 3555(12) |
| N3′ | 6315(4) | 4285(3) | −2246(12) | 7627(3) | 1252(4) | 6187(5) |
| | 1165(1) | 2873(8) | 5467(6) | 11016(8) | 986(5) | 2182(1) |
| | 56(5) | 5359(2) | 3300(3) | 8192(11) | −3462(5) | 2599(9) |
| O2′ | 8089(3) | 3314(3) | 872(13) | 7985(3) | −365(3) | 7459(4) |
| | 591(1) | 288(7) | 5760(6) | 13353(6) | 2421(5) | 2637(1) |
| | 1789(4) | 4433(2) | 2564(3) | 10935(9) | −5405(4) | 5389(7) |
| C11 | — | — | — | — | — | −1662(9) |
| | — | — | — | — | — | 447(2) |
| | — | — | — | — | — | 9134(17) |
| O1a | — | — | — | 9338(3) | 2115(3) | — |
| | — | — | — | 5974(6) | 2653(8) | — |
| | — | — | — | 7647(9) | 2537(4) | — |
| O1b | — | — | — | 8919(3) | — | — |
| | — | — | — | 8124(7) | — | — |
| | — | — | — | 10954(8) | — | — |

* For each atom, the rows of three parameters are, from top to bottom, the fractional coordinates *x*, *y*, and *z*.

## DISCUSSION

All of the biotin enzymes characterized exhibit a two-step, two-site mechanism, but only for transcarboxylase is there experimental data concerning the distance between catalytic sites. A combination of magnetic resonance spectral techniques (4–6) yields a composite picture of pyruvate and propionyl-CoA on transcarboxylase with the methyl carbon of pyruvate 7 ± 1 Å from the methylene carbon of propionyl-CoA. These are the carbons to which $CO_2$ is alternately bound and between which it is transferred.

A simple way to move the biotinyl prosthetic group, and thereby translocate $CO_2$, 7 Å, might involve rotation about one or more of the 10 single bonds in the valeryl-lysyl chain that connects the bicyclic ring of biotin to the backbone of the enzyme. The observed conformations of the vitamers, however, suggest a number of translocation mechanisms that involve rotation about at most two bonds, C2-C6 and C6-C7 of the valeryl chain.

The key rotations were discovered during computer analyses of the observed structures. Carboxybiotinyl models were constructed from the observed conformations of biotin and its methyl ester, sulfone, and *d*-sulfoxide by adding carboxyl groups to their N1′ nitrogens at reasonable bonding distances and angles (unpublished results). The similar, all-*trans*, planar moieties, C7 to C10, of the four heterosulfur molecules were

Table 3. Selected measures of vitamer geometries

| | C2′=O2′, Å | C2′-N1′, Å | C2′-N3′, Å | $\Phi_U$*, ° | $\Phi_H$*, ° | $\Phi_B$*, ° | $d_X$†, Å | $A_B$‡, ° |
|---|---|---|---|---|---|---|---|---|
| Carbobiotin | 1.239(4) | 1.344(4) | 1.351(3) | 1 | 1 | 1 | 0.60 | 119.3 |
| (±)Oxybiotin | 1.269(7) | 1.333(7) | 1.327(5) | 16 | 14 | 15 | 0.58 | 117.7 |
| Selenobiotin | 1.235(10) | 1.358(10) | 1.355(11) | 3 | 4 | 3 | 0.98 | 122.4 |
| Biotin sulfone | 1.223(8) | 1.363(8) | 1.349(8) | 4 | 2 | 4 | 0.69 | 122.2 |
| Biotin-*d*-sulfoxide | 1.233(4) | 1.348(6) | 1.357(6) | 4 | 7 | 6 | 0.89 | 120.1 |
| Biotin methyl ester | 1.236(6) | 1.350(7) | 1.351(6) | 7 | 5 | 6 | 0.87 | 119.4 |
| Biotin (7) | 1.249(6) | 1.332(6) | 1.351(6) | 3 | 3 | 3 | 0.87 | 122.0 |
| Dethiobiotin (8) | 1.244(4) | 1.346(4) | 1.346(4) | 30 | — | — | — | — |
| Azabiotin (12) | 1.243(4) | 1.345(4) | 1.348(5) | 21 | 16 | 18 | 0.56 | 119.4 |

* $\Phi_U$, $\Phi_H$, and $\Phi_B$ are the $\Delta C_s$ asymmetry parameters (see ref. 7) for the ureido, heteroatomic, and bicyclic rings, respectively. Large values point out large departure from ideal mirror symmetry.

† The distance of the heteroatom (S, Se, O, N, and C) from the least-squares plane defined by atoms C2, C3, C4, and C5.

‡ The angle between the planes fit to atoms C2, C3, C4, and C5 and to atoms C2′, N1′, N3′, C4, and C5, respectively.

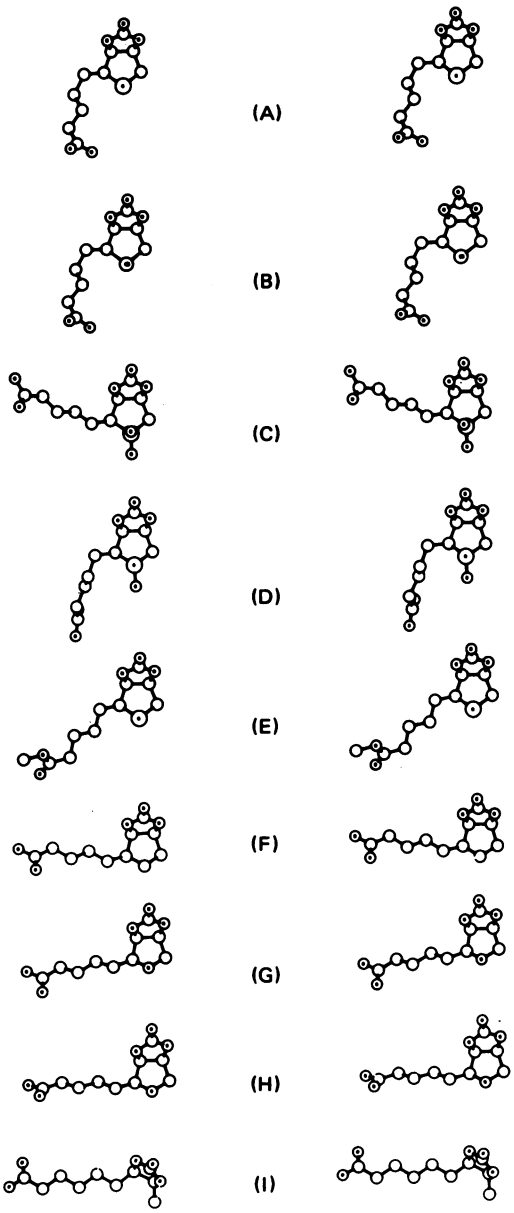



FIG. 1. The biotin vitamers. From top to bottom are (*A*) biotin (ref. 7), (*B*) selenobiotin, (*C*) biotin sulfone, (*D*) biotin-*d*-sulfoxide, (*E*) biotin methyl ester, (*F*) carbobiotin, (*G*) oxybiotin, (*H*) azabiotin (ref. 12), and (*I*) dethiobiotin (ref. 8).

superimposed (Fig. 4) by pairwise fit by a least-squares procedure (17). A *−gauche → trans* twist about the C2-C6 bond and a *+gauche → trans* twist about the C6-C7 bond of biotin would bring its bicyclic ring into approximate congruence with the bicyclic ring of its sulfone, for example. In the process, the hypothetical $CO_2$ carbon is moved 6.9 Å (Table 4).

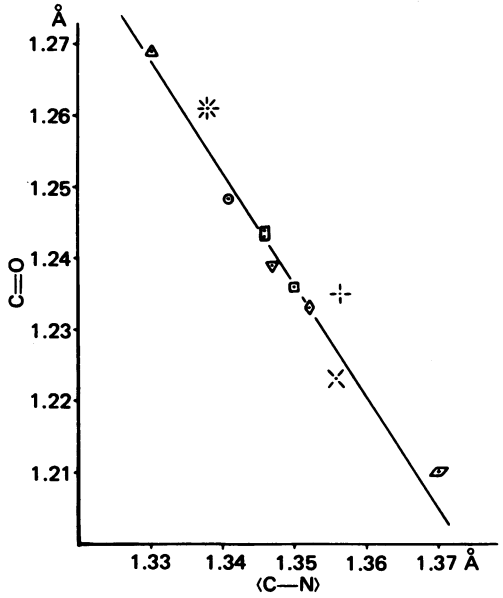



FIG. 2. The C2′=O2′ carbonyl bond length plotted against the averaged C2′-N bond lengths. △, Oxybiotin; *, urea (ref. 14); ○, biotin (ref. 7); □, dethiobiotin (ref. 8) and azabiotin (ref. 12); ▽, carbobiotin; ▫, biotin methyl ester; ◇, biotin-*d*-sulfoxide; +, selenobiotin; X, biotin sulfone; ◇, barbiturate average (ref. 13). The urea and barbiturate values were not used to fit the line $d_{C=O} = 3.36\ Å - 1.57\ d_{\langle C\text{-}N\rangle}$.

The translocation model suggested by the diffraction results has several appealing features. It involves rotation about bonds close to the bicyclic ring moiety, thus allowing translocation events to be controlled close to the $CO_2$ binding site. It allows the amide link in the valeryl-lysyl chain, very probably hydrogen bonded to the enzyme, to be unmoved during the translocation. It is energetically economical because the required movement of the $CO_2$ is effected by rotation about at most two, possibly only one, carbon–carbon single bond. Moreover, the types of rotation required—namely, *+gauche ⇄ trans ⇄ −gauche* twists—have very modest energy requirements (18). An activation energy for translocation could come from various rearrangements of the hydrogen bonding of the ureido function (Fig. 3).

The process of biotin translocation was originally envisioned as a movement of the prosthetic group some 28 Å on the "swinging arm" of the 14-Å lysyl-valeryl chain connecting the coenzyme to the enzyme backbone (1). Magnetic resonance experiments (4–6) require a downward estimate for transcarboxylase to approximately 7 Å, and our studies suggest several mechanisms involving one or two energetically economical bond rotations consistent with the 7 Å figure. There are at least 10 distinct biotin-dependent enzymes, all of which exhibit two-site properties for the half-reactions and all of which presumably involve translocation of $CO_2$. We have outlined a number of possible mechanisms for such translocations.

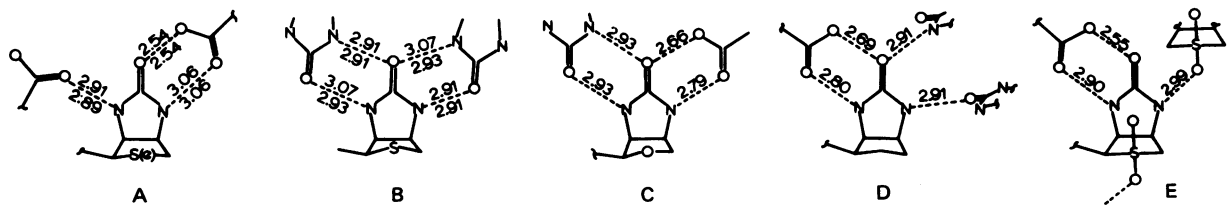



FIG. 3. Hydrogen bonding about the ureido functions of the vitamers. (*A*) Biotin (upper values) and selenobiotin (lower values); (*B*) biotin methyl ester (upper values) and biotin-*d*-sulfoxide (lower values); (*C*) oxybiotin; (*D*) carbobiotin; and (*E*) biotin sulfone. Distances indicated are N···O and O···O values.

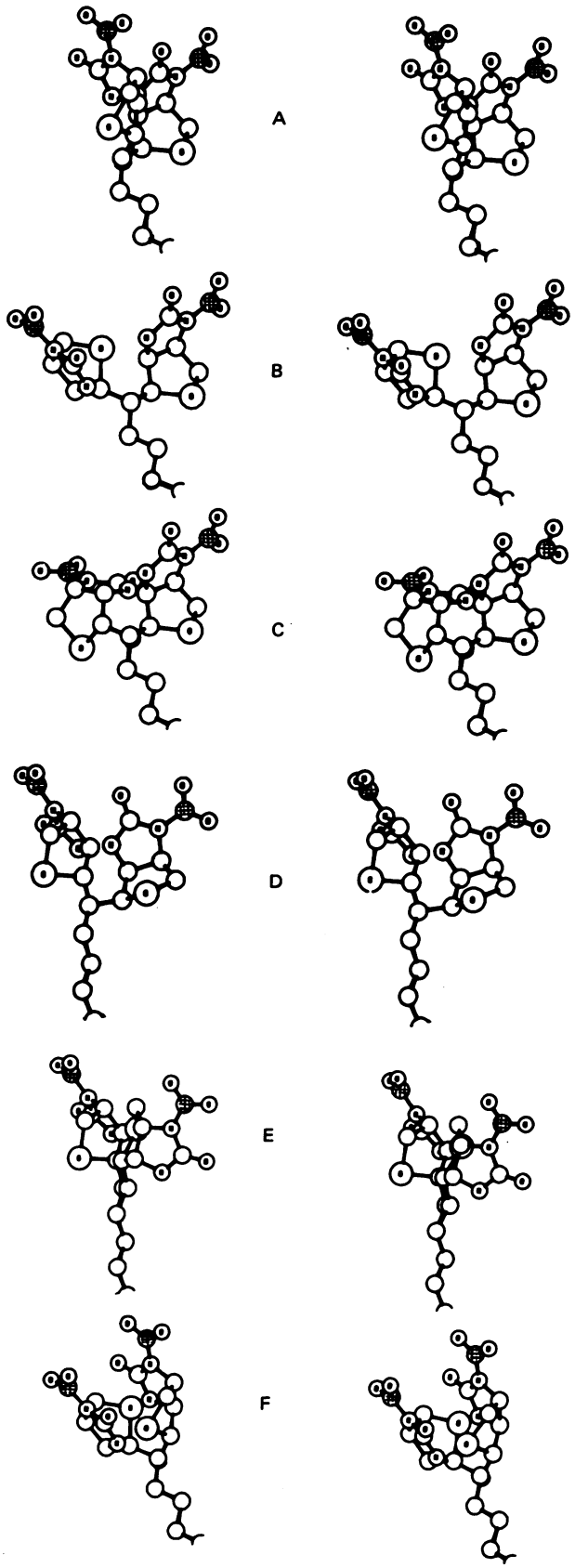



FIG. 4. Translocation scenarios suggested by the diffraction studies and consistent with magnetic resonance spectral studies of transcarboxylase (see text). Refer to Table 4 for the description of the individual translocations. Each diagram is a stereopair.

Table 4. $CO_2$ carbon translocation distances and types relating the sulfur-containing vitamers

| Translocation | Fig. 4* | $d_c$, Å† | ΔC2-C6‡ | $\Delta C_6$-C7§ |
|---|---|---|---|---|
| Biotin → biotin-*d*-sulfoxide | *A* | 5.4 | — | $+g \rightarrow -g$ |
| Biotin → biotin sulfone | *B* | 6.9 | $-g \rightarrow t$ | $+g \rightarrow t$ |
| Biotin → biotin methyl ester | *C* | 6.8 | — | $+g \rightarrow t$ |
| Biotin methyl ester → biotin-*d*-sulfoxide | *D* | 6.6 | — | $t \rightarrow -g$ |
| Biotin methyl ester → biotin sulfone | *E* | 6.9 | $-g \rightarrow t$ | — |
| Biotin sulfone → biotin-*d*-sulfoxide | *F* | 4.4 | $t \rightarrow -g$ | $t \rightarrow -g$ |

* See Fig. 4 for the appropriate stereoview of the translocation.
† The distance the $CO_2$ carbon atom moves during the translocation.
‡ The shift in the S-C2-C6-C7 torsion angle, $+g$ (*+gauche*, ≈60°), $-g$ (*+gauche*, ≈ −60°), $t$ (*trans*, ≈180°).
§ Similarly, the shift in the C2-C6-C7-C8 torsion angle.

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1. Moss, J. & Lane, M. D. (1966) in *Advances in Enzymology and Related Areas of Molecular Biology*, ed. Meister, A. (Interscience, New York), Vol. 35, pp. 321–442.
2. Northrop, D. B. (1969) *J. Biol. Chem.* **244**, 5808–5819.
3. Wood, H. G. & Zwolinski, G. K. (1976) in *CRC Critical Reviews in Biochemistry*, ed. Fasman, G. D. (CRC Press, Cleveland, OH), Vol. 5, pp. 47–122.
4. Fung, C. H., Mildvan, A. S. & Leigh, J. S. (1974) *Biochemistry* **13**, 1160–1169.
5. Fung, C. H., Feldmann, R. J. & Mildvan, A. S. (1976) *Biochemistry* **15**, 75–84.
6. Fung, C. H., Gupta, R. K. & Mildvan, A. S. (1976) *Biochemistry* **15**, 85–92.
7. DeTitta, G. T., Edmonds, J. W., Stallings, W. & Donohue, J. (1976) *J. Am. Chem. Soc.* **98**, 1920–1926.
8. Chen, C. S., Parthasarathy, R. & DeTitta, G. T. (1976) *J. Am. Chem. Soc.* **98**, 4983–4990.
9. Wormser, H. C., Israsena, S., Meiling, M. S., Williams, C. & Perlman, D. (1972) *J. Pharm. Sci.* **61**, 1168–1170.
10. Hofmann, K. (1945) *J. Am. Chem. Soc.* **67**, 694.
11. Bory, S. & Marquet, A. (1976) *Tetrahedron Lett.* (24), 2033–2036.
12. Glick, M. D., Wormser, H. C. & Abramson, H. N. (1977) *Acta Crystallogr. Sect. B* **33**, 1095–1101.
13. Craven, B. M., Cusatis, C., Gartland, G. L. & Vizzini, E. A. (1973) *J. Mol. Struct.* **16**, 331–342.
14. Mullen, D. & Hellner, E. (1978) *Acta Crystallogr. Sect. B* **34**, 1624–1627.
15. Bonnemere, C., Hamilton, J. A., Steinrauf, L. & Knappe, J. (1965) *Biochemistry* **4**, 240–245.
16. Lett, R. & Marquet, A. (1971) *Tetrahedron Lett.* (30), 2855–2858.
17. Nyburg, S. (1974) *Acta Crystallogr. Sect. B.* **30**, 251–253.
18. Hoyland, J. R. (1968) *J. Chem. Phys.* **49**, 2563–2566.