BIOLOGICAL PROPERTIES OF SELENOBIOTIN

Annie Piffeteau, Michel Gaudry and Andrée Marquet

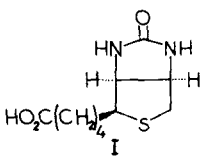
Centre d'Etudes et de Recherche de Chimie Organique Appliquée
2-8 rue H. Dunant 94320 Thiais

Received September 24,1976

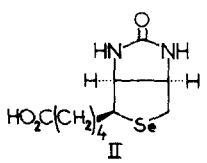
SUMMARY : Selenobiotin is an excellent growth factor, as efficient as biotin in supporting the growth of biotin requiring microorganisms. It is incorporated in carboxylases leading to active "selenocarboxylases". With E. Coli, the activity of the selenoacetyl CoA carboxylase is very similar to that of the normal enzyme.

The toxicity of Selenium is widely documented but its role as an essential micronutrient has been recognized only recently. This has prompted the study of selenoanalogs of natural sulfur containing compounds (1).

An important one is biotin (I), an essential growth factor, which is the prosthetic group of a class of carboxylases (2).



I



II

The first synthesis of selenobiotin (II) has been achieved recently in our laboratory (3) and we report here the comparison of the biological properties of biotin (I) and selenobiotin (II).

According to the numerous studies dealing with the biological properties of biotin analogs, we expected selenobiotin to exhibit either growth promoting or antibiotic activities*. We have observed that this analog is indeed an excellent growth factor.

We have also found that the *in vitro* specific activities of the Acetyl CoA carboxylases of a biotin requiring *E. Coli* strain grown in the presence of biotin or selenobiotin are very similar.

---

* dethiobiotin (4), biotin d sulfoxide (5) and oxybiotin (6) have growth promoting activities whereas biotin sulfone (7), α dehydrobiotin, α methylbiotin (8) exhibit antibiotic properties.

MATERIAL AND METHODS

Bacterial strains

E. Coli C162 (bio B$^-$, His$^-$) and *E. Coli SA291* (His$^-$, deletion gal-ChlA) are generous gifts from Dr P. Cleary. The strains are grown either on complex medium (bactotryptone 10 g/l, yeast extract 5 g/l, NaCl 5 g/l, pH=7.3) or on synthetic medium (KH_2PO_4 13.6 g/l, $(NH_4)_2SO_4$ 2 g/l, $MgSO_4$ 0.2 g/l, $FeSO_4$ 0.5 mg/l, pH=7.0) supplemented with glucose (2 g/l) and L-histidine (35 mg/l). Before autoclaving, the mixture is treated with norit to remove traces of biotin. The biotin or the selenobiotin are then added aseptically.

E. Coli B, the strain used for the biotin carboxylase purification is a gift from Dr G. Cohen. *Lactobacillus Arabinosus 17.5* (ATCC 8014) has been grown according to Wright and Skeggs (9). The growth is measured spectrophotometrically at 570 nm.

Materials

All chemicals were of the highest purity available. The synthesis of (+) selenobiotin has been described previously (3). The d biotin is a gift from the Hoffman La Roche Co (Basel). Acetyl CoA was prepared according to Simon and Shemin (10). The purity was more than 95 % according to the citrate assay after a DEAE cellulose column and desalting over sephadex G10. [^{14}C]$NaHCO_3$ (55 mCi/mmole) was purchased from C.E.A. (Saclay-France).

Protein determination

Protein concentrations were measured by the biuret method (11).

Enzymes purifications and assays

- Biotin carboxylase

Biotin carboxylase from *E. Coli B* was isolated according to Lane (12) and assayed either with [^{14}C] $NaHCO_3$ or spectrophotometrically (12).

- Acetyl CoA carboxylase

The Acetyl CoA carboxylase activity was tested on crude cell free extracts according to Lane (13) with the modification developed recently by Ernst-Fonberg (14).

- Comparison of Acetyl CoA carboxylase activities in *E. Coli C162* grown in the presence of biotin or selenobiotin

The synthetic growth medium, treated with norit is divided in two equal volumes after autoclaving. Biotin or selenobiotin (5 ng/ml) are added to the two flasks which are inoculated with a small amount of a preculture (synthetic medium, biotin 1 ng/ml).

The bacteria are collected during the late exponential phase, centrifuged and frozen at -20° before use.

The cells (0.17-0.20 g dry weight) are sonicated (M.S.E. sonicator ; 0° ; 7 periods of 10 seconds separated by 50 seconds) in 0.8 ml buffer (KH_2PO_4 0.1 M, EDTA 1 mM, $HS-CH_2-CH_2-OH$ 5 mM, glycerol 20 %, pH 7.0). After centifugation (25 minutes, 50000 g, 4°) the supernatant is divided into 0.15 ml fractions which are frozen (-78°, 1 minute) and stored (-20°) before use.

The assays are run in glass scintillation vials (final volume 0.27 ml : tris-HCl pH 7.5 0.12 M 0.12 ml, ATP 50 mM 10 µl, $MgCl_2$ 0.4 M 10 µl, gluthation 0.3 M 5 µl, Bovine Serum Albumin 15 mg/ml 5 µl, Acetyl CoA 10 mM 10 µl, supernatant 100 µl, [^{14}C] $NaHCO_3$ 1500-4000 cpm/nmole 0.2 M 10 µl). After a 20 minutes incubation at 30° the reaction is stopped with 0.1 ml 6NHCl. The content of the vial is taken to dryness according to Ernst-Fonberg (14) (55°, 40 minutes). After careful solubilization, (0.5 ml water, 0.5 ml ethanol, 0.5 ml water) 10 ml of Bray's liquor (15) are added and the vial is counted (SL 30 Intertechnique). The results are corrected for quenching with the external standard method.

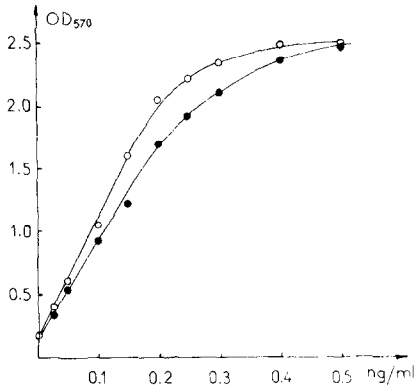


Figure 1 : Growth response of L. Arabinosus 17.5 to increasing amounts of biotin ●—● or selenobiotin O—O . Conditions as Wright and Skeggs (9).

RESULTS AND DISCUSSION

Growth promoting activity of selenobiotin

We have run growth experiments to test selenobiotin using biotin requiring auxotrophs either natural like *Lactobacillus Arabinosus* or induced like *E. Coli* strains SA291 and C162. We selected *E. Coli* since with this microorganism the enzymatic systems dealing with biotin have been studied extensively and make possible a correlation between the growth experiments and the activities of the enzymes at the molecular level.

L. Arabinosus responds only to biotin, biotin d sulfoxide and oxybiotin and the microbiological titration of biotin takes advantage of that specificity (9). Under the titration conditions of Wright and Skeggs we have observed that selenobiotin can fulfil the biotin requirement of that strain with almost the same efficiency (figure 1).

Selenobiotin is also as effective as biotin with *E. Coli C162*. The maximal growth and generation times (52 minutes) are exactly the same for the two compounds when this strain is grown on synthetic medium with biotin (or selenobiotin) concentrations from 5 ng/ml to 1 ng/ml*. Selenobiotin can

* Usually E. Coli is grown on synthetic medium with glucose as carbon source with biotin concentrations ranging from 5 ng/ml to 0.1 ng/ml. 5 ng/ml correspond to conditions under which biotin biosynthesis is completely repressed in the wildstrain (16) and 0.1 ng/ml lead to biotin deficient bacteria with biotin requiring auxotrophs (17). We have tested selenobiotin down to 0.1 ng/ml and we have observed that the *E. Coli C162* grows at this concentration. However at this low concentration it is very difficult to be certain that growth is not due to biotin contamination.

also fulfil the biotin requirement of *E. Coli SA291* at the 1 ng/ml level. But this strain is not very easy to grow and we did not use it for quantitative experiments.

The fact that selenobiotin is a growth factor of *L. Arabinosus* and *E. Coli SA291* proves that it is active *"per se"* and is not transformed into biotin. Indeed, *L. Arabinosus* being a natural biotin auxotroph and *E. Coli SA291* lacking the entire biotin locus are unable to carry out any step of the biotin biosynthesis. Thus, the hypothetical transformation of selenobiotin into an intermediate of the biotin biosynthesis is excluded**.

Selenobiotin is, after oxybiotin≠, the second biotin analog, described so far, with an intrinsic growth promoting activity. Dethiobiotin and biotin d sulfoxide are also active but dethiobiotin is transformed into biotin during the last step of the biosynthesis (2) whereas biotin d sulfoxide, a naturally occuring derivative (5) is *in vivo* reduced to biotin≠≠.

These experiments show that selenobiotin is active as prosthetic group but give no informations on the influence of this replacement on the specific activities of the biotin enzymes.

Activity of "Selenoacetyl CoA carboxylase" of E. Coli

Among the numerous carboxylases from different sources, acetyl CoA carboxylase of *E. Coli* is of great interest and has unique properties. In opposition with other acetyl CoA carboxylases, the *E. Coli* enzyme is active as a monomer and readily dissociates into three active subunits which can be separated and purified as shown by Lane (20) and Vagelos (21). Furthermore, one of these subunits, biotin carboxylase, whose normal substrate is the biotin linked to the protein, can also carboxylate free biotin. This constitutes a good model of the first step of the carboxylation and we have compared selenobiotin and biotin as substrates for purified biotin carboxylase.

The kinetic results show that the biotin and the selenobiotin have very similar behaviour since their K_M* values are identical whereas the

** With *E. Coli C162*, which is unable to transform dethiobiotin into biotin, such a transformation, although highly unlikely could not be rigourously excluded.

≠ The activity of oxybiotin depends on the microorganism involved. Compared with the activity of (d) biotin, that of (dl) oxybiotin ranges from 20 % with *S. Cerevisiae* (6 a-c) 23 % with *L. Casei* (6 a) to 50 % with *L. Arabinosus* (6 a) and *L. Pentosus* (6 d). (dl) oxybiotin is also active with animals : the reponse is 17 % that of (d) biotin with chickens (6 e) and 5 % in curing rat biotin deficiency (6 a).

≠≠ The enzymatic reduction has been studied (18) and the location of the genes responsible for the biosynthesis of the reducing enzymes has been determined (19).

* The K_M derived from the Lineweaver-Burk plots are 0.11 M. Lane has reported a K_M of 0.17 M for biotin (20). According to our own experience, different preparations of enzyme always lead to lower values.

Table I

Comparison of acetyl CoA carboxylase activities≠ with biotin or selenobiotin as prosthetic groups

Biotin	Selenobiotin	$\frac{\text{Selenobiotin}}{\text{Biotin}}$
39	27	0.69
140	75	0.53
47	30	0.65

≠ The activities are expressed as picomoles of Acetyl CoA transformed into malonyl CoA per minute and per mg of protein. Each value is the average of three determinations.

Vmax for selenobiotin is 90 % that of biotin. Lane has tested oxybiotin with biotin carboxylase but he has not reached the K_M and Vmax values. However under the same conditions, (d) oxybiotin is only 20 % as active as (d) biotin (20) compared with 90 % for (d) selenobiotin.

As far as the first step of the carboxylation reaction is concerned, selenobiotin and biotin have about the same activity. To compare their catalytic efficiencies in the complete system we have measured the specific activities of acetyl CoA carboxylase of *E. Coli C162* grown in the presence of biotin or selenobiotin.

The activities (acetyl CoA → malonyl CoA) of cell free extracts have been determined according to Lane (13) and Ernst-Fonberg (14). Under the conditions used for this test, the "seleno-acetyl CoA carboxylase" is 62 % as active as the natural carboxylase (Table I).

As far as we know, the experiments we are reporting constitute the first determination of the specific activity of an active carboxylase with a modified prosthetic group*.

* Lane has studied the *in vitro* incorporation of biotin analogs into the purified apotranscarboxylase of *Propionibacterium Shermanii* (22). He has shown that the purified synthetase that catalyzes this incorporation is very specific for (d) biotin and that, among the compounds tested, only oxybiotin can replace biotin but the holotranscarboxylase obtained in these conditions is inactive (our experiments show that selenobiotin is also a substrate for the corresponding synthetase of *E. Coli*).

REFERENCES

1. a) Stadtman, T.C. (1974) Science, 183, 915-922.
   b) Klayman, D.L., and GÜnther, W.H.H. (1973) Organic Selenium Compounds : their Chemistry and Biology, pp. 579-814, Wiley-Interscience, New-York.
2. a) Moss, J., and Lane, M.D. (1971) in Advances in Enzymology, 35, 321-441.
   b) McCormick, D.B., and Wright, L.D. (1971) in Comprehensive Biochemistry, 21, 81-110, Elsevier, New-York.
   c) Eisenberg, M. (1973) in Advances in Enzymology, 38, 317-372.
3. Bory, S., and Marquet, A. (1976) Tetrahedron Lett., 2033-2036.
4. Tepper, J.P., McCormick, D.B., and Wright, L.D. (1966) J. Biol. Chem., 241, 5734-5735.
5. Melville, D.B., Genghof, D.S., and Lee, J.M. (1954) J. Biol. Chem., 208, 503-512.
6. a) Rubin, S.H., Flower, D., Rosen, F., and Drekter, L. (1945) Arch. Biochem., 8, 79-90.
   b) Hofmann, K., and Winnick, T. (1945) J. Biol. Chem., 160, 449-453.
   c) Axelrod, A.E., Flinn, B.C., and Hofmann, K. (1947) J. Biol. Chem., 169, 195-202.
   d) Krueger, K.K., and Peterson, W.H. (1948) J. Bacteriol. 55, 693-703.
   e) McCoy, R.H., McKibben, J.N., Axelrod, A.E., and Hofmann, K. (1948) J. Biol. Chem., 176, 1319-1326 and 1327-1331.
7. a) Dittmer, K., and Du Vigneaud, V. (1944), Science, 100, 129.
   b) Axelrod, A.E., Dewoody, J., and Hofmann, K. (1946) J. Biol. Chem., 163, 771-772.
8. a) Hanka, L.J., Bergy, M.E., and Kelly, R.B. (1966) Science, 154, 1667- 1668.
   b) Hanka, L.J., Reineke, L.M., and Martin, D.G. (1969) J. Bacteriol., 100, 42-46.
   c) Martin, D.G., Hanka, L.J., and Reineke, L.M. (1971) Tetrahedron Lett., 3791-3794.
9. Wright, L.D., and Skeggs, H.R. (1944) Proc. Soc. Exptl. Biol. Med., 56, 95-98.
10. Simon, E.J., and Shemin, D. (1953) J. Amer. Chem. Soc., 75, 2520.
11. Layne, E. (1957) in Methods in Enzymology, 3, 450-451, Academic Press, New-York.
12. Guchhait, R.B., Polakis, S.E., Dimroth, P., Stoll, E., Moss, J., and Lane M.D. (1974) J. Biol. Chem., 249, 6633-6645.
13. Gregolin, C., Ryder, E., and Lane, M.D. (1968) J. Biol. Chem., 243, 4227-4235.
14. Evers, A.K., Wolpert, J.S., and Ernst-Fonberg, M.L. (1975) Anal. Biochem., 64, 606-608.
15. Bray, G.A. (1960) Anal. Biochem., 1, 279-285.
16. Pai, C.H., and Lichstein, H.C. (1965) Biochim. Biophys. Acta, 100, 28-35.
17. Unpublished results.
18. Cleary, P.P., and Dykhuizen, D. (1974) Biochem. Biophys. Res. Comm., 56, 629-634.
19. Dykhuizen, D. (1973) J. Bacteriol., 115, 662-667.
20. a) Dimroth, P., Guchhait, R.B., Stoll, E., and Lane, M.D. (1970) Proc. Nat. Acad. Sci. U.S.A., 67, 1353-1360.
   b) Guchhait, R.B., Moss, J., Sokolski, W., and Lane, M.D. (1971) Proc. Nat. Acad. Sci. U.S.A., 68, 653-657.
21. Alberts, A.W., and Vagelos, P.R. (1968) Proc. Nat. Acad. Sci. U.S.A., 59, 561-568.
22. Lane, M.D., Young, D.L., and Lynen, F. (1964) J. Biol. Chem., 239, 2858-2864.