Fixation of Carbon Dioxide with Diphenylcarbodiimide as a Model of Biotin Enzyme Active Site and a Weak Base: The Carboxylation of Fluorene under Mild Conditions

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Synopsis. Carbon dioxide was directly fixed into fluorene in the presence of diphenylcarbodiimide (DPC) as a model of a biotin enzyme active site and potassium hydrogencarbonate. It was considered that not only carbon dioxide but also the hydrogencarbonate ion were the carbon source in the presence of DPC. Weak bases such as potassium acetate, potassium propionate, and potassium formate were also effective for carboxylation.

The chemical fixation of carbon dioxide has been widely studied,[1–5] since carbon dioxide is an abundant natural source of carbon. Photosynthesis is known to be the most efficient system for carbon dioxide fixation. However, enzymatic carboxylation without the aid of photoenergy was also known in which biotin enzyme plays an important role.[6,7] It is well known that the active site of biotin is a urea derivative.

We have already reported that active methylene compounds were effectively carboxylated by employing a reagent system, carbon dioxide, diphenylurea or diphenylcarbodiimide (DPC) as a model of biotin active site and potassium carbonate.[8]

In the case of DPC, the reaction occurred even in the presence of water, surprisingly.[8a] The carbonate ion reacts with water to give the hydrogencarbonate ion. Since the hydrogencarbonate ion is a weak base, it is ineffective for a conventional carboxylation reaction. Actually, no carboxylation reaction occurred when potassium hydrogencarbonate was added with diphenylurea. We have found that active methylene compounds can be carboxylated using such weak bases as alkalin metal hydrogencarbonate and DPC. Furthermore, instead of potassium hydrogencarbonate, weak bases such as potassium acetate, potassium propionate, and potassium formate were also effective.

Results and Discussion

It is well known that a strong base, like 1,8-diazabicyclo[5.4.0]undec-7-ene, abstracts a proton from an active methylene compound, producing its carboxylated compound in the presence of carbon dioxide.[9] Since potassium hydrogencarbonate is a weak base, no carboxylation occurred when only potassium hydrogencarbonate was reacted with fluorene under a carbon dioxide atmosphere in DMSO (Table 1). In carboxylation using diphenylurea, a strong base is required since it is believed that the first step is an imine proton abstraction by a strong base. Actually, no carboxylation reaction occurred when a weak base, potassium hydrogencarbonate, was added to diphenylurea. However, when potassium hydrogencarbonate was used with DPC, fluorene was carboxylated and the yield of 9-FLC was as high as 44—47% (Table 1). Sodium hydrogencarbonate was also an effective base.

Table 1. Carboxylation of Fluorene Using a Model of the Biotin Enzyme Active Site[a] and Base under a Carbon Dioxide Atmosphere

Model of the biotin enzyme active site	Base	Water added /mmol	Yield of 9-FLC/%[b]
None	$KHCO_3$	0	0.0
Diphenylurea	$KHCO_3$	0	0.0
Diphenylcarbodiimide	None	0	0.0
Diphenylcarbodiimide	$KHCO_3$	0	43.9, 46.9
Diphenylcarbodiimide[c]	$KHCO_3$	0	49.1
Diphenylcarbodiimide[d]	$KHCO_3$	0	48.5
Diphenylcarbodiimide	$KHCO_3$	10	18.0
Diphenylcarbodiimide	$NaHCO_3$	0	3.7
Diphenylcarbodiimide	$NaHCO_3$	10	11.2

a) Fluorene, 5 mmol; imine, 16.8 mmol; base, 60 mmol; DMSO, 25 ml; reaction temp., 20 °C; reaction time, 2 h. b) The yields were calculated on the basis of fluorene used. c) Under a nitrogen atmosphere. d) Under an air atmosphere.

Table 2. The Solubility of Base

Base	Solubility A[a] H_2O	Solubility B[b] H_2O	The amounts of CO_2 absorption[c] H_2O
	0 (10 mmol)	0 (10 mmol)	0 (10 mmol)
None			16.0 (17.3)
K_2CO_3	0.1 (0.2)	3.7 (8.8)	15.1 (20.0)
$KHCO_3$	0.2 (0.2)	41.0 (19.2)	3.6 (13.0)
$NaHCO_3$	0.2 (0.2)	2.3 (3.3)	17.6 (16.4)
HCOOK	3.6	14.0	31.0
CH_3COOK	3.5	6.8	48.3
C_2H_5COOK	2.1	4.5	52.6

a) Solubility of base in DMSO 100 ml. b) Solubility of base in DPC (67.2 mmol) and DMSO 100 ml. c) The amount of CO_2 absorption (mmol) in the solution of base (240 mmol or 120 mmol), DPC (67.2 mmol), and DMSO 100 ml.

Although the solubilities of carbonate and hydrogencarbonates in DMSO were small, they increased upon the addition of DPC, as shown in Table 2. Especially, the solubility of potassium hydrogencarbonate in DMSO with DPC was as large as 41 mmol/100 ml DMSO, suggesting a reaction of DPC with the base to produce an active intermediate. Upon the addition of water, although the solubilities of potassium carbonate

and sodium hydrogencarbonate increased, the solubility of potassium hydrogencarbonate decreased. The effects of the water addition on the solubility had the same tendency as did the effects of water on carboxylation (Table 1).

In the case of DPC and potassium carbonate, fluorene was carboxylated even in the absence of carbon dioxide, indicating that the carbonate ion was the carbon source. However, the yield of 9-FLC was as low as 6.6%.[8a] In the case of DPC and potassium hydrogencarbonate, the yield of 9-FLC under a nitrogen atmosphere was 49%; the yield under an air atmosphere was also 49%. These values were nearly equal to those under a carbon dioxide atmosphere, clearly indicating that the hydrogencarbonate ion was a carbon source. It was considered that a hydrogencarbonate ion attacked an imine carbon to give anionic intermediates which could transfer carbon dioxide to fluorene.

In biological carboxylation catalyzed by a biotin enzyme, the hydrogencarbonate ion is a carbon source and carboxylation occurs through the activation of a hydrogencarbonate ion. We believe that our result is the first example of a model reaction of biotin enzymatic carboxylation using the hydrogencarbonate ion.

Active methylene compounds other than fluorene were also carboxylated by using a reagent system, DPC, potassium hydrogencarbonate and carbon dioxide. They include indene, indanone, acetophenone, cyclohexanone, 1-tetralone, phenylacetonitrile, and *p*-methoxyacetophenone (Table 3). Higher yields than those using potassium carbonate were obtained in the cases of fluorene, phenylacetonitrile, 1-tetralone, and *p*-methoxyacetophenone. There was no correlation between the pK_a of an active methylene compound and the yield.

Similarly to potassium hydrogencarbonate, potassium acetate, being a weak base, was not an effective base for carboxylation if DPC was not used. However, high yields of more than 50% were obtained by employing a reagent system, carbon dioxide, DPC, and potassium acetate (Table 4). Potassium formate and potassium propionate were also effective, even though the yields were about one half those using potassium acetate. Lithium acetate was not effective.

No carboxylation occurred under a nitrogen atmosphere in the case of potassium acetate, indicating that the acetate ion was not a carbon source, and that only atmospheric carbon dioxide was a carbon source. The amounts of carbon dioxide absorption in the case of potassium acetate are also shown in Table 2. The value (48.3) was fairly larger than 15.1 in the case of potassium carbonate. It is considered that the acetate ion attacks an imine carbon, and that the resulting intermediate easily reacts with carbon dioxide to give carbon dioxide donors.

We have already reported that *o*-substitutions in the phenyl moieties of DPC and diphenylurea were harmful for carboxylation using potassium carbonate. The same result was obtained in the case of potassium hydrogencarbonate, as shown in Table 4. The amounts of carbon dioxide adsorption were not affected by the *o*-substitutions, suggesting that the reaction of carbon dioxide with imine was not blocked by the *o*-substitutions. We considered that *o*-substitutions inhibited the reaction of fluorene with an intermediate including carbon dioxide. However, potassium acetate and potassium propionate were effective bases, even in the case of the *o*-substitution of DPC. We could not clarify any mechanism, however, but speculate two reaction routes. At first, acetate and propionate moieties having a larger space than does the hydrogencarbonate

Table 3. Carboxylation of Active Methylene Compounds Using Potassium Hydrogencarbonate in Carbon Dioxide Atmosphere[a]

Substrate	Yield of carboxylic acid/%[b] (using potassium carbonate)
Indene	21.1 (53.8)
Indanone	44.3 (45.5)
Fluorene	43.9 (35.2)
Acetophenone	4.2 (28.7)
Cyclohexanone	3.6 (14.0)
1-Tetralone	35.9 (21.5)
Phenylacetonitrile	51.5 (29.2)
p-Methoxyacetophenone	20.7 (14.4)

a) Substrate, 5 mmol; diphenylcarbodiimide, 16.8 mmol; $KHCO_3$ 60 mmol or K_2CO_3, 30 mmol; DMSO, 25 ml; reaction temp, 20 °C; reaction time, 2 h. b) The yields of carboxylic acid were calculated on the basis of substrate used. The products were monocarboxylic acids. They were identified by comparision of IR spectra with those of the authentic samples, after esterification with diazomethane, if necessary.

Table 4. Carboxylation of Fluorene Using a Biotin Model and a Weak Base under a Carbon Dioxide Atmosphere[a]

Model of the biotin enzyme active site	Base (Reaction time (h))	Yield of 9-FLC[b] %
None	CH_3COOK (2)	0.0
Diphenylcarbodiimide	CH_3COOK (1)	37.3
Diphenylcarbodiimide	CH_3COOK (2)	52.2, 50.8
Diphenylcarbodiimide	CH_3COOK (3)	52.7, 53.1
Diphenylcarbodiimide	CH_3COOK (5)	52.8
Diphenylcarbodiimide	CH_3COOK (7)	49.6
Diphenylcarbodiimide[c]	CH_3COOK (2)	0.0
Diphenylcarbodiimide	CH_3COOLi (2)	0.0
Diphenylcarbodiimide	CH_3COONa (2)	30.5
Diphenylcarbodiimide	HCOOK (2)	26.5
Diphenylcarbodiimide[c]	HCOOK (2)	8.5
Diphenylcarbodiimide	C_2H_5COOK (2)	27.6, 23.0
Diphenylcarbodiimide[c]	C_2H_5COOK (2)	0.0
Diphenylcarbodiimide	C_6H_5COOK (2)	0.0
o-Cl-DPC[d]	$KHCO_3$ (2)	0.0
o-CH_3-DPC[e]	$KHCO_3$ (2)	0.0
o-Cl-DPC[d]	HCOOK (2)	2.3
o-Cl-DPC[d]	CH_3COOK (2)	48.8, 49.8
o-CH_3-DPC[e]	CH_3COOK (2)	16.9
o-Cl-DPC[d]	C_2H_5COOK (2)	35.8
o-Cl-DPC[d]	C_6H_5COOK (2)	0.0

a) Substrate, 5 mmol; diphenylcarbodiimide, 16.8 mmol; base, 60 mmol; DMSO, 25 ml; reaction temp, 20 °C; reaction time, 2 h. b) The yields of carboxylic acid were calculated on the basis of substrate used. c) Under a nitrogen atmosphere. d) Bis(*o*-chlorophenyl)carbodiimide. e) Bis(*o*-methylphenyl)carbodiimide.

ion may remove the blocking of *o*-substitutions. The second is that the acetate and propionate moieties may activate carbon dioxide.

In any case, we first indicated that weak bases, such as potassium acetate and potassium propionate, are also effective in the case that diphenylcarbodiimide is used as a biotin enzyme active site.

Experimental

DPC and substituted DPC were synthesized according to a method of Campbell et al.[10] Fluorene and DPC were dissolved in DMSO (25 ml); to this solution was added powdered alkali carbonate or alkali hydrogencarbonate. Dry carbon dioxide was then passed into the mixture. The reaction mixture was worked up in the same manner as described previously.[8] The yield of 9-FLC was measured gravimetrically. A reaction under pressure was carried out using a 100 ml magnet-driven autoclave and was treated in the same manner as mentioned above. The solubilities of alkali hydrogencarbonates were determined by titration using aquous HCl (0.01 or 0.001 mol dm^{-3}) after filtration.

The amounts of carbon dioxide absorption in a DMSO solution containing DPC were determined by measuring the weights both before and after carbon dioxide bubbling.

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