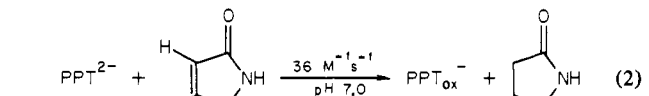
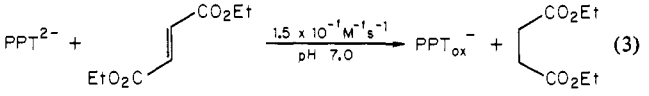
analysis, the hydrolytically stable[12] *N*-alkylnicotinamide mimic, *N*-benzylquinoline-3-carboxamide, was employed. This compound (3×10^{-4} M) in aqueous solution is reduced by PPT^{2-} (1×10^{-5} M) on mixing. The percentage yield of PPT_{ox}^- was determined spectrophotometrically (423 nm) as 100%. The identity of *N*-benzyl-1,4-dihydroquinoline-3-carboxamide was obtained in a preparative experiment and shown to be formed in 100% yield. Flavins do not reduce *N*-alkylnicotinamides but are themselves rapidly reduced by *N*-alkyldihydronicotinamides.

The oxidation of mercaptans by flavins has been studied in detail.[13] The mechanism is thought to be well understood. By use of PPT^{2-} this reaction may be examined in the retrodirection and by this means verification of the accepted mechanism may be sought. Also, disulfide bond reduction by 1,5-dihydroflavin cofactor is an important part of the mechanism of glutathoine reductase, dihydrolipoamide dehydrogenase, and thioredoxin reductase.[14] In studies in aqueous solution, thioglycolic acid disulfide is readily reduced ($k = 2 \times 10^{-2}$ M^{-1} s^{-1}, 30 °C, pH 7) by PPT^{2-} to provide PPT_{ox} (90% yield) plus thioglycolic acid (in ~100% yield based on spectrophotometric assay with 5,5′-dithiobis[2-nitrobenzoic acid]). Reduction of cystine by PPT^{2-} provided PPT_{ox}^- (~100%) and cysteine (90%).

The oxidation of succinic acid by succinic acid dehydrogenase to yield fumaric acid represents one of a class of reversible flavoenzyme oxidations that yield C–C double bonds through the intermediacy of a substrate carbanion species.[1,15] There is great current interest in the mode of two-electron transfer from carbanion to enzyme-bound flavin.[16] Are the electrons transferred one by one to provide radical intermediates, is hydride transfer involved, or does a two-electron transfer occur through the formation of a covalent intermediate followed by an elimination reaction? PPT^{2-} reacts with *N*-methylmaleimide to provide PPT_{ox} in 100% yield. The *N*-methylmaleimide is converted to *N*-methylsuccinimide. At least two intermediates can be detected (pH 7.0) by kinetic measurements. In the reduction of maleimide (4×10^{-4} to 7×10^{-4} M) and diethyl fumarate (2×10^{-3} to 2×10^{-4} M) by PPT^{2-} (10^{-5} M), the reactions followed the first-order rate law to $7t_{1/2}$ or $8t_{1/2}$ and provided PPT_{ox} in 100% of theory (eq 2 and 3). Plots of k_{obsd} vs. [substrate] are linear. With





these substrates only carbon–carbon double bond reduction can be seen, and the reactions are simply first order in PPT^{2-} and substrate. No intermediate can be noted.

The various reactions alluded to herein will be described in detail in forthcoming publications. The results provided at this time serve to indicate the utility of PPT^{2-} as a low-potential flavin analogue.

Acknowledgment. This work was supported by grants from the National Institutes of Health and the National Science Foundation.

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A Carbonate Receptor Model by Macromonocyclic Polyamines and Its Physiological Implications

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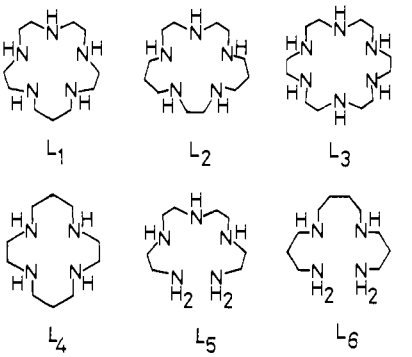
We recently discovered that macromonocyclic pentaamines L_1



and L_2 and hexaamine L_3 form stable 1:1 complexes at neutral pH with polycarboxylates (e.g., citrate, succinate, etc.)[1] and phosphates (e.g., inorganic phosphate, AMP, ADP, ATP, etc.).[2] The 3+ charges by three protons contained in the macrocycles and the favorable orientations of the amine protons for hydrogen bondings make these polyamine ligands suitable model receptors to the biochemically important polyoxy anions. Herein we communicate that these polyamines can take up another polyanion, physiologically essential carbonate, CO_3^{2-}. The results obtained not only add a further step to an emerging new field of anion coordination chemistry but also may provide a possible chemical model of membrane recognition of carbonate that is a key step of respiratory regulation of acid–base balance in our body.[3] Our discovery also may imply that the hemoglobin-coordinated carbonate anion constitutes an considerable part of the CO_2 transported in the blood.

The interaction of carbonate anions with macrocyclic polyamines L_1–L_3 and linear polyamines L_5 and L_6 (spermine) was first demonstrated in paper electrophoresis: in the absence of carbonate ions, the polyamines L_1–L_6 moved to the negative electrode as normal polycations in pH 7 Tris buffer (Figure 1a); when bicarbonate ion was added to the Tris buffer, the speed of the negative shift slowed down except for macrocyclic tetraamine L_4 (Figure 1b); in carbonate buffers (pH 9.3) they moved to the reverse, positive electrode (Figure 1c). A similar peculiar behavior at electrophoresis of L_1–L_3 was earlier observed in polycarboxylate (e.g., citrate) buffers, which led to the discovery of the polyamine complexes with polycarboxylates.[1]

A quantitative measurement of the polyamine–carbonate interaction has been made with an anodic wave polarography in Tris (0.05 M) and borate (0.03–0.075 M) buffers, where diffusion-controlled two-step waves were observed with L_1–L_3 and L_5 (Figure 2).[4] The potentials of the first waves were identical with

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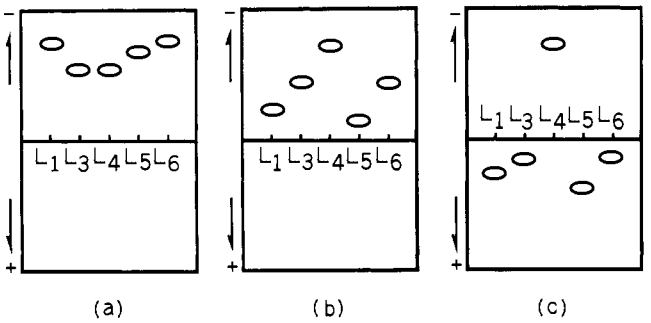
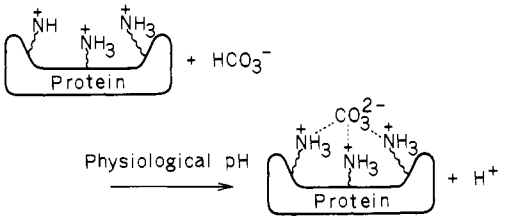


Figure 1. Electrophoretic movement of polyamines in (a) pH 7.2 Tris (0.05 M) buffers, (b) pH 7.2 Tris buffers (0.05 M) with $NaHCO_3$ added (0.1 M), and (c) pH 9.2 carbonate buffers (0.05 M). Conditions: Gelman acetate cellulose sheets, 200–300 V for 15–30 min at 25 °C.

Scheme I



those of the single waves occurring in the absence of carbonate ions. Thus, the first waves were ascriabable to free (i.e., uncomplexed with carbonate) polyamines to yield HgL^{2+} concentrations at the dropping mercury electrode (DME).[5] The second waves with their heights varying with concentration of carbonate ion and pH were best explained by assuming the formation of HgL^{2+} between mercury and polyamine ligands of 1:1 polyamine–carbonate complexes. Under the conditions $[\text{polyamine}]_{total} = 4 \times 10^{-4}$ M << $[\text{carbonate}]_{total} = 0.04 - 0.2$ M of HCO_3^-, the ratios of the second to first wave heights $(i_d)_2/(i_d)_1$ were all proportional to $[\text{carbonate}]_{total}$ at a constant pH (7.20). Further, at constant $[\text{carbonate}]_{total}$ (0.1 M of HCO_3^-), the ratios $(i_d)_2/(i_d)_1$ varied with pH (i.e., the reading of pH meter, a_H) in proportion to $a_H{}^3K_1K_2K_3/(\alpha_H)_L(\alpha_H)_{CO_3}$ in accordance with eq 1, where K_i

$$\frac{(i_d)_2}{(i_d)_1} = \frac{[\text{carbonate complex}]}{[\text{polyamine}]_f} = K\frac{a_H{}^3K_1K_2K_3}{(\alpha_H)_L(\alpha_H)_{CO_3}}[\text{carbonate}]_{total} \quad (1)$$

are the ith protonation constants of polyamines L,[6] $(\alpha_H)_L{}^{-1}$ is the ratio of $[\text{unprotonated amine}]_{uncomplexed}$ to $[\text{total amine}]_{uncomplexed}$, and $(\alpha_H)_{CO_3}{}^{-1}$ is the similar ratio for carbonate. Hence the 1:1 H_3L^{3+}–CO_3^{2-} complex formation constants K were calculable from the $(i_d)_2/(i_d)_1$ ratio (by using eq 1), and the results are summarized in Table I. With L_4, only a one-step wave was seen with or without carbonate. With L_6, the anodic wave was not observed.[7]

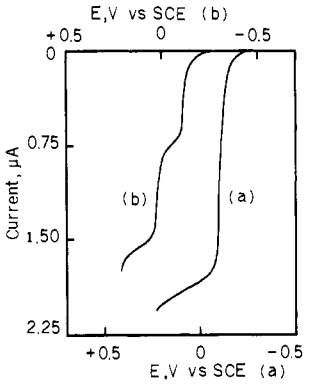


Figure 2. Anodic wave polarograms of L_1 (4×10^{-4} M) (a) without and (b) with $NaHCO_3$ (0.1 M) in Tris buffer (0.1 M) at pH 7.20 and 25 °C.

Table I. Summary of 1:1 Polyamine–Carbonate H_3L^{3+}–CO_3^{2-} Complex Formation Constants K at 25 °C

ligand	K,[a] M^{-1}	pH range measd
L_1	$2.7_8 \times 10^4$	6.5-8.6 (Tris), 9.3 (borate)
L_2	$1.9_0 \times 10^4$	7.0-8.2 (Tris), 9.3-10.0 (borate)
L_3	$5\ 7_0 \times 10^2$	8.0-10.0 (borate)
L_4	no interaction	
L_5	$1.0_7 \times 10^3$	8.2-10.0 (borate)
L_6 (spermine)	strong interaction indicated by electrophoresis	

[a] K is defined as $[H_3L^{3+}\text{-}CO_3^{2-}]/[H_3L^{3+}]\,[CO_3^{2-}]$.

The 1:1 complexes may have structures, where carbonates are bound through either localized anions (on two oxygen atoms) or delocalized anions (on three oxygen atoms) to 3+ ammonium cations via hydrogen bonds. Similar structures have been envisaged for the polyamine–polycarboxylate[1] and polyamine–phosphate complexes.[2] The 2+ charge of macrocyclic tetraamine L_4 (at neutral pH, see its K_i values)[6] presumably is not attractive enough for the carbonate dianion, as it is not for the other biological polyanions.[1,2]

The most significant discovery in the present study is the liberation of a free proton at pH ~7 when carbonates (in HCO_3^- form) interact with polyamines (in H_3L^{3+} form) (see Scheme I). Viewing the polyamines as a site of dense amine residues (e.g., lysine) on carbonate transport proteins, one may regard the present carbonate interactions as a model carbonate uptake in our body. It is a textbook fact in physiology that the acid–base equilibrium of carbonic acid (formed from CO_2 by carbonic anhydrase), together with the selective membrane transport of carbonate anions, is responsible for the production of gastric HCl in the parietal cells, secretion of H^+ in the kidney tubules, and the "chloride shift" in the red blood cells.[3] In our view, however, the fundamental chemistry underlying the production of the strong acid from the weak acid in our body has remained utterly unexplored. Our polyamines thus might offer a useful chemical model.[8]

Further, the present results imply that some portion of CO_2 in the blood may be transported in the form of carbonate bound to positive sites of hemoglobin proteins. The present result also indicates that a biogenic polyamine spermine L_6 is readily carbonated.

(4) A similar anodic polarographic technique was used to determine 1:1 polyamine–polycarboxylate[1] and 1:1 polyamine–phosphate associations[2] in Tris buffers. However, in these complex cases only one-step waves were seen, probably due to the labile nature (i.e., rapid association and dissociation) of the complexes. The association constants were determined from the shifts of $E_{1/2}$ values. In the present carbonate complex case, the dissociation would not occur in the time scale (4–5 s) of the dropping mercury electrode, which accounts for the occurrence of the two waves.

(5) (a) Kodama, M.; Kimura, E. *J. Chem. Soc., Dalton Trans.* (a) **1976**, 2335; (b) **1978**, 1081; (c) *Ibid.* **1980**, 2536.

(6) The protonation constants K_i at 25 °C are 10.64, 9.49, 7.28, 1.71, 1.45 (for L_1), 10.32, 9.62, 7.36, 4.10, 2.38 (for L_2) (Kodama, M.; Kimura, E. *J. Chem. Soc., Dalton Trans.* **1978**, 104), 10.19, 9.23, 8.73, 4.09, ~2, ~1 (L_3),[5c] 11.5, 10.2, <2, <2 (L_4),[5a] 10.36, 9.65, 8.50, 4.70, 2.40 (L_5) (Moss, D. B.; Lin, C.; Rorabacher, D. B. *J. Am. Chem. Soc.* **1973**, *95*, 5179), and 10.25, 6.30 (for CO_3^{2-}).

(7) The resolution of the experimental data merely establishes that three protons are involved in the interaction of polyamines with carbonates. Hence, one may reinterpret the complexes in terms of H_2L^{2+}–HCO_3^- or HL^+–H_2CO_3 (or CO_2).

(8) In relevance to the "chloride shift", it is to be noted that our polyamines do not interact with the chloride anion. Hence, they are selective to the carbonate anion.