Paper Chromatographic and ^{1}H NMR Study of Ion Pair Interactions between *N*-Dodecanoylhistidine Methyl Ester and Dodecyl Dihydrogenphosphate in Chloroform

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Synopsis. A mixture of *N*-dodecanoylhistidine methyl ester (DHOMe) and dodecyl dihydrogenphosphate (DDP) gave a chromatographically distinct complex in chloroform, where the imidazole staining was markedly enhanced and the phosphate staining showed a characteristic delayed appearance. The ^{1}H NMR chemical shift of 8.53 ppm (imidazole C-2H) observed for a 1 : 1 mixture of the two amphiphiles was downfield shifted by nearly 1.0 ppm vis-à-vis DHOMe alone. The data suggest that the complex between DHOMe and DDP in chloroform is stabilized by hydrogen bonding and ion-pair interactions.

The processes which govern molecular assembly are of crucial importance to our understanding of self organization. Water plays a seminal role in biology primarily due to its role in orchestration of hydrophobic interactions[1] that lead to spontaneous protein folding and membrane self assembly. However, it is becoming increasingly clear that just as the polar water molecules govern hydrophobic interactions between amphiphiles, a nonpolar solvent can lead to solvophobic interactions between amphiphilic molecules.[2] Such interactions are relevant to the processes of self organization within the microdomains of the lipid bilayer and the hydrophobic cores of globular proteins. A recent report has demonstrated that an aqueous phosphate bilayer membrane is transformed by addition of $CaCl_2$ to a stoichiometric, water insoluble complex, which retains bilayer characteristics in chloroform and toluene.[3]

We have earlier shown that a mixture of Boc-Asp-NHMe and Boc-His-NHMe forms an intermolecular salt bridge in chloroform.[4] In the present study, we have linked ionizable groups viz. imidazolyl and phosphate to twelve carbon long chains. We designed and synthesized these large amphiphiles in order to find their choice "to ionize or remain neutral" on mixing in an organic solvent of low dielectric constant. We report here that a mixture of *N*-dodecanoylhistidine methyl ester (DHOMe) and dodecyl dihydrogenphosphate (DDP) taken in chloroform gives rise to a chromatographically distinct complex. Proton NMR evidence on the mixture taken in $CDCl_3$ suggests that the complex derives stability from ion-pair interactions between the alkyl phosphate anions and the alkylimidazolium cations.

Experimental

DHOMe was synthesized by coupling of dodecanoic acid and His-OMe in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt). His-OMe was obtained from His-OMe·2HCl by bubbling ammonia as described previously.[4] To a mixture of dodecanoic acid 2.0 g (10 mmol) and His-OMe 1.70 g (10 mmol) taken in *N*-methylpyrrolidinone at 0 °C DCC 2.26 g (10 mmol) and HOBt 1.35 g (10 mmol) were added. The mixture was stirred at 0 °C for 2 h and then at room temperature overnight. Insoluble dicyclohexylurea was filtered and washed with 150 ml of EtOAC. The EtOAc extract was washed with 1 M (1 M=1 mol dm^{-3}) $NaHCO_3$ (40 ml×5), brine (40 ml×5), dried over Na_2SO_4 and evaporated to obtain a light cream solid weighing 3.12 g (89% yield). The product was loaded on silica gel column and eluted with chloroform. This product was negative to ninhydrin and positive for Pauly's reagent.[5] It gave a proton NMR spectrum fully consistent with its structure. DDP and its sodium salts were synthesized using dodecyl alcohol and $POCl_3$ by a method described separately.[6] The detection of phosphate was done by using Goswami and Frey's reagent.[7] Chromatography and stainings were done on Whatman 3. NMR spectra were recorded at 200 MHz. ^{1}H NMR chemical shifts are given relative to tetramethylsilane. DCC and HOBt were obtained from Merck and Sigma respectively.

Results and Discussion

Paper chromatography of DHOMe (Fig. 1a) vs. a mixture of DHOMe and DDP (Fig. 1, b—e) where the stoichiometry of DHOMe: DDP was varied from 1 : 1 to 1 : 10 (w/w), was done using chloroform as the solvent system. The mixture of DHOMe and DDP revealed a trailing behavior for DHOMe (Fig. 1, b—e) which was not the case when DHOMe was chromatographed alone (Fig. 1a).

DPP (1 μg) has R_f=0 (Fig. 1f). As the amount of DDP loaded increased from 1 μg to 50 μg, the lengths of DDP stain increased (as shown by the streaking patterns— Fig. 1, f—k). Nevertheless in all the chromatograms, the phosphate positivity corresponded to R_f less than 0.6 with great intensity at the bottom i.e. at R_f=0.

When paper chromatograms of DHOMe : DDP mixtures in the stoichiometric amounts 0 : 1 to 1 : 10 were run in $CHCl_3$, increase in R_f of phosphate staining could clearly be seen (Fig. 1, l—p). DDP in these mixtures was seen to be carried to the top. Lanes (h—k) represent different amounts of DDP alone and are controls for lanes (m—p) where 5 μg of DHOMe was mixed with the corresponding amounts of DDP. While the control lanes (h—k) show a streaking behavior for phosphate staining, the test lanes where DHOMe and DDP are loaded as a mixture, show phosphate staining with a significantly higher R_f. It is worth noting that the R_f for the highest staining spot has moved up from its value of zero in control lanes (h—k) to nearly 1.0 in test lanes (m—p).

It was interesting to note the difference in the imidazole and phosphate staining of DHOMe : DDP mixtures when compared to DHOMe and DDP taken separately.

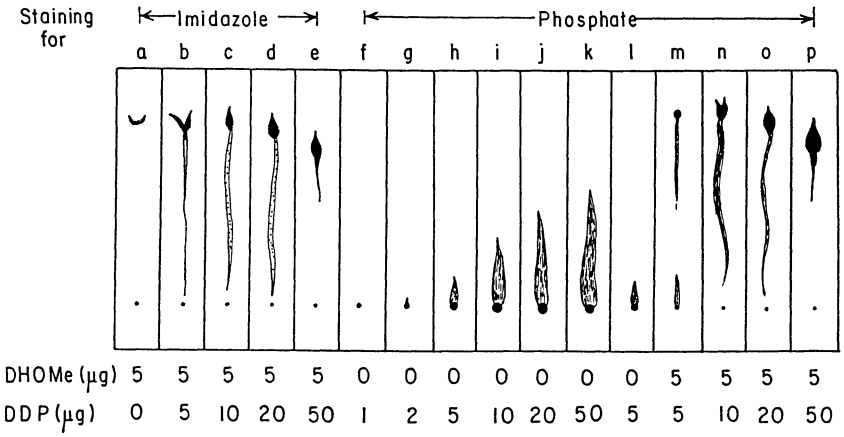

Fig. 1. Chromatographic evidence for complexation of DHOMe and DDP.
Paper chromatograms were run in chloroform and stained with Pauly's reagent (a—e) and Goswami and Frey's reagent (f—p). By comparing the chromatograms of DHOMe and DDP mixture (b—e) and (m—p), it can be seen that the imidazole positivity nearly superimposes the phosphate positivity. While the phosphate staining for DPP alone in lanes f—k was instantaneous, the staining for phosphate in the mixture of DHOMe and DDP (lanes m—p) showed a lag period of several hours.

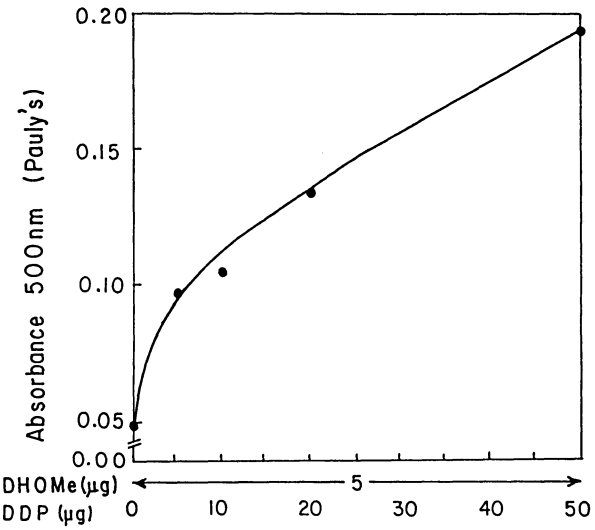

Fig. 2. Intensification of DHOMe staining in presence of DDP.
DHOMe and DDP in the indicated amounts were loaded on Whatman paper and stained with Pauly's reagent. The colored spots were eluted with water (2 ml) and O.D. read at 500 nm. Data have not been collected for DDP points corresponding to 30 and 40 μg respectively.

DHOMe stained more intensely to Pauly's reagent in the presence of DDP than in its absence. As the amount of DDP added to a constant amount of DHOMe (5 μg) was increased from DHOMe : DDP ratio 1 : 0 to 1 : 10 (w/w), progressive increase in staining for imidazole could clearly be seen (Fig. 2). Difference in phosphate staining was also observed for mixture chromatograms when they were compared to pure DDP. Pure DDP chromatograms gave phosphate positive spots immediately (Fig. 1, h—k). DHOMe : DDP mixture chlomatograms were initially negative to phosphate spray (Fig. 1, m—p) and this negative region corresponded to the same site where DHOMe was detected by Pauly's spray in the corresponding chromatograms (Fig. 1, b—e). On keeping overnight, the negatively stained region turned out to be an intensely stained phosphate spot.

The chromatographic evidences suggested that either (a) DHOMe and DDP are complexing or (b) $DHOMe^{+}$ and DDP^{-}/DDP^{2-} are showing coincidental similarity in R_f values and that the two are not actually forming a complex. In order to demonstrate that imidazole positive trails are not accidentally phosphate positive too, sodium salts of DDP were tested for their mobilities in $CHCl_3$. Both mono- and disodium salts of DDP showed $R_f=0$ in $CHCl_3$ solvent system, whereas DDP in presence of DHOMe shows an R_f nearly equal to 1. Thus, it can be concluded that DHOMe and DDP are forming a complex and the altered R_f of DDP in presence of DHOMe is not merely due to its deprotonation.

In order to confirm that mixing of DHOMe and DDP leads to salt bridge formation in $CHCl_3$, ^1H NMR spectra of (a) DHOMe (b) DHOMe : DDP (1 : 1) were recorded in $CDCl_3$. The chemical shifts of C-2H and C-5H resonances of imidazole ring are diagnostic of the state of protonation of imidazole ring.[8,9] As shown in Table 1, the C-2H resonance at 8.53 ppm in the mixture has shifted downfield by 0.99 ppm when compared with the chemical shift of C-2H imidazole in DHOMe alone. Likewise C-5H resonance shows a downfield shift of 0.20 ppm in the mixture. It may be noted that the C-2H resonance of imidazole in the mixture of Boc-His-NHMe and Boc-Asp-NHMe taken in $CDCl_3$ had shown a downfield chemical shift of 0.66 ppm only.[4] The corresponding shift for the intramolecular ion-pair

Table 1. ^{1}H NMR Data for DHOMe vs. DHOMe+DDP (1 : 1) in $CDCl_3$

Resonance	Chemical shifts (ppm)		(b−a)
	DHOMe (48 mM) (a)	DHOMe+DDP (48 mM) (52 mM) (b)	ppm
Imidazole C-2H[a)]	7.54	8.53	0.99
Imidazole C-5H[a)]	6.79	6.99	0.20
Amide NH	7.17, 7.21 (d)[b)]	7.63, 7.67 (d)[b)]	0.46, 0.46

a) Note that the numbering adopted for the imidazole ring of histidine follows recommendation of the IUPAC-IUB commission on Biochemical nomenclature.[10] The C(5)–H referred to in this paper has often been designated as C(4)–5 in the literature. b) 'd' denotes doublet.

interaction in the well-characterized salt bridge in a β turn tetrapeptide viz. Boc–Asp–Pro–Aib–His–NHMe was around 0.81 ppm.[4] Thus the observed chemical shift variation of nearly 1.0 ppm on protonation in the DHOMe : DDP complex described here may be among the highest known in literature on protonation of imidazole. It was interesting to note that in a mixture of DHOMe and DDP, the amide NH of DHOMe was also downfield shifted by 0.46 ppm. This suggested that the phosphate head group of DDP not only contributes proton to the imidazole but that it also establishes a hydrogen bond with the amide NH of DHOMe. In the few instances where histidyl residues are phosphorylated in proteins, the phosphate is known to be transferred to the N-1 of imidozole.[11,12] However, our NMR data do not allow us to confirm if the interaction between DHOMe and DDP involves phosphate and N-1 of histidine imidazole ring. The exact state of ionization of DDP is not known.

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