A functional nickel(II) complex carrier for up-hill transport of a histidine derivative by pH-induced affinity switching

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The square-planar nickel(II) complex of 6,6′-bis(4-hexylbenzoylamino)-2,2′-bipyridine mediates up-hill transport of carbobenzoxyhistidine (Cbz-His) across an organic liquid membrane by affinity switching of the axial coordination of the imidazole unit of Cbz-His to the metal centre.

Selective and active transport of substrates across biomembranes is an essential process in biological systems. As a model of biological membrane transport, carrier-mediated transport across an artificial membrane has been investigated extensively.[1] Since substrate affinity of the carrier should be high in the uptake process but low in the release process, affinity switching of the carrier during the transport process is an effective strategy to achieve active transport.[2] In a previous paper,[3] we reported that the pH-induced affinity switching of a complex-type carrier generated efficient up-hill transport of anions, especially SCN^-, by coupling with symport of H^+. The divalent square-planar nickel(II) complex of 6,6′-bis(4-hexylbenzoylamino)-2,2′-bipyridine (H_2L)[4] which reversibly deprotonates its amide protons, serves as a switch of anion binding to the complex, by decreasing the net charge of the complex from +2 to zero. In this system, preferential axial coordination of SCN^- to the non-deprotonated form of the carrier $[M(H_2L)]^{2+}$ induces selective and efficient up-hill transport of SCN^-. We extended this affinity-switching strategy further to the transport of biologically important substrates which can bind to metal coordination sites. We report here the up-hill transport of an amino acid derivative, carbobenzoxyhistidine (Cbz-His), by pH-induced affinity switching of the nickel(II) complex of H_2L. The carrier complex mediated up-hill transport of Cbz-His *via* the axial coordination of the imidazole unit of Cbz-His. There have appeared only a limited number of reports on the up-hill transport of biologically important molecules.[5]

Electronic spectra of $[Ni(H_2L)][NO_3]_2$ in CH_2Cl_2 at 20 °C showed a π–π^* absorption band at *ca.* 350 nm, which was affected by addition of Cbz-His (containing an imidazole side chain) but not by Cbz-isoleucine (Cbz-Ile).† The spectral change upon addition of Cbz-His was small but clearly seen to occur over one step upon the addition of 2 mol equiv. of Cbz-His (Fig. 1), and the 1 : 2 association constant for Cbz-His, log $K_{Cbz\text{-}His}$, was determined to be 8.30 ± 0.03 (r^2 = 0.997) from the absorbance at 351.4 nm, using a curve-fitting method. Since the observed spectral change for the $[Ni(H_2L)]^{2+}$ was essentially the same as for the strongly coordinating SCN^- anion, the results indicated axial coordination of two imidazole groups to the metal centre (Scheme 1). The spectral change caused by Cbz-His was little affected by the presence of lipophilic NBu_4ClO_4 [log $K_{Cbz\text{-}His}$ = 8.11 ± 0.04 (r^2 = 0.996)]. Therefore, association of Cbz-His was primarily due to the coordination of the imidazole to the metal centre. In the case of the deprotonated complex [NiL], neither Cbz-His nor Cbz-Ile caused any detectable spectral change. The poor interaction of the deprotonated complex with axial ligands is ascribed to its stronger square-planar ligand field compared to the non-deprotonated complex, as indicated previously from the d–d band of its copper(II) analogue.[3]

In two-phase experiments, dichloromethane solutions of $[Ni(H_2L)]^{2+}$ (2.00×10^{-3} mol dm^{-3}) were mixed with the same volume of aqueous Cbz-His solutions (2.00×10^{-3} mol dm^{-3}) at pH 3.0 (Kolthoff's buffer, 0.05 mol dm^{-3} sodium succinate–0.05 mol dm^{-3} sodium tetraborate) or pH 10.0 (McIlvaine's buffer, 0.05 mol dm^{-3} sodium carbonate–0.01 mol dm^{-3} sodium hydrogen carbonate) with or without $NaClO_4$ as an additional salt (2.00×10^{-2} mol dm^{-3}). The results are summarized in Table 1. The major component of the complex in the organic layer is $[Ni(H_2L)]^{2+}$ (78 mol%) after contact with the aqueous pH 3.0 solution, and 10.8% of Cbz-His in the aqueous solution was transferred to the organic layer. Since no transfer of Cbz-His was observed in the absence of complex, the hydrophilic Cbz-His was extracted into the organic phase by $[Ni(H_2L)]^{2+}$. The presence of lipophilic but weakly coordinating ClO_4^- increased the molar fraction of the $[Ni(H_2L)]^{2+}$ to

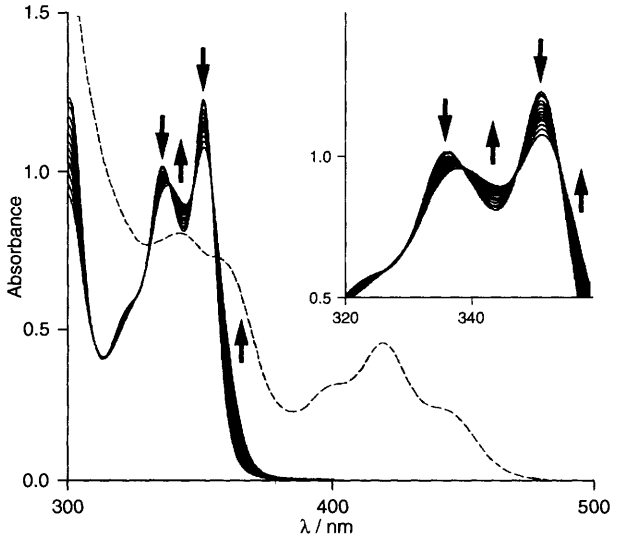
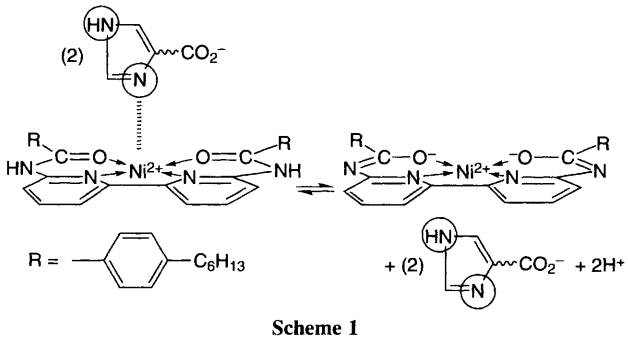


Fig. 1 Spectral change of $[Ni(H_2L)][NO_3]_2$ (5.00×10^{-5} mol dm^{-3}) in CH_2Cl_2 (containing 5% MeOH) upon addition of a CH_2Cl_2 solution of Cbz-His at 20 °C; [Cbz-His] : [complex] molar ratios 0–3.00 : 1. A dotted line indicates the electronic spectra of [NiL] (5.00×10^{-5} mol dm^{-3}) in CH_2Cl_2 (containing 5% MeOH).



Scheme 1

100%, since lipophilic counter anions increase the stability of the non-deprotonated complex in the organic layer. Amounts of the extracted Cbz-His were also increased in the presence of $NaClO_4$, and this synergistic effect might be ascribed to the coextraction of Cbz-His and ClO_4^- into the organic layer. However, the presence of SCN^- (NBu_4 salt) having high coordination ability, in the organic layer greatly reduced the amount of Cbz-His extracted. These results indicated that extraction of Cbz-His is primarily due to the coordination interaction between the imidazole and the metal centre. The complex in the organic layer was exclusively in the fully deprotonated form after contact with the aqueous pH 10.0 solution, and no extraction of Cbz-His was observed. Therefore, it is confirmed that the non-deprotonated complex has a high extraction ability toward Cbz-His but the deprotonated complex has poor extraction ability, and the deprotonation of the amide groups of the complex in the organic layer is controlled by the pH of the aqueous layer.

Table 1 Uptake (%) of Cbz-His (relative to the initial concentration of anion in the buffer solution)[a]

Complex	Additional salt	pH 3.0	pH 10.0
None	$NaClO_4$	0.0	0.0
$[Ni(H_2L)][NO_3]_2$	None	10.8(78)[b]	0.0(0)[b]
	$NaClO_4$	17.0(100)[b]	0.0(0)[b]
	$NaClO_4$[c]	3.0(100)[b]	0.0(0)[b]

[a] Organic phase, CH_2Cl_2 (2 cm^3) containing 2 × 10^{-3} mol dm^{-3} $[Ni(H_2L)][NO_3]_2$; aqueous phase, buffer solution (2 cm^3) containing 2 × 10^{-3} mol dm^{-3} Cbz-His with or without 2 × 10^{-2} mol dm^{-2} of $NaClO_4$. [b] Values in parentheses are the amounts (%) of the non-deprotonated form of the complex in the organic layer after contact with the aqueous solution. [c] The organic solution contained NBu_4SCN {$[NBu_4SCN]$: $[Ni(H_2L)][NO_3]_2$ = 2:1}.

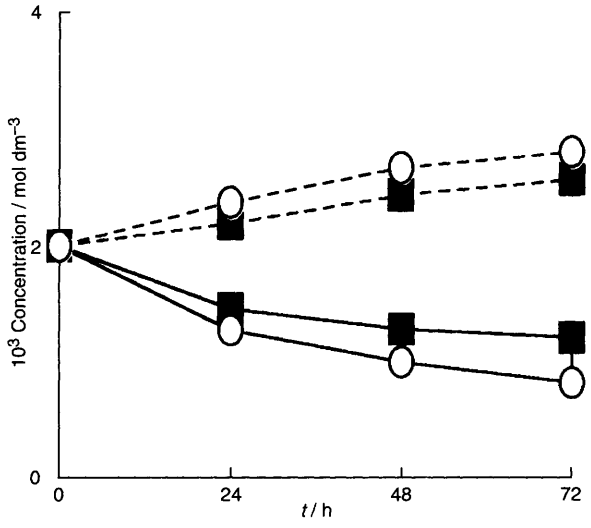


Fig. 2 Time course of the Cbz-His concentration in the aqueous layer I (—) and II (---) during the up-hill transport across the CH_2Cl_2 layer mediated by $[Ni(H_2L)]^{2+}$ (2.0 × 10^{-4} mol dm^{-3}) at 20 °C. Initial concentrations of anions in both aqueous layers were 2 × 10^{-3} mol dm^{-3}. Layer I was at pH 3.0, while the pH of layer II was 6.0 (■) or 10.0 (○).

Based on the above observation, we tested the up-hill transport of Cbz-His at 20 °C. Experimental conditions were essentially the same as those for anion transport, where an organic $[Ni(H_2L)]^{2+}$ (2.0 × 10^{-4} mol dm^{-3}) layer [CH_2Cl_2–MeOH (95/5) 30 cm^3] separated two aqueous buffer solutions I (pH 3.0, 10 cm^3) and II (pH 6.0 or 10.0, 10 cm^3) containing the same concentrations of Cbz-His (2.00 × 10^{-3} mol dm^{-3}) and $NaClO_4$ (2.00 × 10^{-2} mol dm^{-3}). The results shown in Fig. 2 demonstrated that affinity switching of the carrier complex by the pH of external aqueous solutions induced up-hill transport of Cbz-His. No transport of Cbz-His was observed if $[Ni(H_2L)]^{2+}$ was not present in the organic layer or if there was no pH difference between the two aqueous layers. Therefore, it is clear that $[Ni(H_2L)]^{2+}$ is the carrier which induces up-hill transport of Cbz-His by coupling with the pH difference across the membrane. As anticipated from the two-phase experiments, the presence of NaSCN inhibited the transport. Without $NaClO_4$, no practical up-hill transport was observed at all. The role of $NaClO_4$ can best be interpreted as a lipophilic but weak coordinating counter anion that helps extraction of hydrophilic Cbz-His into the organic layer.

In summary, we demonstrated here that the affinity-switching strategy by using the functional complex-type carrier is effective for the up-hill transport of Cbz-His which can coordinate its imidazole unit to the metal. Since many biomolecules have metal coordination sites in their structures, the results presented here open wide applicability of the affinity-switching strategy for the up-hill transport of biologically important molecules. Such studies are now underway in our laboratory.

Footnote

† $[Ni(H_2L)][NO_3]_2$ and [NiL] were synthesized by essentially the same procedure as reported previously,[3] and their structures and purity were confirmed by spectroscopic methods and analyses. Cbz-His was analysed by HPLC.

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