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Polyamino acids and cytochrome C anchored rhodium complexes

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Abstract. Polyamino acids (amino acid = valine, alanine, lysine and arginine) and the protein cytochrome C (Cyt C) have been treated with $[Rh(CO)_2Cl]_2$ (1) to give polymer-supported *cis*-dicarbonyl species. The polymer-supported rhodium complexes, characterised on the basis of infrared and ESCA data, have been found to undergo reversible decarbonylation reaction. The Cyt C-supported rhodium complex acts as a hydrogenation catalyst of low to moderate activity. In the hydrogenation of 3-methyl cyclohexanone no stereoselectivity has been observed.

Keywords. Cytochrome C; polyaminoacids; infrared and ESCA data; hydrogenation catalyst.

1. Introduction

Rhodium complexes anchored on to organic polymers have been extensively investigated as potential catalysts (Bailey and Langer 1981). Here we report the use of selected polyamino acids and Cyt C as support materials for anchoring rhodium *cis*-dicarbonyl species. The ability or otherwise of the pendant functional groups of a polymer, to stabilise partially unsaturated metal centres is an aspect of special interest (Grubbs *et al* 1973; Brintzinger *et al* 1977; Perkins and Vollhardt 1979). One of us earlier reported that (1) supported on polystyrene resin, functionalised with –NHEt– groups, underwent reversible decarbonylation (Bhaduri and Khwaja 1983). This was taken as an indication of the ability of the resin to stabilise unsaturated metal centres by functioning as a chelating ligand. One of the objectives of the work reported here was to find out if the peptide bonds in polyamino acids could act in a similar manner. We also wanted to study the catalytic activities, if any, of the resultant species in catalytic hydrogenation reactions. Since chiral support-materials may provide an environment conducive to asymmetric catalytic hydrogenation reactions, studies with pro-chiral substrates have also been carried out.

2. Results and discussion

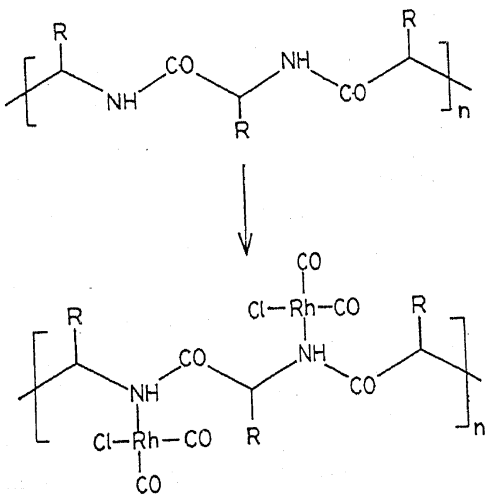
To test the feasibility of interaction of the peptide bond with (1), polyalanine and polyvaline have been treated with (1) in methanol. Repeated washing of the resultant

polymers ensures that they are free of physically adsorbed (1). The infrared spectra in both the cases show two strong bands at 2080 and 2000 cm^{-1}. This indicates the presence of identical rhodium carbonyl species on the polymers in so far as evidence from infrared spectroscopy is concerned. The observed IR frequencies match well with the literature-reported values of similar polymer-supported rhodium *cis* dicarbonyl species (Basset *et al* 1979; Evans and McNulty 1984).

The broad band due to the peptide linkage at 1630 cm^{-1} does not show any observable shift on anchoring of the rhodium species. This may be taken as an indication of interaction through the nitrogen atom of the peptide bond rather than the oxygen atom. Quantitative rhodium and chlorine analyses indicate that the amount of rhodium present is much higher (> 10%) than would be expected had coordination taken place only through the end groups. These results taken together, and in view of the fact that the cleavage of the chloride bridges in (1) by ligands with nitrogen as the donor atom is well established (Lawson and Wilkinson 1965; Bhaduri and Khwaja 1983), interaction and formation of species such as (2) and (3) are proposed for [P-Val] and [P-Ala] respectively (see scheme 1).

Treatment of (1) with the protein Cyt C results in the formation of a similar substance (4). On treatment with (1) the colour of suspended Cyt C in the methanol changes from bright red to dull brown. The organometallic containing protein, (4), is insoluble in all common organic solvents and water. The IR spectrum of (4) in the inorganic carbonyl region is almost identical with that of (2) and (3) (see figure 1). The iron atom of the haem group in Cyt C can be used as an internal standard for quantitative analysis. Energy dispersive analysis by X-ray (EDAX) shows that for one iron atom eighteen rhodium and eighteen chlorine atoms are present. Quantitative rhodium estimation (≈ 15%) by atomic absorption spectroscopy is consistent with these data.

A detailed XPS investigation of rhodium carbonyls on inorganic supports has been reported by Apai *et al* (1987). It has been shown that an assignment of low (0, 1 +) formal oxidation states could be ambiguous in some of these cases. Substantial back-donation from the metal atoms to the carbonyl groups leads to drainage of electron density from the rhodium atoms. Consequently the experimentally measured binding energies of Rh $3d_{5/2}$ levels are higher than would be expected on the basis of formal oxidation states. The XPS spectra of (2)–(4) have been recorded (figure 2) and the



R	Polymer	Polymer-supported species
CH_3	polyalanine	(2)
$CH(CH_3)_2$	polyvaline	(3)
$(CH_2)_4NH_2 \cdot HBr$	polylysine hydrobromide	(5)
$(CH_2)_3NH-C(=NH \cdot HCl)-NH_2$	polyarginine hydrochloride	(6)

Scheme 1.

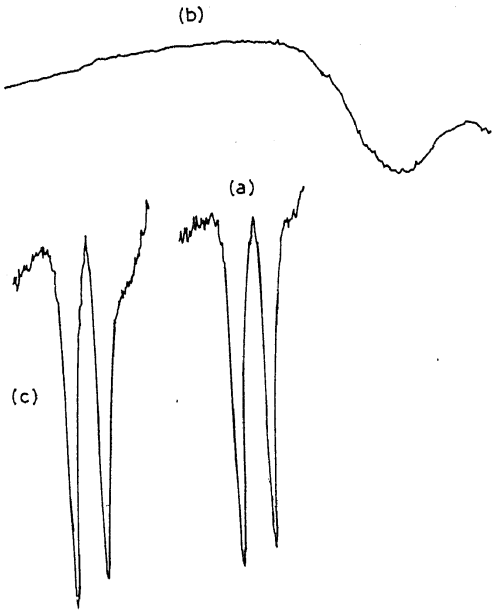


Figure 1. Infrared spectra of (4) as KBr disc. Spectra of (**a**) freshly prepared (4), bands at 2080 and 2000 cm^{-1}, (**b**) (7) i.e. of (4) after using it in catalytic hydrogenation of cyclohexene, and (**c**) (7) recarbonylated by exposing it to CO.

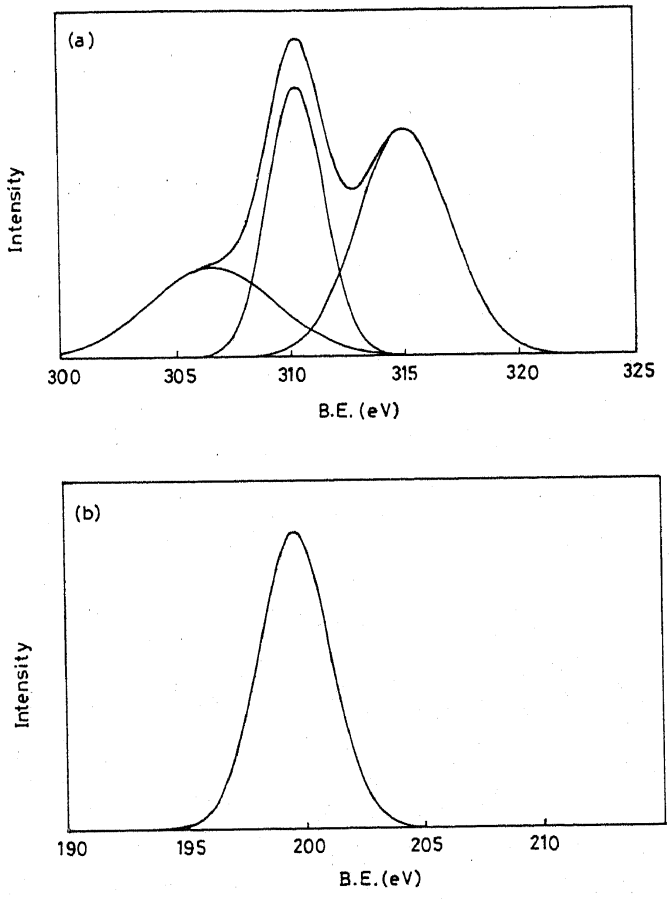


Figure 2. XPS spectra of (4). (**a**) Deconvoluted Rh $3d_{3/2}$ and $3d_{5/2}$. The peak at $\approx$ 306 ev is due to base line problem. (**b**) Deconvoluted $2p$ 'Cl' peak at 199 ev; $2p_{3/2}$ and $2p_{1/2}$ are not resolved due to large line width.

rhodium $3d_{5/2}$ binding energies have been found to be the same within experimental error (FWHM $\approx$ 5 e.v.). The observed binding energy ($\approx$ 310 e.v.) is very close to that of the rhodium geminal dicarbonyl species (310·2 e.v.) as reported by Apai *et al* (1987). The peak at $\approx$ 306 e.v. is considered to be an artifact arising out of skewed base-line rather than signifying the formation of rhodium metal. In the latter case the $3d_{5/2}$ binding energy has been found (Apai *et al* 1987) to be 307·1 e.v. A chlorine 2*p* peak at $\approx$ 199 e.v. ($2p_{3/2}$ and $2p_{1/2}$ could not be resolved due to large line width) is also seen in the XPS spectrum. Similar binding energies for terminal and bridging chlorine atoms have been reported (Apai *et al* 1987). The XPS data are thus consistent with the formulations of (2)–(4) as shown in scheme 1.

Coordination through the side arm is not possible for polyalanine and polyvaline and only the nitrogen atom of the peptide bond interacts with the rhodium centres. However, Cyt C contains other amino acids such as lysine, arginine etc. where the functionalised side arms are certainly capable of similar bonding interactions with the rhodium atoms. Presumably such changes in the coordination environments of rhodium atoms are not reflected in the carbonyl stretching frequencies of the IR or XPS spectra. This is also inferred from the fact that treatments of polylysine hydrobromide and polyarginine hydrochloride with (1) result in the formation of polymer-supported rhodium complexes, (5) and (6), respectively. The IR spectra of (5) and (6) in the inorganic carbonyl regions are identical with that of (2)–(4) for all practical purposes.

Further confirmation of the presence of mononuclear rhodium *cis*-dicarbonyl moieties in (4) comes from the fact that $[Rh(CO)_2Cl]_2$, partially labelled with ^{13}CO, on treatment with Cyt C yields a species whose IR spectrum (figure 3) matches well with the literature reported values of isotopically exchanged rhodium *cis*-dicarbonyl species (Evans and McNulty 1984).

The catalytic activities of species (2) to (6) have been tested for olefin hydrogenation reactions. Initial experiments with cyclohex-2-en-1-one as the substrate established (4) to be the most efficient catalyst. Due to this and the comparatively easy availability of Cyt C as a support material, the ability of (4) to function as a hydrogenation catalyst has been evaluated in some detail. These results are shown in table 1.

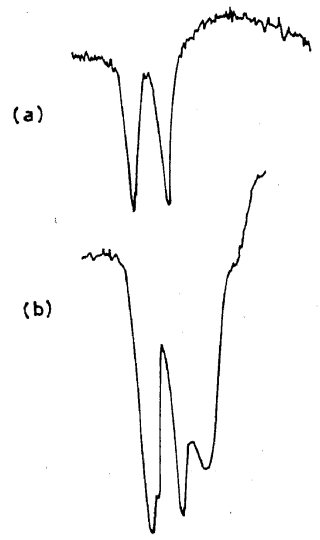
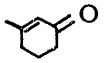
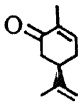
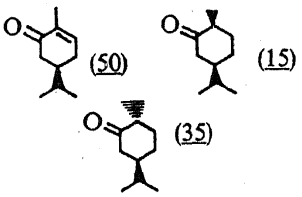


Figure 3. Infrared spectra of (4) as KBr disc before and after isotopic labelling. (**a**) (4) with $\nu(12_{CO})$ at 2080 and 2000 cm^{-1}. (**b**) (4) with $13_{CO}/12_{CO}$ (40:60). New bands at 2060 and 1970 cm^{-1} (Evans and McNulty 1984).

Table 1. Catalysis by 1.

All reactions carried out at 25°C with (4) (3 mg, 4·4 μmol Rh), substrate (4·4 mmol) in methanol (3 ml) unless stated otherwise.

Entry	Substrate	Time(h)	Conversion(%)	Product(s)
(i)		40	100	
(ii)		24	0[a], 50[b], 100[c]	
(iii)		24	100[d]	
(iv)		24	50	
(v)		24	25	
(vi)		24	10[d]	
(vii)		168	100[d]	
(viii)	$PhCH=C(NHAc)CO_2H$	72	10	$PhCH_2CH(NHAc)CO_2H$
(ix)		72	100[d,e]	 (50) (15) (35)

[a]In octane (5 ml); [b]in propan-2-ol (5 ml); [c]with (7) (3 mg, 4·4 μmol Rh) as the catalyst; [d]in EtOAc (5 ml); [e]relative amounts of (2R, 5R) and (2S, 5R) of 5-isopropyl-2-methyl cyclohexanone determined on the basis of ^{1}H nmr and optical rotation measurements.

As can be seen from entries (iii), (iv), (vii) and (ix) the catalytic activity of (4) is limited to the hydrogenation of the olefinic functionality – carbonyl groups are not affected. Experiments under identical conditions with rhodium (5%) on carbon as the catalyst show that in these cases in addition to the hydrogenation of the olefinic double bonds, reductions of the carbonyl group also take place. This may be taken as an indication that under the catalytic conditions generation of finely divided rhodium metal on the polymer-support does *not* occur.

Use of methanol as a solvent in the hydrogenation of cyclohex-2-en-1-one results in the formation of the ketal. Ketal formation is catalysed by (4). This aspect however has not been studied any further and ethyl acetate has been used as the solvent.

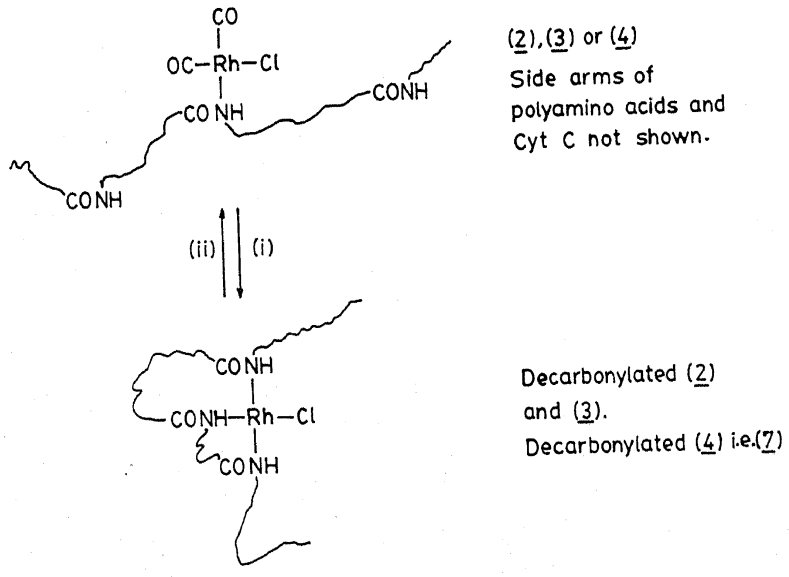
The steric bulks of the substrates seem to have adverse effects on the efficiencies of the catalytic system. This is apparent from the entries for 3-methyl cyclohexenone and cyclohexenone where for the former considerably long reaction time is required for complete conversion. The diffusion barrier that exists between an insoluble polymer matrix and the bulk solvent is known to have an adverse effect on the rate of catalytic hydrogenation and other reactions (Grubbs *et al* 1973; Bailey and Langer 1981; Bhaduri *et al* 1981; Pittman 1982). With bulky substrates such effects are expected to be more pronounced (Grubbs *et al* 1973).

Solvent polarity also has a noticable effect on the catalytic activity of (4) – no hydrogenation of cyclohexene is observed in a non-polar solvent like *n*-octane. It is well known that polymer-supported catalysts do not function efficiently in non-swelling solvents. The amino acid composition of the protein Cyt C, approximately 33% amino acids with polar charged side-chains and 23% with polar non-charged side-chains, suggests that swelling of the biopolymer would take place only in polar solvents. The lack of catalytic activity in *n*-octane is therefore not surprising.

Due to the inherent chirality of the protein-support in (4) the ability, if any, of this catalyst to effect asymmetric hydrogenation reactions has been evaluated. For the two pro-chiral substrates, 3-methyl cyclohexenone and α-acetamidocinnamic acid [entries (vii) and (viii)], the results are disappointing, the observed catalytic efficiencies, as judged by time taken for measurable conversions, are very low and low, respectively. For the former substrate no optical induction is achieved. The reduction of (5R)-carvone is limited mainly to the hydrogenation of the exocyclic double bond – no dihydrocarvone can be seen in the product mixture. Since hydrogenation of the endocyclic double bond is sterically more demanding than the exocyclic one, the diffusional barrier is probably responsible for the observed regioselectivity.

Infrared spectra of (4) at the end of the catalytic runs show the complete disappearance of the carbonyl bands. However, on exposure to carbon monoxide the carbonyl bands reappear without any apparent loss in intensity or shift in the band positions (figure 1). In other words (4) undergoes reversible decarbonylation indicating stabilisation of the decarbonylated rhodium complex rather than formation of finely dispersed rhodium metal. Similar reversible decarbonylation is also observed with species (2) and (3). Reversible decarbonylation with -NHEt group-containing polystyrene resin-supported rhodium complexes have also been reported (Bhaduri and Khwaja 1983).

As shown in scheme 2, it is proposed that on decarbonylation of (4) a species such as



(i) H_2 or N_2, 25°C, CH_3OH or $CH_3CO_2C_2H_5$, 24 h.

(ii) CO, 25°C, CH_3OH or $CH_3CO_2C_2H_5$, 48 h.

Scheme 2.

(7) is formed where chelation by the polymer backbone stabilises the decarbonylated rhodium centres. In these species tetra- rather than tri-coordination is assumed around the metal atoms, in view of the fact that for most rhodium (I) complexes in solution such a coordination environment is encountered. Although not shown explicitly, in (7) coordination by the side arms of the amino acid residues such as aspargine, glutamine etc. are certainly highly probable. In the XPS spectrum of (7) there is a drastic reduction in the rhodium 3*d* peak intensities. This probably indicates that due to chelation by the polymer backbone as shown in scheme 2, the rhodium atoms are deeply buried within the polymer matrix. Since, on treatment with hydrogen, (2) and (3) also undergo reversible decarbonylation, chelation by peptide nitrogen atoms in (7) is assumed.

Decarbonylation occurs not only during catalytic hydrogenation experiments but may also be effected by simply stirring a suspension of (4) with a polar solvent for 24 h under an atmosphere of argon. In this case also the original CO bands are regenerated when the decarbonylated material is left under carbon monoxide. Since treatment with hydrogen is not essential for effecting the loss of carbonyl ligands, the formation of hydridic species on decarbonylation is not proposed.

3. Experimental

3.1 *General techniques and materials*

All reactions and manipulations were carried out under an atmosphere of dry nitrogen or argon unless stated otherwise. Poly-l-alanine, poly-l-valine, poly-l-lysine hydrobromide, poly-l-arginine hydrochloride and horse heart cytochrome C were bought from Sigma Chemicals. The olefinic and α, β-unsaturated carbonyl substrates used for hydrogenation studies were obtained from Aldrich and used without further purification. The rhodium chloro carbonyl dimer, (1), was synthesised according to the literature procedure (McCleverty and Wilkinson 1966). The isotopic labelling of (1) was carried out by stirring it in *n*-hexane under an atmosphere of $^{13}CO/^{12}CO$ (40:60) at 25°C for 48 h.

Infrared and nmr spectra were recorded on a PE781 and Bruker 80 MHz FT instrument. A Shimadzu GC 9A and JASCO DIP 140 polarimeter were used for routine gas-chromatograhic analyses and optical rotation measurements. The XPS spectra were recorded at the Regional Sophisticated Instrumentation Centre (Madras) on a VG scientific instrument. The EDAX analyses were carried out by Dr S Sunshine at Northwestern University. An atomic absorption spectrophotometer, IL 751, was used for rhodium analyses.

3.2 *Synthesis of* (2)–(6)

The reaction between (1) and Cyt C to give (4) was carried out in the following manner. Cyt C (25 mg, 0·002 mmol) was stirred with (1) (10 mg, 0·025 mmol) in methanol (5 ml) for 0·5 h at 25°C. The brown powder was filtered off, repeatedly washed with methanol, and dried under vacuum.

The other polymer-supported species (2), (3), (5) and (6) were prepared in a similar manner. Due to the solubility of (6) in methanol, at the end of the reaction it was precipitated out by the addition of dichloromethane.

3.3 *Catalytic experiments*

These experiments were carried out in an appropriate solvent (10 ml) under an atmosphere of hydrogen (760 mm of Hg) at 25°C over different time intervals. The extent of conversion was measured by gas chromatographic analyses. The recarbonylation of the used catalyst was carried out by stirring a methanolic suspension at 25°C under an atmosphere of carbon monoxide for 48 h.

Acknowledgement

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