Syntheses of Silica Gel Bound Hemin, Biliverdin, and Bilirubin

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3-Aminopropylsilated silica gel bound hemin, biliverdin, and bilirubin were synthesized by reacting 3-aminopropyl silated silica gel with hemin, biliverdin and bilirubin respectively. The aspects of binding of bilirubin to amino group of 3-aminopropylsilated silica gel were studied using the above synthetic silica immobilized hemin, biliverdin and bilirubin, and oxodipyrromethene.

1. Introduction

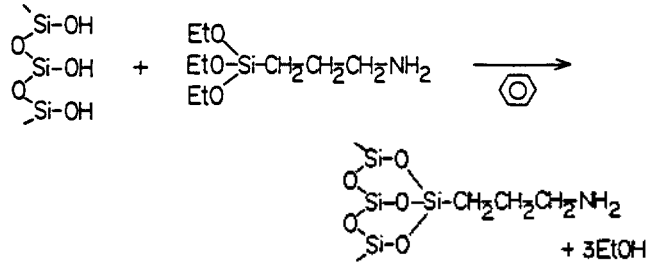
It is known that bilirubin complexed to serum albumin is transported to the liver, where it is rendered more soluble by attachment of sugar residues to its propionate side chains. The conjugates of bilirubin, bilirubin diglucuronide and monoglucuronides are excreted into bile. Knowledge of the exact nature of the binding between bilirubin and albumin is incomplete,[1] although several types have been suggested: covalent bonding,[2,3] hydrophobic and electostatic bonding,[4] and salts linkage[5] and hydrogen bonding.[5,6]

In order to explore aspects of binding to polymer and their surfaces as models for studies of the properties and reactivities of bilirubin, biliverdin, and protoporphyrin in biological systems, we initiated an investigation of 3-aminopropylsilated silica gel bound hemin, biliverdin, and bilirubin.

2. Results and Discussion

Preparation of 3-Aminopropylsilated Silica Gel. Silica-immobilized 3-aminopropylsilated silica gel[7] was prepared by reacting 3-aminopropyltriethoxysilane (Petrarch System, Inc.) with Baker silica gel (40–140 mesh) in benzene. Schematic reaction is written below (see Scheme 1). White solid was extracted with benzene in soxhlet apparatus for 2 hrs and dried for 2 hrs at 100.°C/0.05 mmHg.

Ir spectrum of the solid 3-aminopropylsilated silica gel KBr matrix shows that amino and methylene groups are present. No uv and visible absorption of the solid sample KBr matrix is observed. (a solid KBr disc was made just like an ir disc, see Table 1).



Scheme 1.

Association of Hemin, Biliverdin, and Bilirubin to 3-Aminopropylsilated Silica Gel. As a preminary test for the synthetic work of hemin-biliverdin-, and bilirubin-loaded silica

TABLE 1: IR and UV data of Silica Gel Bound Hemin, Biliverdin, and Bilirubin

Compds	IR	UV
3-Aminopropyl silated silica gel(APS)	3440(SiO_2, NH_2) 2940(CH) 1630(SiO_2) 1100(SiOH) 800	No abs in visible
Hemin	1710(COO) 940(OH)	550(λ_{max})
APS-Hemin	3400(SiO_2) 3060(CH=) 2500(NH_3+ overtone) 2000(NH_3+ overtone) 1650(CO_2) 1210-1020 800	660(sh) 590(sh) 494(sh) 386(λ_{max})
Biliverdin (BV)	3420(NH) 1705(CO_2 1600(CH+)	662(sh) 378(λ_{max})
APS-BV	3440(NH) 2000(NH_3+ overtone) 1700(CO_2) 2080 800	590 350
Bilirubin (BR)	3420(NH) 1700(CO_2) 1650 1615 940	470(λ_{max})
APS-BR	3440(NH) 2500(NH_3+ overtone) 1670(CO_2) 1630	470(sh) 400(λ_{max})

* by using solid KBr matrix

TABLE 2: Association Percentage of Hemin to 3-Aminopropylsilated silica Gel against Time Interval

No	Time(sec)	Abs(at 400 nm)	% Change
1	0	0.76	0
2	160	0.52	32
3	365	0.40	47
4	580	0 33	57
5	790	0.28	63
6	997	0.25	67
7	1210	0.25	67

TABLE 3: Association Percentage of BV to 3-Aminopropylsilated Silica Gel against Time Interval

No	Time(Sec)	Abs(at 380 nm)	% Change
1	0	0.88	0
2	160	0.60	32
3	377	0.47	47
4	590	0.38	57
5	799	0.33	63
6	1012	0.30	66
7	1229	0.28	68

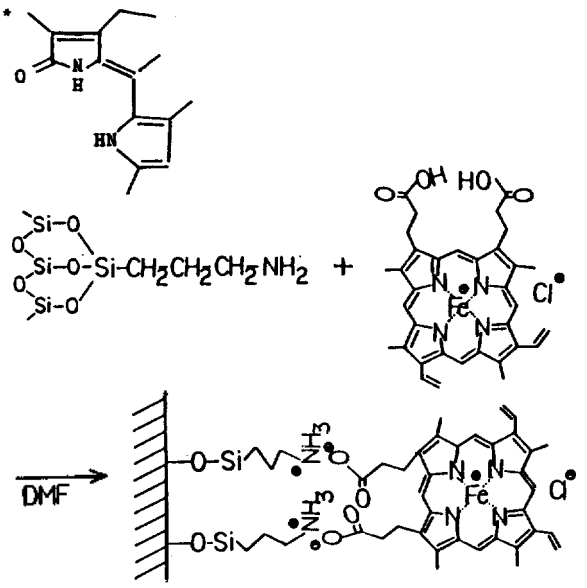
TABLE 4: Association Percentage of BR to 3-Aminopropylsilated Silica Gel against Time Interval

No	Time(sec)	Abs(at 450)	% Change
1	0	0.61	0
2	122	0.47	23
3	330	0.38	38
4	550	0.32	48
5	757	0.27	56
6	971	0.23	62
7	1182	0.19	69
8	2108	0.11	82

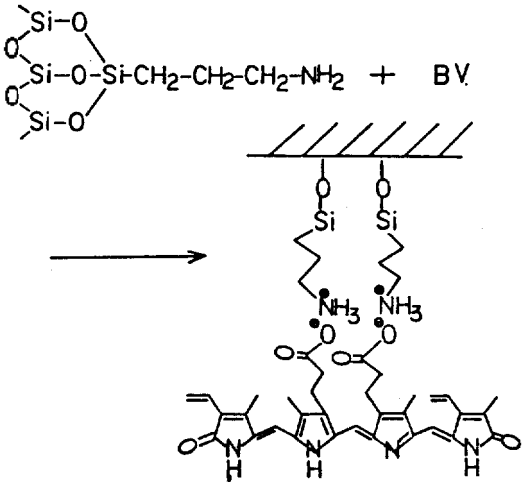
TABLE 5: Association Percentage of Oxodipyrromethene* to 3-Aminopropylsilated Silica Gel against Time Interval

No	Time(Sec)	Abs(at 390 nm)	% Change
1	0	1.01	0
2	153	0.96	4.9
3	373	0.95	5.9
4	590	0.94	6.9
5	800	0.93	8.0
6	1028	0.93	8.0

gel, and reactivity properties of bilirubin, biliverdin, and hemin for 3-aminopropylsilated silica gel, the following experiments were carried out. A solution of appropriate concentration of hemin (Bilirubin or Biliverdin, Abs = 0.9) in MeOH/$CHCl_3$ (1:1, 3 m*l*) [$CHCl_3$ only or $CHCl_3$/MeOH (8:2) for bilirubin, $CHCl_3$ only for dipyrroles] was added to a uv cuvette containing a small magnetic bar. To the solution was added 3-aminopropylsilated silica gel and the mixture was stirred and the concentration of pigment in the supernatant monitored every 30 sec by uv absorbance. The solid took on the color of the solution during the reaction. The results are shown in Tables 2, 3, 4, and 5.



Scheme 2.

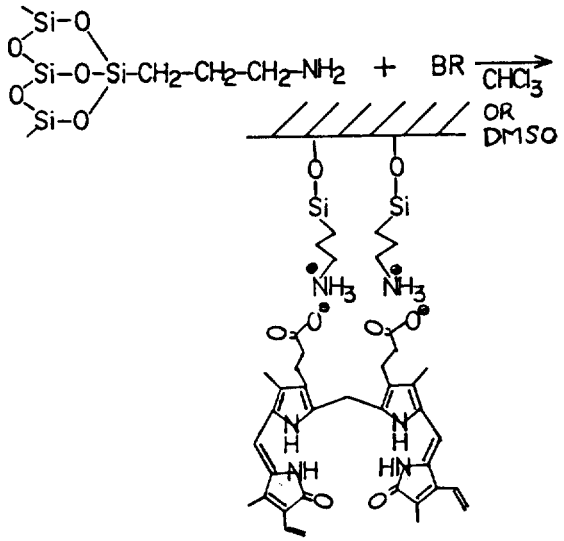


Scheme 3.

These experiments show that hemin, biliverdin, and bilirubin with carboxylic groups can be picked up easily by amino groups of 3-aminopropylsilated silica gel. Reates of the titration of hemin and biliverdin to amino group of 3-aminopropylsilated silica gel are same, comparing the case of 600 sec (57 % change for both). But the rate of bilirubin are a little slow (48 %) for 600 sec). Probably it is due to intramolecular hydrogen bond of bilirubin.

The pyrromethenone without a carboxylic group can not be picked up easily by 3-aminopropylsilated silica gel, although the concentration of the dipyrrloe in the supernatant changed slowly. Probably this is due to hydrogen bonding association, not to the ionic bonding, or to the adsorption of dipyrrole on silica gel itself.

Syntheses of 3-Aminopropylsilated Silica Gel Bound Hemin, Biliverdin, and Bilirubin. 3-Aminopropylsilated silica gel bound hemin (APS-Hemin) was synthesized by reacting 3-aminopropylsilated silica gel with hemin in DMF. After 20 min stirring, the solid was filtered and rinsed (see Scheme 2). The ir and UV spectral data of the product were given in Table 1. 3-Aminopropylsilated silica gel bound biliverdin (APS-BV) was also prepared by same method in $CHCl_3$/MeOH (1:1) (see Scheme 3). 3-Aminopropylsilated silica gel bound bilirubin



Scheme 4.

TABLE 6: Dissociation of Hemin Loaded Silica Gel in DMF

1	0	0	0.24
2	10	0.01	0.43
3	20	0.02	0.49
4	30	0.03	0.55
5	60	0.05	0.70
6	$+N(Et)_3$ 0.05m*l*	0.07	0.56

(APS-BR) was prepared in $CHCl_3$ or DMSO (see Scheme 4). Ir data show that the bonding type is ionic (salt linkage).

Dissociation of Silica Gel Bound Hemin, Biliverdin, and Bilirubin. When hemin loaded silica gel (10 mg) was stirred in DMF (3 m*l*) in uv cuvette by using magnetic bar, hemin was dissociated from the ionic bound hemin silica gel (see Table 6). When a little amount of triethylamine (0.05 m*l*) was added into the reaction cuvette after 60 min mixing, hemin concentration of the supernatant was deceased. Probably the hemin would be destroyed by adding triethylamine. Distortion of the absorption spectra of the supernatant solution after the addition of triethylamine supports that this is.

Dissociation of BV and BR from silica gel bound BV and BR were shown in Table 7 and 8. Addition of triethylamine to the dissociated mixture increases the dissociation of BR and BV, probably because of proton transfer to triethylamine from the ionic salts.

3. Experimental

General. Hemin and bilirubin were purchased from Eastman Organic Chemicals and Matheson respectively. Biliverdin was prepared by the reported method.[8] Oxodipyrromethene was also prepared by known method.[9] Silica gel (40-140 mesh) was purchased from Baker and 3-aminopropyltriethoxysilane was purchased from Petrarch System Inc. Solvents were reagents grade.

Uv spectra were run in solid sample KBr matrix on a Cary 219 spectrophotometer. Solid KBr disc was prepared by mixing solid sample (*ca* 10 mg) and dried KBr (20 mg) and then

TABLE 7: Dissociation of Biliverdin loaded Silica Gel in DMF

No	Time(min)	Abs(at 650 nm)
1	0	0.10
2	40	0.34
3	50	0.44
4	60	0.58
5	$+N(Et)_3$ 0.05 m*l*	0.64

TABLE 8: Dissociation of Bilirubin from Bilirubin Loaded Silica Gel in DMF

No	Time(min)	Abs(at 450 nm)
1	0	0.15
2	10	0.46
3	20	0.63
4	30	0.73
5	$+N(Et)_3$ 0.05m*l*	>2.0

pressing just like ir KBr disc. IR spectra were run is KBr disc on a Perkin-Elmer 599 spectrometer.

Preparation of 3-Aminopropysilated Silica Gel. To 5.100g of silica gel (40-140 mesh) was added 30 m*l* of a 10 % benzene solution of 3-aminopropyltriethoxysilane. The slurry was stirred for 30 min. The slurry was filtered with a soxhlet apparatus for 2 hrs, and dried for 2 hrs at 100 °C/0.05 mmHg using a drying pistol. White solid (5.6623g, 551.4 mg was loaded) was obtained.

Synthesis of 3-Aminopropylsilated Silica Gel Bound Hemin. To 3-aminopropylsilated silica gel (250 mg, 0.2 mmole of NH_2 group) in 2 m*l* of DMF, was added dropwise a solution of hemin in DMF (hemin 163 mg, DMF 3 m*l*). The mixture was stirred for 20 min and then filtered through a sintered funnel. Rinsed with DMF twice. Dried at 100 °C/0.05 mmHg for 2 hrs. Product (black) (0.2685 g, 18mg loaded) was obtained.

Synthesis of 3-Aminopropylsilated Silica Gel Bound Biliverdin. To 3-aminopropylsilated silica gel (200 mg, 0.16 mmole of NH_2 goup) in 5 m*l* of $CHCl_3$/MeOH (1:1) was added dropwise a solution of biliverdin (48 mg, 0.16 mmole) in 10 m*l* of $CHCl_3$/MeOH(1:1). The mixture was stirred for 10 min. The solid took on the blue color of the solution. The mixture was centrifuged and washed with $CHCl_3$/MeOH(1:1) twice. The blue black solid was dried (210 mg).

Synthesis of 3-Aminoprpylsilated Silica Gel Bound Bilirubin. To a slurry of 3-aminopropylsilated silica gel (269 mg) in DMSO (2 m*l*) was added a solution of bilirubin (143 mg) in DMSO (15 m*l*). The mixture was stirred for 20 min and then centrifuged. The solid was washed twice with DMSO and dried at 100 °C/0.05 mmHg for 4 hrs. An orange solid (260 mg) was obtained. The same results were obtained when chloroform was used for the solvent.

References

(1) R. Brodersen in "Bilirubin," K. P. M. Heirwegh and Brown, Eds., CRC Press, Boca Raton, FL, (1982).

(2) C. C. Kuenzle, N. Gitzelmann-Cumarasamy and K. J. Wilson,

J. Biol. Chem., **251**, 801 (1976).
(3) T-W. Wu, *Israel J. Chem.*, **23**, 241 (1983).
(4) C. Jacobsen, *Eur. J. Biochem.*, **27**, 513 (1972).
(5) J. Jacobsen, *Int. J. Peptide Protein Res.*, **9**, 235 (1977).
(6) J. M. Van der Eijk, R. J. M. Nolte, V. E. M. Richterw, and W. Dren th, *Biopolymers*, **19**, 445 (1980).
(7) T. G. Waddell, D. E. Leyden, and M. T. Debello, *J. Amer. Chem. Soc.*, **103**, 5303 (1981).
(8) A. F. McDonagh and L. A. Palma, *Biochem. J.*, **189**, 193 (1980).
(9) D. A. Lightner adn Y-T. Park, *Tetrahedron* **35**, 463 (1979).

The Oxidation of Hydrazobenzene by Oxygen Catalysed by Co(3MeOsalen) in Methanol

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The oxidation of hydrazobenzene by oxygen in methanol solution is catalysed by Co(3MeOsalen) which is a synthetic oxygen carrier. The products are *trans*-azobenzene and water. The rate of the reaction has been studied spectrophotometrically and the rate law established. A mechanism involving a ternary complex of catalyst, hydrazobenzene and oxygen has been proposed.

1. Introduction

It has been known since the observations of Pfeiffer and his co-workers in 1933[1] that cobalt(II) Schiff's base complexes *e.g.* Co(salen), Figure 1, form reversible complexes with oxygen. There has been considerable recent interest in these compounds because of their relationship to the natural iron-containing oxygen carriers hemoglobin and myoglobin.[2] Hemoglobin and myoglobin consist of an iron-porphyrin complex, the haem group, embedded in the protein which provides one axial ligand, an imidazole group, to the iron. On oxygenation the sixth coordination site of the iron accepts the dioxygen ligand. Thus the ligand field about the iron in oxyhemoglobin is approximately octahedral.[3]

Cobalt(II) Schiff's base complexes are square planar with a low spin d^7 electron configuration. The binding of an axial, fifth ligand leads to a ground state with the unpaired electron in the d_z^2 orbital. This electron configuration is a necessary prerequisite for the binding of dioxygen.[4] Drago and Corden[5], in their spin-pairing model for the binding of dioxygen, presented evidence

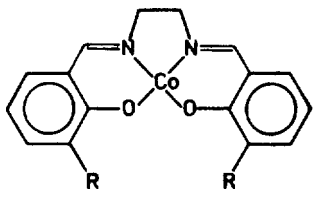

Figure 1. Structure of Schiff-base complexes. R = H, Co(salen); $R = CH_3O$, Co(3MeOsalen).

that the odd electron in the oxygenated complex is in the dioxygen π^* orbital, but that the extent of electron transfer from cobalt to oxygen is dependent on the nature of the equatorial and axial ligands. In the extreme case of complete electron transfer the complex could be formulated as cobalt(III)-superoxide. The change in the electronic structure of oxygen from the relatively unreactive triplet ground state to a doublet coordinated species, with a weakened 0-0 bond bearing fractional negative charge, could be expected to enhance the reactivity of oxygen in radical or nucleophilic reactions.

Cobalt(II) Schiff's base complexes with added axial ligands have been shown to catalyse the oxidation by oxygen of secondary alcohols[6] to ketones, and of phenols[7] to quinones. The structurally related bis(dimethylglyoximato) cobalt(II) has been reported to catalyse the oxidation of hydrazobenzene to azobenzene in the presence of triphenylphosphine.[8] Kinetic studies[6,7] have shown that in the oxidation of alcohols and phenols the transition state is a ternary complex of dioxygen, cobalt catalyst (including axial base) and substrate. Thus the reactions resemble an enzyme-catalysed process in which the two substrates, dioxygen and the organic molecule, are brought together by the catalyst. In this paper we show that the oxidation of hydrazobenzene catalysed by Co(3MeOsalen), Figure 1, follows a similar kinetic scheme, providing evidence of saturation of the catalyst by the substrate as an enzyme catalysed reaction.

Autoxidation of hydrazobenzene was first described in 1901 by Manchot and Herzog.[9] This, and later work[10,11] has shown