Journal of Chromatography, 196 (1980) 379–389
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,869

MODEL STUDIES OF THE REACTIONS AND NON-SPECIFIC ADSORPTIVE PROPERTIES OF CYANOGEN BROMIDE-ACTIVATED MACROPOROUS AGAROSE

J. F. KENNEDY* and J. A. BARNES
Department of Chemistry, University of Birmingham, Birmingham B15 2TT (Great Britain)
and
J. B. MATTHEWS
Department of Oral Pathology, University of Birmingham, Birmingham B15 2TJ (Great Britain)
(Received February 18th, 1980)

SUMMARY

Dns derivatization of the acid-hydrolysed and unhydrolysed amine derivatives arising from the reaction of cyanogen bromide-activated Sepharose® and N-α-acetyl L-ornithine reveal the production of arginine, confirming that the amine–Sepharose reaction product is an O-substituted isourea. Thin-layer chromatography of the Dns derivatized unhydrolysed filtrate from the reaction indicated the formation of a number of derivatives; this suggested that the isourea bonds can be cleaved by amine nucleophiles in the pH range 8–9.5. The cationic groups, introduced into the cyanogen bromide-activated Sepharose concomitantly with the coupling of amine ligands, were explored by assessing the adsorption of amino acids and peptides onto a cyanogen bromide-activated Sepharose 4B–sheep anti-human immunoglobulin G immunoadsorbent. Solutions of mixed amino acids and mixed peptides were loaded serially onto columns of the immunoadsorbent which were then subjected to a number of successive adsorption–desorption cycles. The adsorption pattern of both the amino acids and the peptides suggested that both ionic and non-ionic interactions are involved in the adsorption process. Such results aid explanation of the undesirable, non-specific adsorption phenomena which, although occurring to a significant extent in immunoadsorption, are frequently overlooked.

INTRODUCTION

The cyanogen bromide activated form of agarose is at present the most favoured and widely used water-insoluble matrix in affinity chromatography. However, the precise total structures of CNBr-activated agarose and the endogenous functional groups are unknown, hence the molecular interactions of compounds containing free amino groups and water-insoluble CNBr-activated polysaccharides are poorly understood. Axèn *et al.*[1] gave a hypothetical interpretation of the chemistry

0021-9673/80/0000–0000/$02.25

of the activation process and the coupling reaction (Fig. 1), in the case of Sephadex®, a cross-linked dextran[1]. Activation of Sephadex with CNBr resulted in the formation of reactive cyclic imidocarbonate groups and neutral carbamate groups (Fig. 1, I and II, respectively).

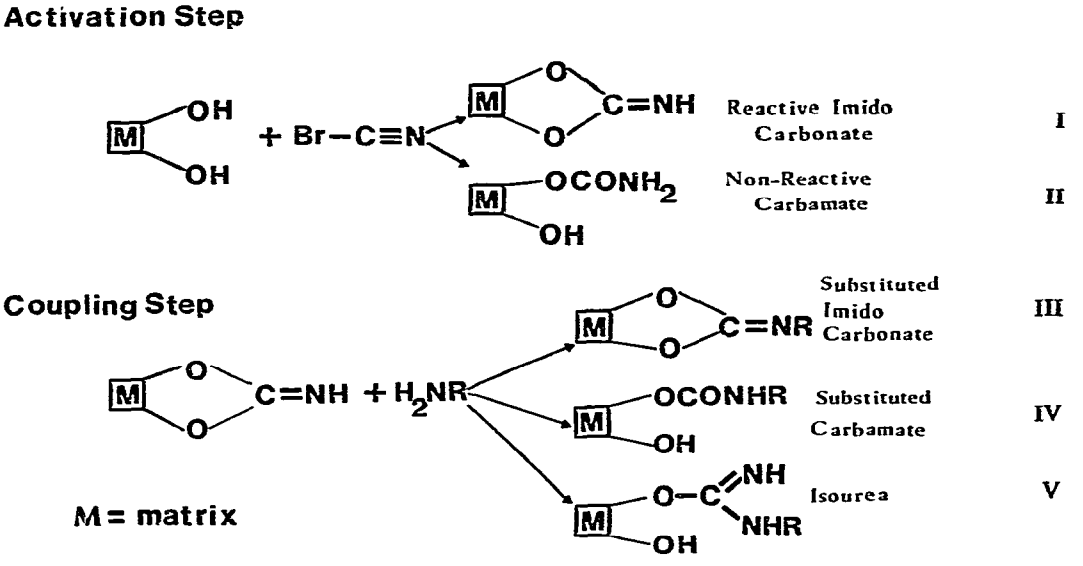


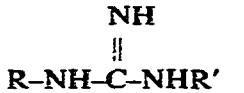
Fig. 1. Proposed mechanism for the CNBr-activation of polysaccharides and the coupling of amines to the cyclic imidocarbonate.

Bartling *et al.*[2] deduced a similar mechanism for the activation of cellulose with CNBr from infrared spectroscopy of the activated matrices and the observed liberation of ammonia in the coupling reaction. Ahrgren *et al.*[3] showed that the reaction of cyanogen bromide with methyl 4,6-O-benzylidene-α-D-glucopyranoside yielded the *trans*-2,3-cyclic carbonate and the 2- and 3-carbamate. It has also been postulated that the activation of dextran or Sephadex with CNBr probably generates the 2,3- or 3,4-linked cyclic imidocarbonates[4,5], *e.g.* Fig. 1 (I). The CNBr activation of D-galactans such as agarose which contain no vicinal hydroxy groups was assumed to proceed through the 4,6-cyclic and interchain imidocarbonates[1,4]. Axèn and Ernback[6] also postulated three derivatives arising from the coupling reaction between amino groups and the activated polysaccharide (Fig. 1): N-substituted imidocarbonate (III), N-substituted carbamate (IV) and N-substituted isourea (V). The same structures were believed to arise when other hydroxylic polymers were used.

Recent studies[2,3,7,8] support the view that the isourea derivative (Fig. 1, V) is the main product arising from the coupling reaction between the amino groups and CNBr-activated carbohydrate. Sevenson[7], using isoelectric focusing, demonstrated that the changes in net charge resulting in the modification of subtilisin coupled to CNBr-activated starch oligosaccharides was consistent with the formation of N-substituted isourea (Fig. 1, V). Wilchek *et al.*[8] investigated the nature of the complex between CNBr-activated Sepharose and amines and demonstrated that the amine–

Sepharose is also an O-Sepharose-N-substituted isourea. Further supporting evidence was supplied by Nishikawa and Bailon[9] who compared the titrimetric analysis of agarose gels with their elemental nitrogen analysis and found that more nitrogen was obtained in the gel derivative than could be accounted for by titratable functional groups. The high pK_a value associated with the isourea linkage was also observed by the latter workers and confirmed by Wilchek[10].

The coupling of alkyl or arylamines to CNBr-activated agarose is accompanied by the concomitant introduction of cationic groups into the activated gel resulting in strong ion exchangers with a pK_a of about 10 for a basic amidine nitrogen[11]. This gives rise to significant non-specific adsorption of proteins. Furthermore, there are many reports in the literature of the ligand–matrix bond instability. Cuatrecasas and Parikh[12] demonstrated the instability of the product derived from coupling L-alanine to the CNBr-activated agarose. Hofstee[13,14] and Junowicz and Charm[15] have also reported the instability of the linkage between alkyl amines and CNBr-activated agarose. Model studies with the 4-nitroanilide and 6-aminohexanoic acid coupled to CNBr-activated Sepharose showed that either the ligand itself, or guanidine, or urea derivatives were released depending upon the pH and composition of the eluent[16,17]. Wilchek *et al.*[8] found that amines coupled to CNBr-activated Sepharose were released, by amine-containing buffers, as N_1, N_2 disubstituted guanidines:



Since it is mechanistically possible for certain eluents to solubilise the covalently coupled ligand, this study was undertaken to assess the effect of ammonia as a potential eluent in immunoadsorption chromatography. It was also our purpose to investigate alternative methods for characterizing the complex between CNBr-activated Sepharose and amines using Dns derivatization techniques. Owing to the complex nature of the reaction of CNBr-activated agarose and amine ligands some of the functional groups generated serve as sites for non-specific adsorption. In this paper we therefore also describe the adsorption of amino acids and di- and tripeptides to a CNBr-activated Sepharose 4B–sheep anti-human immunoglobulin G (IgG) immunoadsorbent column. The results provide some insights into the nature of non-specific adsorption effects in immunoadsorption chromatography.

MATERIALS AND METHODS

N-α-Acetyl L-ornithine, N-α-acetyl L-arginine and citrulline were purchased from Aldrich (Milwaukee, WI, U.S.A.). N-α-Acetyl citrulline was prepared by treating citrulline dissolved in 0.1 *M* sodium bicarbonate with acetic anhydride[8]. Paper chromatography (PC) used for the identification of guanidine and guanidine derivatives was performed in three different solvent systems[8]: (a), 1-butanol–acetic acid–water (4:1:5); (b), 1-propanol–0.5 *M* ammonium hydroxide (7:3); (c), 1-butanol-pyridine-acetic acid–water (15:10:3:12). Dns-chloride and polyamide sheets for thin-layer chromatography (TLC) were purchased from BDH (Poole, Great Britain). The developing buffers used for the polyamide sheets were all of AnalaR grade: (1), 1.5% formic

acid; (2), toluene–acetic acid (9:1); (3) ethyl acetate–methanol–acetic acid (20:1:1). CNBr-Sepharose 4B was purchased from Pharmacia (Uppsala, Sweden). The amino acid standard solution 2.5 μmole/ml was purchased from Pierce (Rockford, IL, U.S.A.). The peptides were bought from Sigma (St. Louis, MO, U.S.A.) and the materials used for preparing the buffers and salt solutions were of reagent grade. Chromatography columns GA 10 × 15 (15 × 1.0 cm) were obtained from Wright Scientific (Kenley, Great Britain). The Dns derivatization of the amino acid standards and the hydrolysed and unhydrolysed derivatives were performed by the method of Hartley[18].

Assessment of ammonia treated cyanogen bromide-activated Sepharose

Scheme 1. Ammonia–Sepharose was prepared by reacting CNBr-activated Sepharose 4B (0.5 g dry gel, swollen and washed with 10^{-3} *M* hydrochloric acid and equilibrated with 0.9% sodium chloride) with 0.5 *M* ammonium hydroxide adjusted to pH 9.5 with hydrochloric acid (5 ml) for 24 h at room temperature. Aliquots (1 ml) were removed after 3 and 24 h and filtered and the filtrates were concentrated by rotary evaporation under vacuum then analysed by PC in three solvent systems, and, after Dns-derivatization, by TLC on polyamide sheets (5 × 5 cm) in three solvent systems.

Scheme 2. To ammonia–Sepharose equilibrated with and suspended in 0.1 *M* sodium bicarbonate buffer pH 8.3 (2.0 ml) N-α-acetyl L-ornithine (287.5 μmoles) dissolved in the same sodium bicarbonate buffer (5.0 ml) was added. The mixture was tumbled for 24 h at room temperature. Aliquots (1 ml) of the filtrate were removed after 15 h and 24 h incubation and their pH values measured, and the aliquots were then concentrated and hydrolysed and portions were Dns-derivatized. After hydrolysis of the filtrates with 6 *M* hydrochloric acid for 24 h, the Dns derivatized hydrolysed samples were analysed by two-dimensional TLC on polyamide sheets and amino acid analyses were performed on the acid-hydrolysed and the unhydrolysed concentrated filtrates.

Ammonia hydrolysis of the CNBr-Sepharose–alkyl amine conjugates

Scheme 3. To 6 ml swollen CNBr-activated Sepharose 4B, equilibrated with 0.1 *M* sodium bicarbonate buffer pH 8.3, N-α-acetyl L-ornithine (60 μmoles) dissolved in sodium bicarbonate buffer (5 ml) was added and the mixture tumbled for 12 h. The gel was then filtered and washed with sodium bicarbonate buffer. After further filtering, the gel was sucked dry and 0.5 *M* ammonium hydroxide pH 9.5 (5.0 ml) was added to the gel. The mixture was tumbled for 24 h at room temperature, then filtered and aliquots (1 ml) of the filtrate were concentrated, hydrolysed with 6 *M* hydrochloric acid for 24 h and then Dns derivatized. Further aliquots of the filtrate (unhydrolysed) were concentrated and analysed by PC and by TLC on polyamide sheets. Amino acid analysis was conducted on the concentrated hydrolysed filtrate.

Immunoadsorbent column preparation

CNBr-activated Sepharose 4B was swollen in and washed with 10^{-3} *M* hydrochloric acid to remove dextran and lactose stabilizers, filtered and equilibrated in 0.2 *M* sodium citrate buffer pH 6.5. The immunoglobulin G (IgG), pre-dialysed against the 0.2 *M* citrate buffer, was added to a suitable amount of gel (1 g dry weight)

suspended in citrate buffer (6 ml, approximately twice the volume of swollen gel) and tumbled for 2 h at room temperature. After filtering, the gel was washed twice with citrate buffer and then lightly stirred magnetically in 1 *M* ethanolamine pH 9.5 (approximately twice the volume of the gel) for 2 h at room temperature to block residual un-used active groups. After filtering, the gel was washed with 0.15 *M* phosphate buffered saline pH 7.2.

After degassing, the gel (1 g dry gel, approximately 3 ml swollen per column) was packed in the column and the flow-rate of the phosphate-buffered saline ambient buffer was adjusted to 20 ml/h. The 3 ml immunoadsorbent columns consisted of 2.61 mg and 9.9 mg sheep anti-human IgG per ml gel for the amino acid and peptide adsorption test columns respectively. All the washings from the gel and the column effluents were retained and monitored spectrophotometrically.

Before loading each column with mixed amino acids or mixed peptides, the columns were washed with 0.5 *M* ammonia then equilibrated with 0.15 *M* phosphate buffered saline pH 7.2. Each column was loaded serially with 250 μl (625 nmole) amino acid standard solution and 0.5 ml 40 μg/ml (90–123 nmole) mixed peptide solutions respectively. The columns were then washed with phosphate buffered saline until the absorbance of the amino acid eluents at 280 nm was negligible (<0.005) and the absorbance of the peptide wash at 320 nm was also small (<0.005). After loading, the columns were eluted with 0.5 *M* ammonia. The ammonia eluents from the amino acid column were monitored at 280 nm while the eluents from the peptide column were monitored at 220 nm. The pooled ammonia eluents from each adsorption–desorption cycle were quantitated on a Locarte Automatic amino acid analyser of the University of Birmingham Macromolecular Analysis Service.

RESULTS

In order to determine the derivatives arising from the reaction between CNBr-activated Sepharose and amines it was useful to employ simple amines which yield characteristic products. The production of an isourea derivative when either ammonia or N-α-acetyl ornithine was employed as the ligand confirms that ammonia–Sepharose is an O-substituted isourea. The adsorption and elution behaviour of peptides and amino acids on CNBr-activated Sepharose helped to characterise the non-specific interactions in terms of ionic or hydrophobic properties.

PC of the underivatized filtrate, from the reaction of ammonium hydroxide on ammonia–Sepharose (*Scheme 1*), in three solvent systems revealed a spot which migrated with the same R_F value as guanidine, and gave the same colour when sprayed with the modified Sakaguchi reagent[19]. Two-dimensional TLC of the Dns derivatives of the concentrated filtrates revealed a spot on polyamide sheets which migrated like guanidine. Ammonia was the only product revealed by amino acid analysis of the filtrate. A guanidine product would suggest that the ammonia–Sepharose conjugate is an O-substituted isourea.

TLC of the acid-hydrolysed Dns derivatized filtrate from the reaction of N-α-acetyl L-ornithine with ammonia treated CNBr-activated Sepharose (*Scheme 2*) revealed spots which migrated like arginine and L-ornithine whereas TLC of the non-hydrolysed filtrates revealed only N-α-acetyl L-ornithine. Amino acid analysis of the non-hydrolysed filtrate from the 15 h reaction revealed 176.5 μmole of N-α-acetyl

TABLE I

YIELDS OF PRODUCTS FROM THE REACTION OF AMMONIA WITH N-α-ACETYL ORNITHINE-TREATED CNBr-ACTIVATED SEPHAROSE AND OF N-α-ACETYL ORNITHINE WITH AMMONIA-TREATED CNBr-ACTIVATED SEPHAROSE

Experiment scheme	*Filtrate incubation time (h)*	*Filtrate pH*	*Sample type**	*N-α-Acetyl-ornithine added (μmole)*	*Arginine (μmole)*	*N-α-Acetyl-ornithine (μmole)*	*Ornithine (μmole)*	*Lysine like? (μmole)*
1	24	9.5	NH	0.0	0.00	0.0	0.0	0.0
2	15	8.8	NH	287.5	0.00	176.5	0.0	0.0
2	24	9.0	NH	287.5	0.00	70.2	0.0	0.0
2	15	8.8	H	287.5	0.25	0.0	218.0	7.5
2	24	9.0	H	287.5	0.08	0.0	73.0	2.6
3	24	9.1	H	60	0.15	0.0	3.0	0.0
3	24	9.1	H (filtrate with few gel beads)	60	1.00	0.0	5.5	0.0

* NH=non-hydrolysed filtrate; H = acid hydrolysed filtrate.

L-ornithine whereas the hydrolysed filtrate from the 15 h reaction showed 218 μmole of L-ornithine and 0.25 μmole of arginine (Table I). There was also a lysine-like peak equivalent to 7.5 and 2.6 μmole derived from the 15 h and 24 h hydrolysed filtrates respectively. The absence of any detectable arginine, in the starting material and in the acid hydrolysate of N-α-acetyl L-ornithine, would suggest an O-substituted isourea linkage of ammonia–Sepharose. Nevertheless, the presence of the lysine-like peak would also suggest a complex hydrolysis mechanism.

PC of the concentrated unhydrolysed filtrate from the reaction of ammonia with N-α-acetyl L-ornithine–CNBr-activated Sepharose conjubate (*Scheme 3*) revealed a spot which migrated with a R_F value the same as arginine and which gave the same pink colour when sprayed with the modified Sakaguchi reagent. Amino acid analysis of the acid hydrolysed 24 h filtrate indicated 0.15 μmole of arginine and 3.0 μmole of L-ornithine. When the hydrolysed filtrate contained a few beads of derivatized Sepharose, the yield of arginine was 1.0 μmole. TLC of the acid hydrolysed, Dns derivatized filtrate also revealed spots which migrated with arginine and ornithine. However, TLC of the unhydrolysed filtrate showed a number of spots as indicated in Fig. 2.

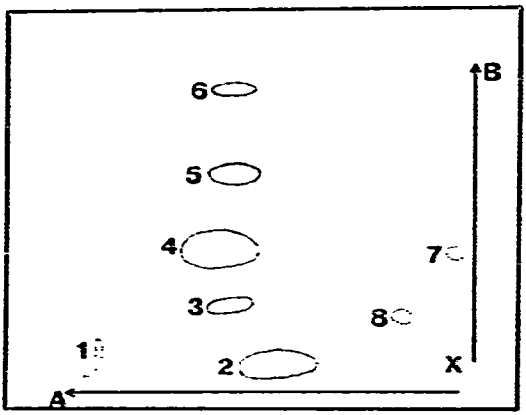


Fig. 2. Tracing of chromatogram of the unhydrolysed filtrate from the reaction of ammonia with N-α-acetyl ornithine–CNBr-activated Sepharose conjugate. X = the origin of the sample (non-hydrolysed filtrate from *Scheme 3*); A = development in the first dimension in buffer 1; B = development in the second dimension in buffer 3; 1 = Dns-arginine; 2 = Dns-OH; 3 = unresolved; 4 = Dns-N-α-acetyl ornithine; 5 = Dns-N-α-acetyl citrulline; 6 = Dns-amine; 7 = Dns-ornithine; 8 = unidentified.

Spot 1 migrated similarly to Dns-arginine. Spot 2 compared to Dns-OH while spot 3 was unidentified. Spot 4 occupied the same position as a Dns-N-α-acetyl L-ornithine standard while spot 5 compared to Dns-N-α-acetyl citrulline and spot 6 to Dns-amine. Spot 7 had the same migration position as a Dns-L-ornithine standard but spot 8 was unidentified.

The adsorption pattern of amino acids and peptides on CNBr-activated Sepharose–sheep anti-human IgC immunoadsorbent columns is illustrated in Tables II and III respectively. Loading the columns with amino acids and peptide showed that the adsorption profile is much more distinct for amino acids (Table II) than for the peptides employed (Table III).

TABLE II

ADSORPTION OF AMINO ACIDS BY SEPHAROSE-IMMOBILISED SHEEP ANTI-HUMAN IgG

Amino acid applied	*Percent of applied amino acid adsorbed per cycle*		
	Cycle 1	*Cycle 2*	*Cycle 3*
L-Aspartic acid	55.6	0.0	0.0
L-Threonine	6.8	4.6	2.1
L-Serine	71.6	0.0	0.0
L-Glutamic acid	42.0	0.0	0.0
L-Proline	58.0	0.0	0.0
Glycine	8.8	1.9	0.0
L-Cystine	9.6	5.9	2.6
L-Valine	5.2	0.0	0.0
L-Methionine	5.2	1.0	0.0
L-Isoleucine	5.6	0.0	0.0
L-Leucine	9.6	0.0	0.0
L-Tyrosine	3.6	0.0	0.0
L-Phenylalanine	10.0	2.4	0.0
L-Histidine	9.0	1.4	0.0
L-Lysine	10.4	2.8	Not resolved
L-Arginine	20.8	7.3	Not resolved

TABLE III

ADSORPTION OF PEPTIDES BY SEPHAROSE-IMMOBILIZED SHEEP ANTI-HUMAN IgG

Peptide applied	*Percent of applied peptide adsorbed per cycle*					
	Cycle 1	*Cycle 2*	*Cycle 3*	*Cycle 4*	*Cycle 5*	*Cycle 6*
Glycyl–L-aspartic acid	0.0	0.0	0.0	0.0	0.0	0.0
N-Glycyl–L-serine	39.0	20.0	18.3	0.0	0.0	0.0
Glycyl–glycyl–glycine	0.0	0.0	0.0	0.0	0.0	0.0
N-Glycyl–L-phenylalanine	10.0	3.0	0.0	0.0	0.0	0.0
L-Phenylalanine–glycine	6.0	0.0	0.0	0.0	0.0	0.0

DISCUSSION

The detection of a guanidine product, by TLC and PC, in the non-hydrolysed filtrate obtained from the reaction of ammonium hydroxide on ammonia treated CNBr-activated Sepharose, and the detection of arginine by TLC and amino acid analysis of the acid-hydrolysed, Dns derivatized filtrate from the reaction of N-α-acetyl L-ornithine with ammonia–Sepharose conjugate, confirm that the ammonia–Sepharose conjugate is an O-substituted isourea. TLC of the unhydrolysed filtrate from the reaction of 0.5 *M* ammonia, with the CNBr-activated Sepharose–N-α-acetyl L-ornithine conjugate revealed a number of hydrolysis products.

The results illustrated in Figs. 2 and 3 indicate the possible release of arginine (V), ornithine (VI), N-α-acetyl ornithine (VII), N-α-acetyl citrulline (VIII) and citrulline (IX) from N-α-acetyl ornithine–Sepharose (IV). The appearance of these derivatives confirm that the coupling of the amine ligands proceed via the cyanate ester intermediate (II), formed by the reaction of cyanogen bromide on Sepharose (I),

which is more reactive than the imidocarbonate derivative (Fig. 1, I). The weakness of the arginine spot (Fig. 2) would suggest that cleavage of the O–C bond of O-substituted amine is perhaps less favoured under these conditions whereas the C–NH bond is more labile. These results support the findings of Wilchek *et al.*[8], Tesser *et al.*[17] and Oka and Topper[20] who reported the release of substituted guanidines by ammonia from conjugates formed between ligands of the RNH_2 type and CNBr-activated Sepharose (Fig. 3, III).

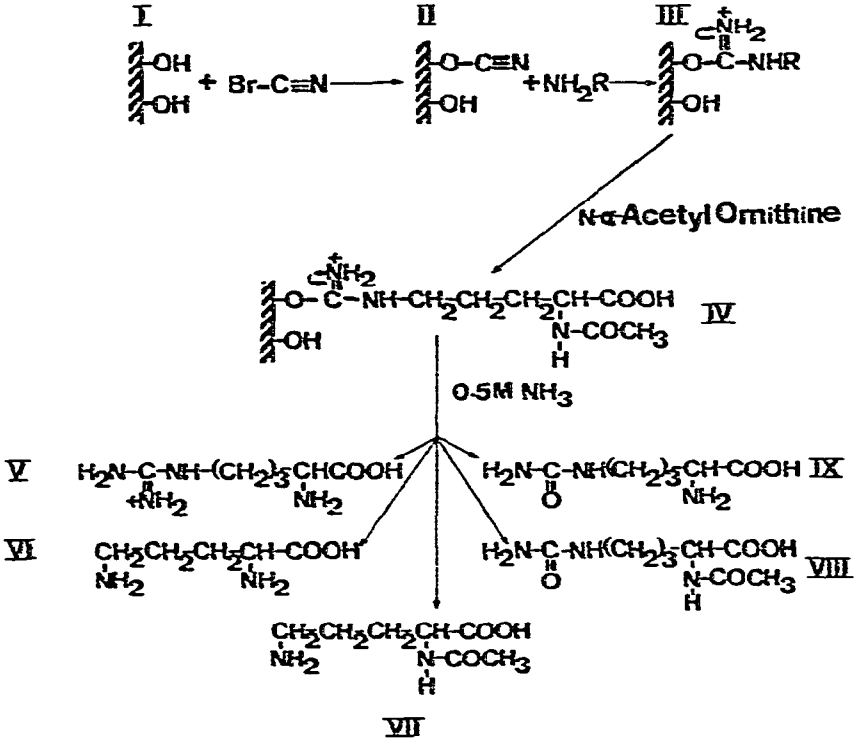


Fig. 3. Reaction sequences for the CNBr-activation of Sepharose and the coupling of the isocyanate product with amines. Nucleophilic attack by amino groups on the conjugates releases N_1,N_2-di-substituted guanidines and substituted ureas.

Contemporary evidence suggests that the predominance of the cyclic imidocarbonate derivatives, postulated by the earlier workers who explored the mechanism of the reaction between amines and CNBr-activated Sephadex and cellulose, does not hold for CNBr-activated agarose. The molecular structure of dextran and cellulose affords the formation of 5-membered imidocarbonate rings whereas the agarose structure (devoid of vicinal hydroxy groups) only allows for the formation of 6-membered rings. Since 5-membered rings may be more energetically favoured than 6-membered rings, the possible predominance of reactive imidocarbonate derivatives, prepared by CNBr activation of Sephadex and cellulose, is conceivable. In contrast, the cyanate ester (*e.g.* Fig. 3, II) intermediates yielding the more stable isourea derivative (Fig. 3, III) of primary amines are the main active species in CNBr-activated

Sepharose. Our results confirm that the coupling of aliphatic amines to CNBr-activated Sepharose yield isourea linkages which further react with amines to produce N_1,N_2-disubstituted guanidines and substituted ureas.

The adsorption characteristics of amino acids and peptides on the test immunoadsorbents served as simple models for exploring the cationic charge distribution[8–11] on the surfaces of the adsorbent. By relying on the charged characteristics and the stereochemical features of the functional groups we were able to assess protein–protein interactions with ionic adsorbents. The adsorption of the hydroxymonoamino-monocarboxylic α-amino acids, L-serine and L-threonine (72% and 7% respectively), are particularly interesting. The outstanding difference in these molecules is the presence of the methyl group adjacent to the hydroxy group in L-threonine. The hydrophobic methyl group would not be favourably adsorbed on the hydrophilic Sepharose gel beads. The ten-fold difference in the adsorption of L-threonine could be partially attributed to steric hindrance by the methyl group and to possible hydrogen bonding with the lone pair electrons on the hydroxy group oxygen atom. The mono-amino-dicarboxylic α-amino acids, L-aspartic and L-glutamid acids showed 56% and 42% adsorption respectively. This reflects the importance of the contribution of the charged polar groups at pH 6–7 to the adsorption process.

The sulphur-containing amino acids, L-cysteine and L-methionine, revealed 9.6 and 5.2% adsorption respectively. The sulphur atom of methionine situated between a CH_2 and CH_3 group is not alkali labile and its contribution to the acidic properties of the molecule is some 50% less than that of the thiol group sulphur atom. Among the cyclic and aromatic amino acids L-phenylalanine, L-tyrosine and L-proline, 10.0, 3.6 and 58.0% respectively were adsorbed. The weakly acidic hydroxyl group of the L-tyrosine aromatic ring appears to decrease the ionization of the carboxylic acid group by resonance stabilization. In contrast, the amino acid L-proline containing the pyrolidine ring and the imidine nitrogen is substantially ionized at neutral pH.

Of the four dipeptides and one tripeptide investigated, only glycyl–L-serine showed any significant adsorption under these conditions (Table III). This seems to be consistent with the high adsorption of L-serine (71.6%) in the first cycle (Table II). It is noticeable that the highest adsorption of peptides and amino acids is associated with the first cycle. This is probably reflecting some binding by unblocked active groups in the immunoadsorbents. There is also a saturation effect from the species being loaded. As the adventitious cationic charges in the immunoadsorbent become neutralised by undesorbed molecules of opposite charge, the tendency for further adsorption to take place is reduced. These results indicate that contributions from ionic and some non-ionic effects may be concomitantly implicated in the adsorption process. Such combined effects have been observed by Yon[21], Hofstee[22], Er-El *et al.*[23] and Kennedy *et al.*[24]. Clearly, once a non-specific adsorption has occurred, the nature of the adsorbed species may disadvantageously cause a modification of the matrix surface, both from the viewpoints of non-specific and specific adsorption. The initial non-specific adsorption of aromatic and/or ionic species could well amplify non-specific adsorption.

In practice, we have found 0.5 *M* ammonia an efficient eluent in immunoadsorption chromatography[24]. However, caution should be exercised in its use with different types of proteins and the coupled gel should not be stored in buffers containing amines.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the kind interest and encouragement of Dr. A. R. Bradwell in the work. We thank the Endowment Fund Medical Research Committee, University of Birmingham, for a research grant to J.A.B.

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