Specific Dimerization of the Light Chains of Human Immunoglobulin

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1. The light chains of human immunoglobulin were allowed to dimerize *in vitro* on removal of the dispersing agents acetic acid or urea. 2. On electrophoresis in polyacrylamide gel at pH 8·8 the dimers yielded up to nine regularly spaced bands. This approximates to the number of electrophoretic components known to occur among the monomers. 3. Single electrophoretic components of the dimers were isolated from the gel, dissociated into monomers, and subjected as such to electrophoresis in urea-containing gels. Each gave two adjacent bands. 4. Similarly, after all the light chains as monomers had been subjected to electrophoresis in urea-containing gels, single electrophoretic components were isolated and allowed to dimerize. When examined now as dimers in the absence of urea, each component gave two adjacent bands. 5. These findings are explicable on the following basis. (*a*) The dimerization of the light chains is specific, at least inasmuch as it occurs between monomers of the same electrophoretic mobilities. (*b*) With the buffer constant, different light chains undergo different changes in net charge on being transferred from urea-containing to urea-free solution; in this way two different chains of the same initial charge can acquire a charge difference of 1. 6. Experiments with Bence-Jones proteins and other homogeneous light chains gave results substantiating the conclusions (*a*) and (*b*).

The nomenclature used in this paper is that recommended by the World Health Organisation (1964).

The light polypeptide chains of mammalian immunoglobulins are separated into multiple components by electrophoresis in urea-containing starch gels at neutral pH (Cohen & Porter, 1964). The chains under these conditions exist as monomers (Pain, 1963), with molecular weights varying within the range 22000–24000 (inferred from studies on Bence-Jones proteins; e.g. Wikler, Titani, Shinoda & Putnam, 1967). Human light chains show ten components, each of which is seen on antigenic analysis to contain both κ and λ types (Cohen & Gordon, 1965). The difference in net charge between adjacent components is probably 1 (Feinstein, 1966). Thus, if the net charge on the most cathodic component is q, that on the adjacent one is $q-1$, on the next $q-2$ and so on up to the most anodic component with a charge of $q-9$. This statement would apply to the average charges if there was a distribution of fractional charges within each component.

On the removal of urea or other dispersing solute from a solution containing all the different light

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chains, dimerization occurs (Pain, 1963; Gally & Edelman, 1964). The question arises whether there is any specificity in this reaction. The maximum specificity would involve dimerization between chains of identical primary structures. The present paper deals partially with the problem by considering whether or not the dimerization is electrophoretically specific, i.e. between components of the same electrophoretic mobilities.

Random dimerization would yield approximately twice as many differently charged entities as specific dimerization. Let us consider, for example, the dimerization of human light chains occurring on the removal of urea from a solution at neutral pH, and assume for simplicity that the net charges per chain remain unaltered. A random process would give dimers with 19 different net charges $2q$, $2q-1$, $2q-2$, ... $2q-18$. This compares with ten differently charged components to be expected from specific dimerization. Any changes in net charges per chain accompanying removal of the urea would alter the details but not the substance of this argument.

A further consequence of random dimerization is that the dimeric population of a single intermediate charge would contain many different electrophoretic components of the monomers.

We have investigated the dimerization of human light chains by two methods. First, the number of electrophoretic components among the dimers was examined. Secondly, single electrophoretic components of the dimers were prepared, dissociated into monomers, and examined by a second electrophoresis in the presence of urea. Our results indicated specific dimerization, but were complicated by the finding that different light chains can undergo different changes in net charge on being transferred from urea-containing to urea-free solution. To clarify matters, simple mixtures of homogeneous light chains from Bence-Jones proteins and myeloma proteins were investigated.

MATERIALS AND METHODS

Materials. Analytical-grade urea (British Drug Houses Ltd., Poole, Dorset) was used without further purification, the solutions always being freshly prepared. 2-Mercaptoethanol was redistilled within 1 month of use. Iodoacetamide was recrystallized from ethanol and iodoacetic acid from diethyl ether. Hydrolysed starch was obtained from Connaught Medical Research Laboratories (Toronto, Ont., Canada).

Normal light chains. Human IgG was obtained from Commonwealth Serum Laboratories (Melbourne, Vic., Australia) in the form of Cohn's fraction II-1,2 from pooled plasma. Alkylated light chains were prepared from it by the following steps, based on the procedure of Fleischman, Pain & Porter (1962). For reduction, IgG (25 mg./ml.) was allowed to react with 0·5 M-2-mercaptoethanol in 0·02 M-sodium phosphate, pH 7·7, at room temperature for 30 min. For alkylation, an equal volume of 0·55 M-iodoacetamide was added and allowed to react at room temperature for 15 min., with the pH maintained between 7 and 8 with 2 M-NaOH. The protein was transferred into the dissociating solvent, 0·75 M-acetic acid, by passage through Sephadex G-25, and finally the light chains were separated by passage through Sephadex G-100 equilibrated with 0·75 M-acetic acid, at room temperature. The yield of light chains, as measured by the E_{280} value, was 25%.

For electrophoresis as dimers the light chains were concentrated by pressure dialysis to approx. 20 mg./ml. with transfer to 0·05 M-glycine–NaOH buffer, pH 8·8. When required in monomeric form they were concentrated to the same extent, with transfer to 7 M-urea in the same buffer. Pressure dialysis was carried out in the cold-room, with a pressure of 560 mm. Hg across $\frac{8}{32}$ in. cellophan tubing (Visking); the recoveries of light chains exceeded 90%.

Homogeneous or 'monoclonal' light chains. These consisted of three κ-chain Bence-Jones proteins (Ken, Mor and Ree), a κ-chain of a myeloma serum protein (Cor) and a λ-chain of a homogeneous serum protein (Daw) from a patient with cryptogenic hyperglobulinaemia.

The Bence-Jones proteins were prepared from urine by precipitation with 3·0 M-$(NH_4)_2SO_4$, gradient elution from DEAE-cellulose with 0–0·5 M-NaCl in 0·01 M-tris–HCl buffer, pH 8·8, and finally passage through Sephadex G-100 in 0·05 M-glycine–NaOH buffer, pH 8·8, to remove any aggregate. They were reduced and alkylated under the same conditions as was IgG (see above), and the reagents were removed by passing the protein through Sephadex

G-25 equilibrated with the buffer to be used for electrophoresis. In some experiments iodoacetate was used as the alkylating agent. Before the reduction both protein Ken and protein Mor were found to migrate through Sephadex G-100 in 0·75 M-acetic acid entirely as dimers, indicating that they consisted entirely of disulphide-bonded dimers (Gally & Edelman, 1964).

The protein Daw, a euglobulin, was prepared from serum by precipitation, first by dialysis against 0·0175 M-sodium phosphate buffer, pH 6·2, and secondly by re-solution in 0·1 M-NaCl and addition of Na_2SO_4 to a final concn. of 0·9 M. The protein Cor was separated from plasma by gradient elution from DEAE-cellulose with 0·0175–0·2 M-sodium phosphate buffer, pH 6·2. Alkylated light chains were prepared from proteins Daw and Cor by conventional techniques (Fleischman *et al.* 1962) and made available for the present work in the form of freeze-dried powders.

The conditions used for reduction and alkylation of light chains in this work affect only a single half-cystine residue involved in an interchain disulphide bond. The two intrachain disulphide bonds are unaffected. The half-cystine residue is C-terminal in κ-chains and next to the C-terminus in λ-chains. The interchain bond links heavy and light chains in whole immunoglobulin molecules, and light chains in those light chain dimers having an interchain disulphide bond. There is no evidence of a disulphide bond between the light chains in whole immunoglobulin molecules (Fleischman, Porter & Press, 1963; Gally & Edelman, 1964; Milstein, 1966).

Electrophoresis. This was carried out at room temperature in starch gels containing urea, and in polyacrylamide gels both with and without urea. Horizontal Perspex trays (25 cm. × 12·5 cm. × 0·6 cm.) were used. Samples were loaded into slots of maximum volume 0·06 ml. The 0·05 M-glycine–NaOH buffer, pH 8·8, was used for the gels and inner buffer tanks (except where stated otherwise), and 0·3 M-boric acid–0·6 M-NaOH buffer, pH 9·5, for the electrode vessels. The final pH of gels containing 7 M- or 8 M-urea was 7·5–7·8.

Electrophoresis in urea-containing starch gels, and staining with Amido Black, were as described by Smithies (1959) and Poulik (1966). Running conditions were 4 v/cm. for 20 hr.

The polyacrylamide gels (Raymond, 1962; Davis, 1964; Ornstein, 1964) were 7% (w/v) in acrylamide and 0·14% (w/v) in the cross-linking methylenebisacrylamide. They were allowed to polymerize in a tray with a cover containing slot-formers, with the catalysts ammonium persulphate (2 mM) and tetramethylethylenediamine (4 mM). The same procedure sufficed when urea was incorporated in the gel. The reagents were from Eastman Organic Chemicals (Rochester, N.Y., U.S.A.). In view of possible reactions of persulphate with proteins (Brewer, 1967), some gels were subjected to a voltage gradient of 4 v/cm. for 4 hr. before the sample was applied, so that all the persulphate would lie anodic to the protein. Another preliminary treatment sometimes used was washing of the gel to remove the catalysts and any non-cross-linked polyacrylamide. Neither treatment made any difference to the electrophoretic patterns. Running conditions were 4 v/cm. for 16 hr. in the absence of urea and 5 v/cm. for 20 hr. in the presence of urea. (If the catalysts are not removed before electrophoresis the resistance of the gel, and hence the voltage gradient for a given current, increases as the catalysts

migrate into the buffer tanks. In these cases the current was set so as to give the required final voltage gradient, and the time of electrophoresis lengthened to allow for the initial lower gradient.) The gels were stained with Amido Black and cleared electrophoretically (Ferris, Easterling & Budd, 1962).

To prepare individual electrophoretic components of normal light chains, three or more samples were run in parallel with the slots loaded with the maximum amount of protein (2 mg.) compatible with good resolution. After electrophoresis a central strip was cut out and stained to locate the bands in the adjoining strips; migrations from different slots were sufficiently uniform to allow this. Bands were cut out, frozen and thawed in the case of starch gels, macerated, suspended in the glycine–NaOH buffer and centrifuged. The elution was repeated once and the pooled eluates were concentrated by pressure dialysis in $\frac{8}{32}$ in. cellophan bags.

Ultracentrifugal analysis. This was carried out in a Spinco model E ultracentrifuge.

RESULTS

Dispersity of the light chains. Reduced and alkylated light chains, both normal and homogeneous, existed in 0·75 M-acetic acid entirely as monomers. This is the same result as found by Gally & Edelman (1964) for light chains in 0·5 M-propionic acid, and was demonstrated in the same way, namely by comparing their mobility on Sephadex G-100 with that of disulphide-bonded dimers.

The dispersity of light chains in the presence of urea was also investigated by chromatography on Sephadex G-100. The protein was applied, at the concentrations used for electrophoresis, on to a column equilibrated with the urea-containing buffer, and run at room temperature. Normal light chains revealed some aggregate larger than dimers, amounting to about 15% of the protein under the standard conditions (Fig. 1). This was not eliminated by increasing the concentration of urea to 9 M, nor by minor variations in the preparative method. It is likely that the aggregate did not migrate further than the immediate vicinity of the slots during electrophoresis; there was regularly some staining for protein in the vicinity of the slots, and this accords with the known sieving properties of the gels (Smithies, 1959; Ornstein, 1964). The homogeneous light chains after reduction and alkylation contained only monomers in both 7 M- and 8 M-urea. A urea concentration of 7 M was chosen for electrophoresis because it allowed dialysis to take place in the cold-room, and gave the same electrophoretic patterns as did 8 M- and 9 M-urea.

Normal light chains dialysed into the 0·05 M-glycine–sodium hydroxide buffer, pH 8·8, from either 0·75 M-acetic acid or urea-containing solutions, gave a symmetrical ultracentrifugal peak with a
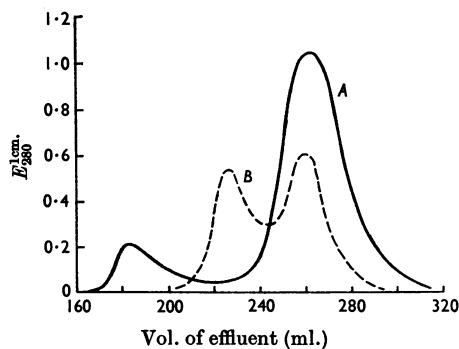

Fig. 1. Dispersity of light chains in 7 M-urea–0·05 M-glycine–NaOH buffer, pH 7·7. Samples were applied to a column (3 cm. × 80 cm.) of Sephadex G-100 equilibrated with this solvent system, and run at room temperature. The curves have been redrawn from records provided by an automatic monitor (LKB Uvicord). Curve A (———), reduced and alkylated normal light chains, 50 mg. applied in a volume of 2·2 ml.; curve B (----), an equimolar mixture of untreated protein Ken (a disulphide-bonded dimer) and reduced and alkylated protein Ken, 20 mg. applied in a volume of 4·5 ml. The small leading peak shown by the normal light chains is seen on comparison with curve B to consist of aggregates larger than dimers.
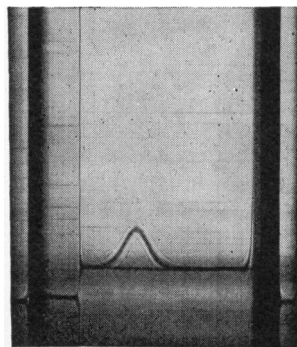

Fig. 2. Ultracentrifugal pattern given by normal light chains dialysed from 7 M-urea–0·05 M-glycine–NaOH buffer, pH 7·7, into 0·05 M-glycine–NaOH buffer, pH 8·8. Conditions were as follows: concentration, 7 mg./ml.; speed, 59 780 rev./min.; temperature, 20°; photograph, 77 min. after reaching full speed; phase-plate angle, 70°. Sedimentation was from left to right. The sedimentation coefficient ($S_{20,w}$) was 3·2 s.

sedimentation coefficient ($S_{20,w}$ 3·2 s) indicative of dimers (Fig. 2). The protein Ree was partially precipitated on being dialysed from 0·75 M-acetic acid into the glycine buffer; ultracentrifugation of the supernatant after the removal of the precipitate showed dimers and a very small amount of larger

aggregate. The other homogeneous light chains yielded only dimers under these conditions.

A final problem was whether dimers of alkylated light chains, held together only by non-covalent bonds, persist in the dimeric form during electrophoresis through urea-free polyacrylamide gels. That they do is suggested by the finding that the mobilities of proteins Ken and Mor were not altered by reduction and alkylation with iodoacetamide. (In the absence of reduction the dimeric state in these proteins was stabilized by an interchain disulphide bond.) This remained true at the highest concentration of polyacrylamide tested (15%, w/v). In contrast, in the presence of 7M-urea in a 7% polyacrylamide gel, dimers of proteins Ken and Mor migrated at only 0·8 of the rate of the reduced and alkylated (and, in urea, monomeric) forms.

Electrophoretic patterns of normal light chains. The monomers gave banded patterns in urea-containing starch or polyacrylamide gels, in accord with the results of Cohen & Porter (1964). Up to ten regularly spaced bands were visible, with no appreciable staining between them.

Dimerization was allowed to proceed on the removal, by dialysis in the cold-room, of either acetic acid or urea. The dimers gave a reproducible banded pattern on electrophoresis in polyacrylamide gel (Plate 1*A*). Up to nine bands were visible, with considerable background staining between them. Increasing the distance of migration up to threefold failed to resolve further bands amidst this background. No satisfactory patterns were obtained in starch gels.

[In the results given below the electrophoretic components of both monomers and dimers are identified by numbering from the cathode, starting with the first component to be seen consistently. Numbers for the dimers are prefixed with D. Both sets of numbers are tentative. Thus a faint band was sometimes seen cathodic to the monomeric band 1, and we give below a reason for believing that there is at least one dimeric band cathodic to band D1. Our numbering of the monomers corresponds with that of Cohen & Porter (1964), as the light chain Daw migrated in position 4 in both schemes.]

The dimeric components D3, D4 and D5 were each eluted from the polyacrylamide gel and concentrated by pressure dialysis against 7M-urea in 0·05M-glycine–sodium hydroxide buffer, pH 7·7. They were thus dissociated into monomers. When submitted to electrophoresis in the presence of urea, in either starch or polyacrylamide, each component consistently gave two adjacent bands (Plate 1*B*). Dimer D3 gave rise to monomeric components 3 and 4, dimer D4 to monomeric components 4 and 5, and dimer D5 to monomeric components 5 and 6. In each case the more anodic

of the monomeric components contained the greater amount of protein. Much smaller amounts of a third band closer to the anode than the other two were seen inconsistently.

To assess whether or not this result was related to carbamoylation by cyanate derived from the urea (Stark, Stein & Moore, 1960), the electrophoretic examinations were repeated at intervals. Prolonged incubation in urea (about 6 weeks in the refrigerator) was required to give patterns with an average displacement of one band anodically. On this basis attack by cyanate could not have been sufficient to account for two instead of one principal monomeric components in the original patterns.

We argue below that the above findings are consistent with specific dimerization, provided that there are differences in the changes in charge undergone by different light chains during transfer between urea-free and urea-containing solutions. The following experiment demonstrated these differences. Monomers of all the light chains were submitted to electrophoresis in a urea-containing starch gel, and the components 4, 5 and 6 were eluted. Each of these was concentrated by pressure dialysis against urea-free glycine buffer, thereby being allowed to dimerize. When examined by electrophoresis in polyacrylamide in the absence of urea, each component gave rise to two, and only two, bands: component 4 to bands D3 and D4, component 5 to bands D4 and D5, and component 6 to bands D5 and D6 (Plate 2). In this case the more cathodic band in each pair contained the greater amount of protein.

The following electrophoretic components were eluted for antigenic analysis: monomeric components 3 and 4 obtained by running dimeric component D3 on a starch–urea gel, and dimers D3 and D4 obtained by running monomeric component 4 on a polyacrylamide gel. They were examined on Ouchterlony plates by using rabbit anti-(human IgG) and rabbit anti-(human κ-chain). No antigenic differences were detected among the four samples, and all appeared to contain both κ- and λ-chains.

Electrophoretic patterns of homogeneous light chains. Reduced and alkylated homogeneous light chains were examined in parallel with normal light chains. Fig. 3 summarizes the results and demonstrates three points.

First, the distance between adjacent bands in the pattern of dimeric normal light chains corresponded to a charge difference of 2, since it equalled the differences both between Daw and Daw($-$) chains and between Ken and Ken($-$) chains. [The suffix ($-$) indicates an extra negative charge per chain due to alkylation with iodoacetate instead of iodoacetamide (Feinstein, 1966).]

Secondly, in two cases a small difference in mobility was demonstrated between two light chains
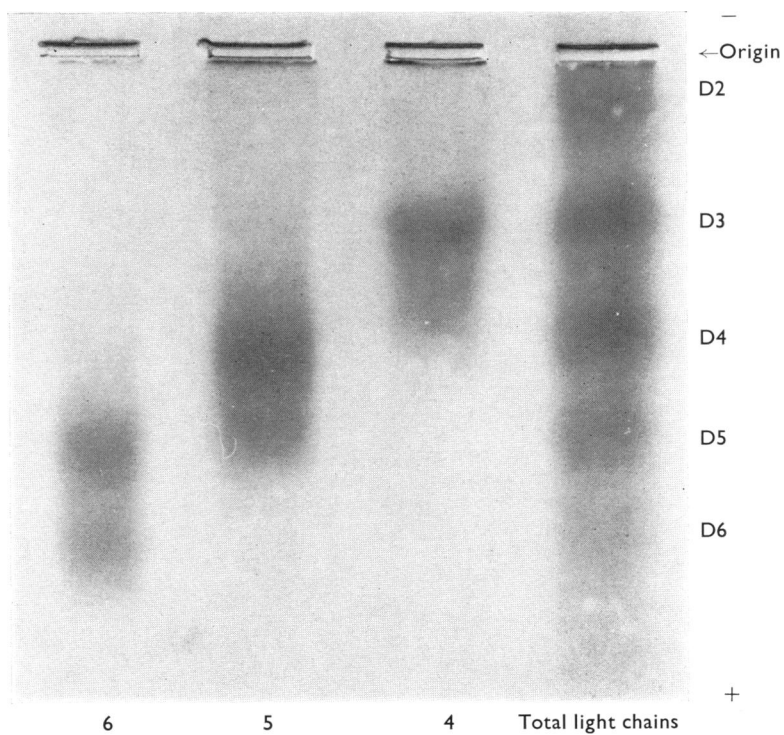
(A)
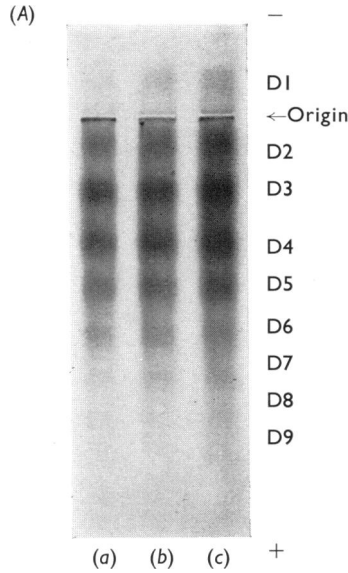
EXPLANATION OF PLATE 1(A)

Electrophoretic patterns given by dimers of reduced and alkylated normal light chains in a polyacrylamide gel containing 0·05 M-glycine–NaOH buffer, pH 8·8. Approximate amounts of protein placed in the slots were: (a) 1 mg.; (b) 1·5 mg.; (c) 2 mg. The components are numbered from the cathode.

(B)
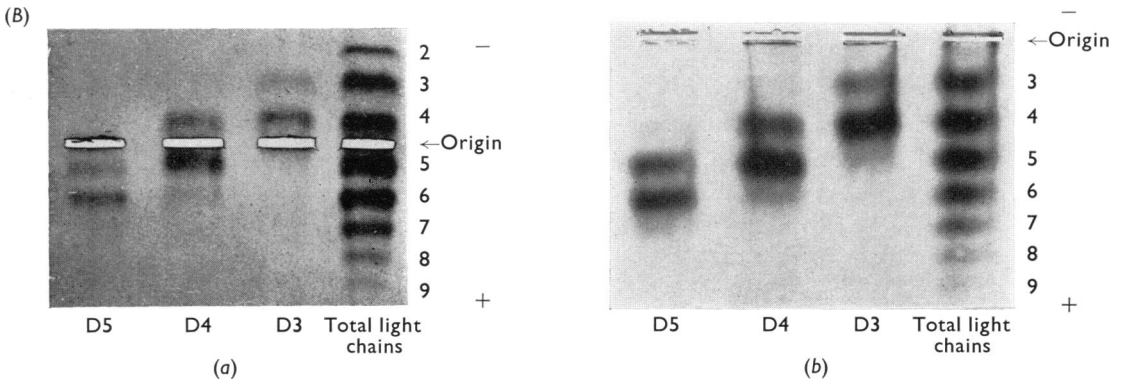
EXPLANATION OF PLATE 1(B)

Electrophoretic patterns given by monomers of reduced and alkylated normal light chains in (a) starch and (b) polyacrylamide gels, containing 7 M-urea–0·05 M-glycine–NaOH buffer, pH 7·5–7·8. The slots were loaded with monomers obtained from all the light chains or from one of the dimeric components, D3, D4, or D5, as indicated. The numbering of the monomeric components is from the cathode.

←Origin

D2

D3

D4

D5

D6

6   5   4   Total light chains

EXPLANATION OF PLATE 2

Electrophoretic patterns given by dimers of normal light chains in a polyacrylamide gel containing 0·05 M-glycine–NaOH buffer, pH 8·8. The dimers were obtained from all the monomers, or from one of the monomeric components, 4, 5 or 6, as indicated.

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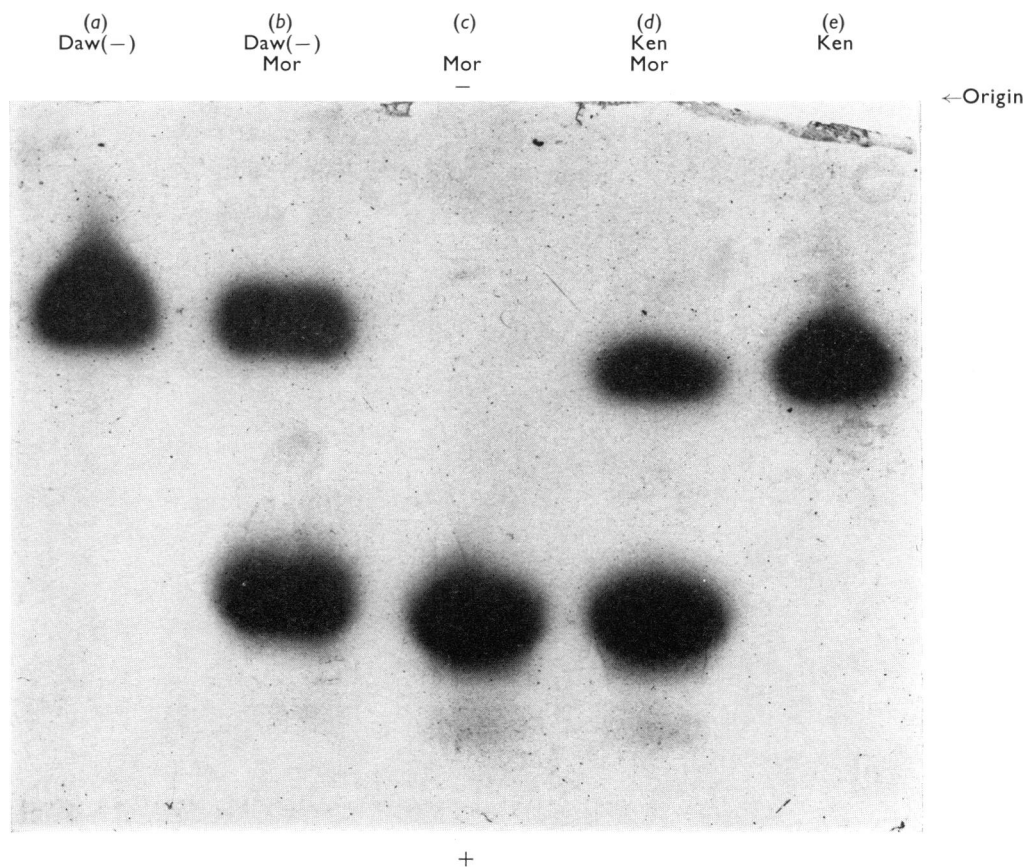
EXPLANATION OF PLATE 3

Electrophoretic patterns given by dimers of homogeneous light chains in a polyacrylamide gel containing 0·01 M-tris–HCl buffer, pH 8·0. Samples (a), (c) and (e) were not treated apart from reduction and alkylation. In samples (b) and (d) dimerization had taken place in mixed solution as the chains were transferred by dialysis from 0·75 M-acetic acid to the tris–HCl buffer.

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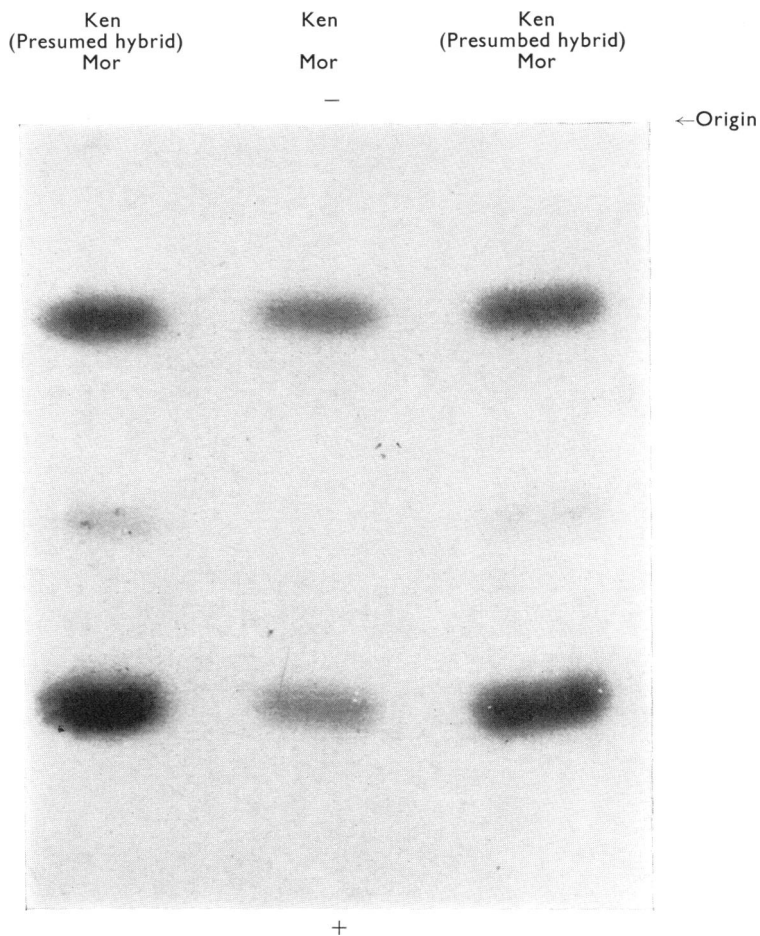
EXPLANATION OF PLATE 4

Electrophoretic patterns given by disulphide-bonded dimers of homogeneous light chains. Electrophoretic conditions were as given in Plate 3. The centre sample consisted of untreated proteins Ken and Mor, mixed immediately before application to the gel. The peripheral samples were duplicates and consisted of those dimers that formed in mixed solution and acquired an interchain disulphide bond. Experimental details are given in the text.
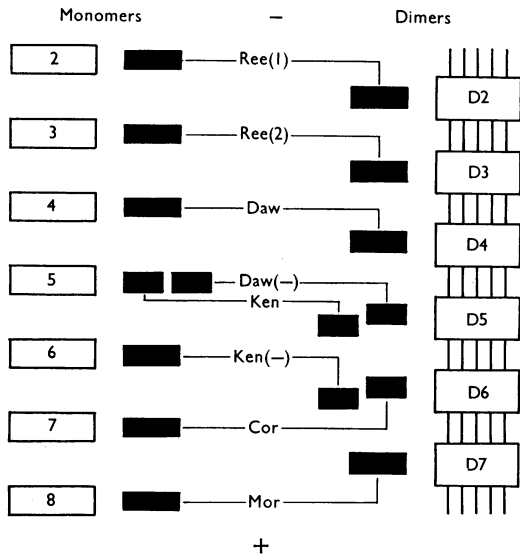
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Fig. 3. Electrophoretic patterns given by homogeneous light chains in polyacrylamide gels: a composite diagram derived from 14 patterns. The monomers were examined in urea-containing gels and the dimers in urea-free gels containing 0·05 M-glycine–NaOH buffer, pH 8·8. The numbered outer blocks indicate the positions of normal light-chain components, monomers on the left and dimers on the right. The suffix (−) indicates alkylation with iodoacetate instead of iodoacetamide. The protein Ree gave two components in both monomeric and dimeric forms. Additional components among the other proteins were present in only low concentrations and are not shown. For the monomers, correspondence between the bands given by normal light chains and those given by homogeneous chains was precise and easy to evaluate. For the dimers the homogeneous chains could be allocated to one zone or another but they were not always in the middle of the zone. The small differences in mobility between homogeneous chains within the dimer D5 and dimer D6 zones were reproducible.

Fig. 4. Separation of monomers from disulphide-bonded dimers by passage through Sephadex G-100 equilibrated with 0·75 M-acetic acid. The column (5 cm. × 83 cm.) was run at room temperature. The curves have been redrawn from tracings provided by an automatic monitor (LKB Uvicord). Curve 1 (———), an equimolar mixture of proteins Ken and Mor applied to the column immediately after reduction with 0·5 M-mercaptoethanol; curve 2 (– – – –), the mixed proteins delivered by run 1, after an intervening rise in pH had led to dimerization and the formation of interchain disulphide bonds.

whose mobilities as dimers corresponded to a single band in the normal pattern: Daw(−) and Ken chains, and Cor and Ken(−) chains.

Thirdly, the relative mobilities of some pairs of samples revealed differences in charge per chain that were unequal in the monomeric and dimeric forms. This was readily seen with Ken(−) and Cor chains. As monomers, Cor had one more negative charge, whereas as dimers Ken(−) appeared to have a fractionally greater negative charge. The difference in charge per chain between Daw and Mor chains also decreased by 1 as a concomitant of dimerization.

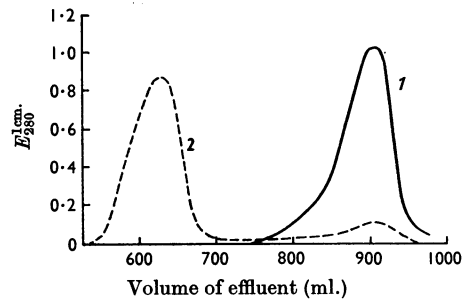The following experiment demonstrated specific dimerization of two different light chains in a mixture. It was performed ten times, with all combinations of two among the five homogeneous light chains available. Samples of two different reduced and alkylated homogeneous light chains were mixed in equimolar amounts, transferred into 0·75 M-acetic acid by passage through Sephadex G-25 and allowed to stand for 24 hr. at room temperature. (In runs through Sephadex G-100 equilibrated with 0·75 M-acetic acid, taking place over the same time and at the same temperature, all the reduced and alkylated chains were shown to exist as monomers.) The total protein concentration at this stage was approx. 1 mg./ml. The chains were then allowed to dimerize by transfer by dialysis in the cold-room into an alkaline buffer (0·05 M-glycine–sodium hydroxide buffer, pH 8·8, or 0·01 M-tris–hydrochloric acid buffer, pH 8·0) suitable for electrophoresis. The transfer was complete after 2 days. Finally they were concentrated five- to ten-fold by ultrafiltration, and examined by electrophoresis in polyacrylamide gel. Specificity of dimerization appeared always to have been absolute. Even with the gel maximally loaded with protein no hybrid dimer could be seen (Plate 3).

In a final experiment the dimers formed in a mixed solution of light chains were encouraged to form an interchain disulphide bond. Dimers thus stabilized were characterized electrophoretically, eliminating the possibility of artifacts due to dissociation in the polyacrylamide gel. The proteins Ken and Mor (in the form of disulphide-bonded dimers obtained originally from the urines) were mixed in equimolar amounts, reduced by

exposure to 0·5M-2-mercaptoethanol at room temperature for 30min., and passed at room temperature through Sephadex G-100 (5cm. × 83cm.) equilibrated with 0·75M-acetic acid. The passage through Sephadex transferred the protein into the acid, removed the mercaptoethanol and provided a check on the completeness of dissociation into monomers (Fig. 4). The total protein concentration was then 0·7mg./ml. Next the protein was dialysed for 4 days in the cold-room against 0·01M-tris–hydrochloric acid buffer, pH8·0; for the last 24hr. the external solution was saturated with oxygen. Stabilized and non-stabilized dimers were separated by concentrating the protein by ultrafiltration and passing it through the same Sephadex G-100 column, equilibrated with 0·75M-acetic acid, as was used previously. In this run 87% of the protein was eluted in the same position as were the proteins Ken and Mor in their original forms, indicating dimers stabilized by an interchain disulphide bond. There were no larger aggregates. The dimers were transferred by pressure-dialysis into 0·01M-tris–hydrochloric acid buffer, pH8·0, and examined by electrophoresis in polyacrylamide gel (Plate 4). Here for the first time evidence of a hybrid dimer was obtained. Most of the protein migrated as two bands in the positions of the untreated proteins Ken and Mor. But approximately midway between these was a faint band whose intensity suggested a protein content less than 10% of the total. Random dimerization in a mixture of two types of chain would yield half the protein in the form of hybrid dimer.

DISCUSSION

The clearest demonstration of specific dimerization was obtained when two different homogeneous chains, reduced and alkylated, were allowed to dimerize in a mixture. There was apparently a complete absence of hybrid dimer. The only other explanation of electrophoretic patterns such as that in Plate 3 is that dimers secured only by non-covalent bonds dissociated in the polyacrylamide gel. Against this possibility is the fact that the mobilities of the proteins Ken and Mor were unaltered when their interchain disulphide bonds were reduced and alkylated with iodoacetamide; any dissociation would have been accompanied by an increased rate of migration, at least in the denser gels. Dissociation in the gel of the re-formed dimers was ruled out altogether in the experiment in which they were stabilized by interchain disulphide bonds. Certainly a small amount of (probable) hybrid was seen here, and 13% of the chains were not characterized electrophoretically because they failed to become disulphide-bonded and were removed on the Sephadex G-100 column

(Fig. 4). But even an assumption that all these chains formed hybrid dimers left us with less than 25% of hybrids, compared with 50% to be expected from random dimerization. This experiment is thought to confirm the predominantly specific nature of dimerization, but at the same time it reveals that some associations that would otherwise be transient can be stabilized by interchain disulphide bonds.

Instead of extrapolating the behaviour of a small number of homogeneous chains to the entire population of normal light chains, with probably at least hundreds of different sequences (e.g. Cohen & Milstein, 1967), we examined the independent evidence for electrophoretically specific dimerization within this population. First it is emphasized that all the dimers examined had dimerized in vitro, and did not represent the survival of pairings established in vivo during the synthesis of immunoglobulin.

For the reason given in the introduction, the fact that the dimers showed approximately the same number of electrophoretic bands as the monomers suggests that the dimerization had been electrophoretically specific. A similar electrophoretic pattern has been reported for dimers of rabbit light chains (Small, Reisfeld & Dray, 1965). A problem arises in interpreting the staining between the bands. No additional bands were demonstrated on prolonging the electrophoresis, so we are left with the possibilities of a spread of charges from each band (so that the centres simply represent modes) or of binding to the gel. The former explanation is consistent with the minor differences in mobility shown by different homogeneous chains corresponding to one normal band (Fig. 3).

The charge difference of 2 represented by a spacing of one band in the normal dimeric pattern is also consistent with electrophoretically specific dimerization.

Better evidence of specific dimerization is provided by the monomers obtained from individual electrophoretic components of the dimers. The fact that the great bulk of these monomers gave regularly two adjacent electrophoretic bands, the more anodic containing regularly the greater amount of protein, is incompatible with random dimerization. The appearance of these two adjacent bands could have been due to many dimers having involved chains with a difference in net charge of 1. However, there is no apparent reason for such odd pairings being preferred. Another explanation follows from considering possible alterations in charge undergone in the reaction dimer $\xrightleftharpoons{\text{urea}}$ monomer. Such alterations could reflect: (1) the change in pH due to the urea; (2) changes in pK values of ionizing residues due to the urea; (3) changes in pK values of
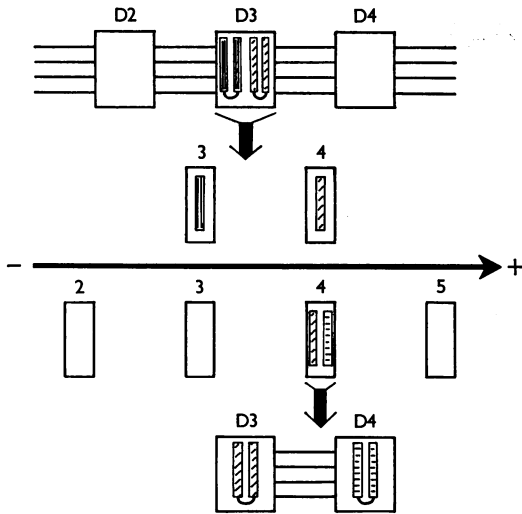
Fig. 5. Some relationships among the electrophoretic components of dimers and monomers of normal light chains. The postulated behaviour of three types of chain is shown. No difference in mobility within a single band is implied, nor is any direct comparison intended between the mobilities of monomers and dimers.

ionizing residues due to transfer from the interior to the exterior of the protein when the dimer is cleaved or the chains unfolded. In a heterogeneous population the changes in charge on different chains could vary. The result in Plate 1(B) could then be explained as follows. (1) The dimerization of normal light chains is specific at least inasmuch as it occurs between monomers with the same electrophoretic mobilities. (2) The dimerization is accompanied by an average change in charge of x, whose value is immaterial, and by a range of changes in charge of $x \pm \frac{1}{2}$.

This means, as required by the result in Plate 1(B), that two dimers with the same net charge can give rise in urea to monomers with charges either equal or differing by 1. The interpretation of the result in Plate 1(B) is shown diagrammatically in the upper part of Fig. 5.

It has been possible to verify several predictions arising from this hypothesis.

(1) One should be able to demonstrate, from a monomeric population of normal chains with the same charges, the formation of dimers with differences in charge per chain of up to 1. This result is shown in Plate 2, and the interpretation in the lower part of Fig. 5.

(2) The number of dimeric electrophoretic components to be expected from ten monomeric components is eleven. The number actually observed was nine, which was thought to be

tolerably close because of the difficulty in defining peripheral bands. An extrapolation of the result in Plate 2 would yield a dimeric component cathodic to that now labelled D1.

(3) Homogeneous light chains, such as Bence-Jones proteins, when mixed in monomeric form and allowed to dimerize, should do so with at least the degree of specificity required by the hypothesis. We have discussed this result above.

(4) Given a sufficient number of homogeneous chains, one should be able to demonstrate the different changes in charge postulated to accompany dimerization. This was readily done, as illustrated in Fig. 3.

There is no simple correlation between the change in charge accompanying dimerization and the principal division of light chains into κ and λ types. This has been shown by antigenic analyses of light chains fractionated by successive electrophoretic runs (the rationale of which can be followed from Fig. 5), and by the behaviour of the homogeneous light chains available.

Specificity of dimerization was demonstrated only as judged by electrophoretic mobilities. No attempt was made to invoke other possible criteria: the κ and λ types, the antigenic subtypes within these (e.g. Nachman, Engle & Stein, 1965), and the allotypes. Further, the methods used indicated only a preponderance of specificity in the dimerization of normal chains, and would not have detected a small proportion of hybrids. Given the likely complexity of the light-chain population (reviewed by Cohen & Milstein, 1967) it would be incredible if every variation in primary structure were to be reflected in specificity of dimerization.

Even with these reservations the specificity shown is a remarkable and unexpected property. In one relatively simple situation in Plate 3 two different κ-chains, presumably with much similarity in sequence and tertiary structure, sought out their homologous partners with a precision reminiscent of antibody specificity, but with the obvious difference that union occurred between units with the same rather than complementary conformation. The dictating conformations must reside in the N-terminal halves of the chains, as it is only here that sufficient variation in sequence occurs (Hilschmann & Craig, 1965; Milstein, 1966; Titani, Whitly & Putnam, 1966). Any further speculation about the mechanism involved is hindered by ignorance of the tertiary structure of the chains.

The phenomenon we describe might be of biological importance. It is likely that light chains are normally synthesized in greater numbers than are heavy chains. Thus the light chains appearing in normal urine are a newly synthesized product not derived from the breakdown of whole immunoglobulin molecules (Stevenson, 1962; Gordon,

Eisen & Vaughan, 1966), and an excess of light chains is synthesized by antibody-producing cells in tissue culture (Shapiro, Scharff, Maizel & Uhr, 1966a). Free light chains probably represent an intermediate product in the synthesis of immunoglobulin (Shapiro, Scharff, Maizel & Uhr, 1966b; Askonas & Williamson, 1967). If the free light chains within a cell can have more than one kind of sequence, and if they dimerize before joining the heavy chains, then specificity of dimerization could be important in determining the final composition of immunoglobulin molecules.

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