A Determination of the Sub-units of Arachin by Osmometry

ARACHINS A, B AND A1

By M. P. TOMBS AND M. LOWE

Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford

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1. Osmotic pressure determinations of dissociated arachins are a particularly suitable method for determination of the number of sub-units in the protein, because they yield a number-average molecular weight. 2. Arachin, in 8M-urea-0.1M-sulphite, produces 12 sub-units from the form of molecular weight 345 000. 3. When the urea concentration is varied the molecules became fully dissociated at 6M-urea-0.1M-sulphite. Although sulphite is necessary to break disulphide bridges, concentrations greater than 0.1M cause a re-aggregation of the sub-units. Similar results were obtained in guanidine solutions. 4. A new form of arachin has been discovered, A1, migrating more rapidly than arachin A. 5. The *N*-terminal residues of arachin have been re-investigated on more highly purified samples: they are glycine, valine and (iso)leucine in the proportions 4:1:1. 6. The three forms of arachin have the structure (B) $\beta_4\gamma\delta$, (A) $\alpha_2\beta_2\gamma\delta$ and (A1) $\alpha_4\gamma\delta$, for the forms of molecular weight 170 000. 7. Dissociation in 8M-urea produces some fragments, detected by gel electrophoresis, which appear to be dimers of the type α -S-S- β , β -S-S- β , held together by disulphide bonds.

Arachin, the major protein of the groundnut, *Arachis hypogaea*, is a well-characterized protein, with a molecular weight about 330 000 (Johnson & Shooter, 1950) and the ability to dissociate reversibly to the so-called 'monomer' form of molecular weight 180 000 and irreversibly to still smaller fragments at extremes of pH.

Not all groundnuts contain the same arachin, and at least two forms of the protein exist. They can be distinguished by polyacrylamide-gel electrophoresis, arachin A migrating slightly ahead of arachin B at pH 8.6 (Tombs, 1963, 1965*a*). The two proteins had very similar amino acid compositions and general properties, but electrophoresis of the dissociated proteins showed a difference in their sub-unit composition. Arachin B produced three chains, β , γ and δ ; arachin A produced almost equal amounts of α and β chains, as well as the γ and δ chains.

The data available suggested that the structure of arachin B was probably $\beta_8\gamma_2\delta_2$ chains, and that of arachin A $\alpha_4\beta_4\gamma_2\delta_2$ for the forms of molecular weight 330 000. There were several uncertainties, however, especially in the numbers of γ and δ chains, two of each being the minimum possible.

We have now used osmometry to determine the total number of sub-units, and re-investigated the *N*-terminal residues of arachin. Also, there was clearly a possibility that a form of the protein

containing a preponderance of α chains might exist, and a search has been made for it.

EXPERIMENTAL

Materials. Arachin was prepared as previously described; an extract of groundnut meal was brought to 40% saturation with $(\text{NH}_4)_2\text{SO}_4$, and the precipitate regarded as crude arachin. Alternatively arachin was isolated from the extract by Ca^{2+} pptn. Both preparations were further purified by filtration on G100 Sephadex. This removed a small amount of non-protein material and a trace of low-molecular-weight protein.

African groundnut varieties were a gift from Mr W. E. Stonebridge, Agricultural Research Station, Kano, Nigeria. The Virginia nuts were the same as used previously.

Osmometry. The osmometer used was a commercial version (Nash-Thompson Ltd.) of that described by Rowe & Abrahams (1957). Membranes were made from collodion as described by them. This osmometer measures the rate of solvent transfer across the membrane, and before each run a check was made to ascertain that zero response (a galvanometer deflexion) corresponded to zero applied pressure, with solvent on both sides of the membrane. This provided a check on the sensitivity of the machine, and an estimate of the zero error (± 0.2 mm. H_2O). After a series of measurements, usually on a range of protein concentrations from 0.1 to 2% (w/v), solvent was again placed on both sides of the membrane, and the zero response measured again. This provided a check on possible membrane permeation, which did not occur.

Concentrations. Since many measurements were made in

8M-urea solution, and the procedure involved dialysis, this presented problems in the determination of protein concentration. The ultraviolet extinction at 278 m μ was used, with specific extinction coefficients separately determined. The sample of arachin used contained 18.4% of nitrogen by micro-Kjeldahl, after correction for moisture content, and, in 8M-urea-0.1M-sulphite, $E_{1\text{cm}}^{1\%}$ was 7.98 (mean of 6); in 6M-guanidine hydrochloride-0.1M sulphite $E_{1\text{cm}}^{1\%}$ was 7.85 (mean of 6) with an estimated error ± 0.1 . We assumed that E was not affected by urea concentration in the range 4–8M: the error introduced is unlikely to have been significant.

The presence of sulphite had no effect on the absorptions. Generally, approx. 2% solutions of arachin were made in the appropriate solvent, and dialysed against it overnight. There was negligible loss of ultraviolet-absorbing material from the sac. Then a series of dilutions were made of the contents of the sac. A complete set of determinations required 80mg. of protein.

Electrophoresis. This was performed in polyacrylamide gels, as described previously (Tombs, 1965a,b). Unless otherwise stated a discontinuous buffer system was used, with tris-citrate (0.075M-tris-0.0025M-citric acid, pH 8.6) in the gels and sodium borate (0.03M-sodium borate, pH 8.6) in the buffer tanks.

Determination of thiols and disulphide. Disulphides were determined by reaction with sulphite and *p*-chloromercuribenzoate according to the equations:

In a typical experiment 31.4mg. of arachin in 12ml. of 4M-urea was caused to react with excess of sulphite and *p*-chloromercuribenzoate. Unchanged mercuribenzoate was then determined by back-titration with dithizone in carbon tetrachloride by the method of Fridovich & Handler (1957).

Disulphide splitting with dithiodiglycol was carried out by the method of Smithies (1965) in the presence of mercaptoethanol;

100mg. of arachin in 5ml. of 8M-urea-tris-citrate was incubated with 0.01ml. of dithiodiglycol and 0.004ml. of mercaptoethanol at 37° for 2hr.

N-Terminal analyses. These were done by the phenylthiohydantoin method, substantially as described by Fraenkel-Conrat, Harris & Levy (1955) and Tombs (1965a). Arachin was allowed to react with 3ml. of isothiocyanatobenzene in 80ml. of 10M-formamide, pH 9.0, excess of reagent extracted with cyclohexane and the substituted protein precipitated by dialysis against water. The precipitate was then suspended in 100ml. of N-HCl and refluxed for 1hr. Finally the phenylthiohydantoin derivatives were extracted with ethyl acetate, concentrated by rotary evaporation and examined by chromatography on Whatman no. 1 paper. Two solvent systems were used: pyridine-heptane (2:3, v/v) and the upper layer from butanol-formic acid-heptane (2:1:2, by vol.). Markers containing phenylthiohydantoin-glycine, -valine and -leucine in the molar proportions 4:1:1, 3:2:1 and 3:1:2 were run for comparison. Two detection methods were used: iodine-azide reagent (Fraenkel-Conrat *et al.* 1955)

and permanganate (2N-KMnO₄ in N-H₂SO₄) followed by washing in tap water (Hanson & Whittaker, 1958). The latter method is said to be more quantitative. The chromatograms were subsequently scanned in a Joyce Loebel microdensitometer.

RESULTS

Osmotic pressures. The osmotic pressure, and hence the number-average molecular weight of arachin, was determined in a variety of dissociating solvents. To correct for non-ideality it was necessary to extrapolate the data to zero concentration of protein, and where appropriate results were fitted to the standard equation (1).

$$\pi/c = \frac{RT}{M} (1 + Bc), \quad (1)$$

where M is a number-average molecular weight here and elsewhere. Plots of π/c against c for a series of urea concentrations are shown in Fig. 1, and values of M in Table 1. RT has the value 2.52×10^5 for pressures in cm. water, at 25° with concentrations in g./100ml. of solution. Except where the π/c vs. c plot showed obvious curvature results were fitted to the regression line $y = a + bx$, where $y = \pi/c$, $x = c$ and $B = b/a$. The errors shown in Table 1 are the residual standard deviations of y on x . The main objective of the osmotic pressure measurement was to determine the total number of sub-units in arachin. This can be calculated from the number-average molecular weight of the products of dissociation and the molecular weight of

Fig. 1. π/c against c plots for arachin in the solvent indicated, all in the presence of 0.1M-sodium sulphite.

Table 1. *Osmotic pressure results with arachin in various solvents*

Solvent	$\bar{M}_n$	ν (330 000g)	ν (345 000g)	B	B^*
4M-Urea-0.1M-SO ₃ ²⁻	60570 ± 3000	5.45	5.69	0.08	8
6M-Urea-0.1M-SO ₃ ²⁻	30030 ± 1200	10.99	11.49	0.04	4
8M-Urea-0.1M-SO ₃ ²⁻	30030 ± 200	10.99	11.49	0.18	18
8M-Urea-tris-citrate	51766 ± 2000	6.38	6.66	0.27	27
8M-Urea (initial)	71000	4.65	4.86	—	—
8M-Urea (7 days)	74100	4.45	4.65	—	—
8M-Urea-0.05M-SO ₃ ²⁻	32300	10.20	10.68	—	—
8M-Urea-0.5M-SO ₃ ²⁻	45000	7.33	7.67	—	—
8M-Urea-0.1M-SO ₃ ²⁻	90000	3.67	3.83	—	—
8M-Urea (after reaction with dithiodiglycol)	33820 ± 1100	9.76	10.20	0.38	38
4M-Guanidine-HCl	66840 ± 5000	4.94	5.16	0.63	63
4M-Guanidine-HCl-0.1M-SO ₃ ²⁻	28700 ± 400	11.49	12.0	0.31	31
4M-Guanidine-HCl-0.5M-SO ₃ ²⁻	52500	6.29	6.56	—	—
6M-Guanidine-HCl-0.1M-SO ₃ ²⁻	30430 ± 2000	10.80	11.33	0.83	83

the intact molecule (M_i). Consider a molecule containing, for example, three different types of chain, dissociating according to $\alpha, \gamma, \delta, \rightarrow x\alpha + y\gamma + z\delta$.

If the system is completely dissociated the number-average molecular weight, which is the quantity determined by osmotic pressure measurements, is:

$$M_d = \frac{xM_\alpha + yM_\gamma + zM_\delta}{x + y + z} \quad (2)$$

where M_α etc. are the molecular weights of the individual sub-units. However,

$$M_i = xM_\alpha + yM_\gamma + zM_\delta \quad (3)$$

and therefore

$$\frac{M_i}{M_d} = x + y + z = \nu$$

where ν is the total number of fragments of the original molecule produced in the dissociation.

If the dissociation is partial, for example if a proportion (h) of the intact molecule remains, it can easily be shown that

$$\frac{M_i}{M_d} = (x + y + z)(1 - h) = \nu(1 - h)$$

again giving the number of particles produced, on average, from the original intact molecule.

In general, the ratio M_i/M_d can always be used to determine the average number of fragments produced; ν need not be integral and can indeed be less than 1, if aggregation occurs. Particularly if the value of M_d for the fully dissociated protein can be obtained, the total number of sub-units can be found, however many kinds of sub-unit there are.

To calculate ν , the value of M_i is necessary.

According to Johnson & Shooter (1950) the 'dimer' form has molecular weight 330 000 by sedimentation-diffusion methods. They also give a value of 180 000 for the monomer, implying a value of 360 000 for the 'dimer'. The mean of these values (345 000) has been used for the calculations for Figs. 3 and 4; values calculated from both 330 000 and 345 000 are shown in Table 1.

To dissociate a protein fully it is necessary not only to disrupt non-covalent links such as hydrogen or 'hydrophobic' bonds, but also to minimize electrostatic interactions and to break interchain disulphide bonds. For this reason most measurements were made in the presence of 0.1M-sodium sulphite, which splits disulphide bonds, especially those between chains (Cecil, 1963), and also provides sufficient ionic strength to minimize Donnan-equilibria effects. Urea and guanidine hydrochloride were chosen as dissociating solvents: Fig. 3 shows that ν was 5.5 at 4M-urea, rose to 11.5 at 6M-urea and remained at that value on raising the concentration to 8M-urea. In both 4M- and 6M-guanidine a value 11.5-12.0 was obtained: these results suggest that ν had a maximum value 11.5-12.0 and that the molecule was fully dissociated under these conditions. There was no indication that more powerful dissociating conditions would produce an increase in ν .

We also investigated the effect of varying sulphite concentration. Some π/c vs. c plots are given in Fig. 2, and they show obvious curvature. At 0.05M- and 0.5M-sulphite there was aggregation on dilution of the protein; at 1.0M-sulphite the opposite occurred and there was clearly dissociation on dilution. The extrapolations shown in Fig. 2 were used to estimate M_d for the protein at concentrations high enough to avoid dilution effects

Fig. 2. π/c against c plots, all in 8M-urea with various sulphite concentrations (as shown). Broken lines are approximate extrapolations of the flat part of the curves.

Fig. 3. Plot of M_i/M_d (ν) against urea (○) or guanidine hydrochloride (●) concentration. All points were determined in the presence of 0.1 M-sodium sulphite.

and the value of ν so calculated is shown in Fig. 4. This Figure also shows ν in the absence of sulphite, and a value for 4M-guanidine-0.5M-sulphite. The same aggregating effect seemed to occur in both urea and guanidine.

The sharp increase in ν on adding sulphite up to 0.1 M was strong evidence for the presence of inter-chain disulphide bonds but the sharp decrease in ν at higher sulphite concentrations was unexpected.

Fig. 4. Variation of M_i/M_d (ν) with sulphite concentration. All points were measured in 8M-urea (○) or 4M-guanidine hydrochloride (●).

The curve suggested, however, that ν reaches a maximum of 12.

Values of B calculated from eqn. 1 are also shown in Table 1 and B^* is its value when converted into c.g.s. units (pressures in dynes/cm.² and concentrations in g./ml.). Values from 3 to 10 (4M-, 6M-urea) can be interpreted as indicating roughly spherical molecules, but the higher values in guanidine and 8M-urea solution suggested greater asymmetry.

N-Terminal amino acids. The *N*-terminal amino acids of arachin were determined by Cater, Naismith, Thompson & Ure (1957), who found glutamate, valine, leucine and glycine; Tombs (1965a) found glycine, valine and glutamate by the DNP method, and glycine, glutamate and isoleucine by the phenylthiohydantoin method. By both methods glycine was the major *N*-terminal residue. In the present sample of arachin, glutamate was no longer *N*-terminal, and spots due to glycine and two others were seen on the chromatogram. The main difference between this sample and those used previously was that it had been purified by Sephadex filtration. A sample of glycinin, a similar protein from soya beans, also lost an apparent *N*-terminal glutamate on Sephadex filtration (M. P. Tombs, unpublished work). This suggests that the *N*-terminal glutamate previously reported was an artifact, possibly due to contamination by a peptide or free glutamic acid.

The difficulty of resolving phenylthiohydantoin-valine, -leucine and -isoleucine is well known, and

Table 2. Ratio of the N-terminal residues of arachin: chromatography of phenylthiohydantoin derivatives

Results are in arbitrary units, and are the means of the number of determinations indicated.

	Glycine	Valine	Leucine	No. of determinations
Arachin	4.0	1.1	1.3	9
Standard mixture (4:1:1)	4.0	1.1	1.2	5
Standard mixture (3:2:1)	3.0	1.6	0.9	5
Standard mixture (3:1:2)	3.0	1.1	1.7	5

the two minor spots were identified as two of these three, almost certainly valine, and either leucine or isoleucine. The ratio of the three N-terminal residues was determined by comparison with marker mixtures of phenylthiohydantoin-glycine, -valine and -leucine in the proportions 4:1:1, 3:1:2 and 3:2:1. These were the only three possibilities and, on inspection, the arachin N-terminal residues clearly corresponded to the proportions 4:1:1. However, the chromatogram was also scanned. The results are shown in Table 2, where the data are in arbitrary units based on the height of the peaks in the scan, and again show that the proportions 4:1:1 corresponded most closely to the N-terminal residues of arachin.

Arachin in 8M-urea. Treatment with 8M-urea produces substantial dissociation of arachin, with values of ν ranging from 4.9 in unbuffered urea to 6.4 in urea buffered with tris-citrate. This nevertheless represents only a partial dissociation, and the further increase in ν on treatment with sulphite showed the presence of interchain disulphide bonds.

It is possible to calculate the number of such links; thus M_2 in 8M-urea-tris-citrate is 51800; M_s , the number-average molecular weight of the fully dissociated sub-units, is 28700. There are therefore 51800/28700, i.e. 1.8 chains, on the average in the product of urea dissociation. Since at least one disulphide bond is required to form a dimer, two to form a trimer and so on, there is therefore at least an average of 0.8 interchain disulphide bond in the products of dissociation.

Similar calculations for unbuffered 8M-urea (pH 7.3) showed ν of 4.9 and an average of 2.5 sub-unit chains in the dissociation product, with at least 1.5 interchain disulphide bonds; 4M-guanidine hydrochloride gave similar values.

A direct determination of the number of disulphide bonds with sulphite and mercuribenzoate gave a value of 11.4 disulphide bonds/330 000g. of protein in 8M-urea. No attempt was made to exclude oxygen from the arachin solution.

Fig. 5. Electrophoretic analysis of arachin. (1) Arachin in 8M-urea. (2) The same sample, but after reaction with dithiodiglycol, both run in 8M-urea-tris-citrate in a 12% gel. (3) Arachin in tris-citrate buffer. (4) The same sample treated with sodium sulphite: both run in tris-citrate in a 10% gel. (6) Arachin dissolved in 8M-urea, then dialysed into tris-citrate. (5) The same sample, after reaction with sulphite and mercuric chloride before dialysis.

Partly dissociated arachin, for example in tris-citrate buffers, contains traces of the sub-units as detected by electrophoresis (Tombs 1965a) and also forms which are intermediate in mobility between the sub-units and the monomer form. This is illustrated in Fig. 5, where the species in question are labelled ' $\alpha\beta$ and β_2 dimer'. From their mobility and general behaviour they appeared to contain two of the main sub-unit chains.

Fig. 5 shows electrophoretic patterns of arachin dissolved in 8M-urea-tris-citrate and run in the same solvent, and after dialysis into tris-citrate, and running in that solvent. In both cases, the $\alpha\beta$ and β_2 dimer forms were prominent, though there was also some general aggregation. Fig. 5 also shows the effect of three treatments (sulphite, sulphite and mercuric chloride and a disulphide-interchange reaction with dithiodiglycol), each designed to split disulphide bonds. In each case the ' β_2 and $\alpha\beta$ dimer' zones were greatly reduced in intensity and the α

Fig. 6. Electrophoretic analysis of the total protein from individual groundnuts. Tris-citrate buffer, 7.5% acrylamide gels. The difference between the nuts is shown most clearly in samples 31 and 32.

Fig. 7. Electrophoretic analysis of the same samples as 31 and 32 in Fig. 6, but after depolymerization and in 10% gels. V is a typical Virginia nut.

and β chains appeared or were intensified. The γ and δ zones were unaffected. Some aggregate still remained at the origins, however. A determination of M_d after reaction with dithiodiglycol (Table 1) showed ν to be 10.2, and the ratio M_d/M_s became 1.2, indicating that 0.2 interchain disulphide bond

remained in the dissociation products. The reaction was therefore not complete, in agreement with the electrophoretic pattern.

All these results, however, showed clearly that the β_2 and the ' $\alpha\beta$ dimer' forms contain interchain disulphide bonds, and that the γ and δ chains were apparently not involved.

Arachin A1. Two electrophoretically distinct forms of arachin, B and A, have been described previously (Tombs, 1963, 1965a) in a Virginia strain of groundnut. Further search among African strains has now been made, and another arachin migrating faster than arachin A has been discovered. Electrophoretic patterns of the total protein from single nuts are shown in Fig. 6, and show the three types of arachin. Nuts containing only one arachin seem to be very rare, and sample 31 (Fig. 6) shows an extreme case of the presence of A1, compared with an extreme B. It was notable that in this case both the 'dimer' and the monomer forms migrated more rapidly than the other types. Fig. 7 shows an electrophoretic pattern of the same two nuts after dissociation with formamide and mercaptoethanol (Tombs, 1965a). Clearly arachin A1 contains more α chain than the other two types.

DISCUSSION

Osmometry has an advantage in determining the total number of sub-units in a protein because it yields number-average molecular weights. These have several advantages, particularly that M_w/M_n permits the determination of the total number of fragments produced on dissociation no matter how many different sorts of fragments there are. This is useful where a protein is known to contain more than one type of chain. A similar point has been made by Kawahara & Tanford (1966).

We estimate that the overall error of our determination, including the uncertainty of M_d , was of the order of ± 0.5 sub-unit. For the fully dissociated protein ν must be integral and, in its 'dimer' form, even. The maximum value of ν in urea and guanidine solution, and a range of sulphite concentrations, was 12. The nature of the curves in Figs. 3 and 4 also offers some evidence that more extreme dissociating conditions would not have produced a higher value, although it is not easy to prove conclusively that a protein is fully dissociated. We conclude that arachin in its 'dimer' form of molecular weight 345 000 contains 12 sub-units, and the 'monomer' six.

To avoid confusion the form previously called 'dimer' will now be called the dodecamer, and the 'monomer' will be called the hexamer.

The ratios of the N-terminal residues showed that the hexamer contains four chains with N-terminal glycine, one with N-terminal valine and one with

N-terminal (iso)leucine. It does not follow directly from this that there are only three types of chain (there could be six), and in fact there are either three or four, depending on the variety of arachin.

Thus Tombs (1965a) showed that by electrophoresis four zones could be detected in dissociated arachin A, called α , β , γ and δ chains, but only three in arachin B (β , γ and δ chains). Mainly from electrophoresis results it appeared that arachin B had the minimum probable structure $\beta_4\gamma\delta$ and arachin A $\alpha_2\beta_2\gamma\delta$, whereas both α and β chains had *N*-terminal glycine. We have now found some African varieties which have an arachin (A1) containing only α , γ and δ chains, migrating faster than A.

Thus in arachin B there are six chains to be distributed amongst three kinds, in the proportions 4:1:1. The possible structures are $\beta_4\gamma\delta$, $\beta_3\gamma_2\delta$ and $\beta_2\gamma_3\delta$, but it is impossible to account for the existence of the other forms of arachin unless the structure is $\beta_4\gamma\delta$, making arachin A $\alpha_2\beta_2\gamma\delta$ and arachin A1 $\alpha_4\gamma\delta$. Tombs (1965a) showed also that the α and β chains make up the major part of the arachin molecule, so that the proposed structures are the only ones consistent with the results.

There are five possible hexamers ($\beta_4\gamma\delta$, $\beta_3\alpha\gamma\delta$, $\beta_2\alpha_2\gamma\delta$, $\beta_2\alpha_3\gamma\delta$ and $\alpha_4\gamma\delta$) but only three of these have been detected. In contrast, in the similar case of lactic dehydrogenase, where two types of sub-unit combine to form tetramers, all five possible forms occur (Fritz & Jacobson, 1965). The structures of the dodecamers are $\beta_3\gamma_2\delta_2$, $\beta_2\alpha_2\gamma_2\delta_2$, $\beta_4\alpha_4\gamma_2\delta_2$, $\beta_2\alpha_6\gamma_2\delta_2$ and $\alpha_8\gamma_2\delta_2$.

Behaviour in 8M-urea and disulphide bonds. The intact molecule does not contain interchain disulphide bonds (Tombs, 1965a), but that there are interchain disulphide links in 8M-urea is evident both from the effect of sulphite and dithiodiglycol in raising ν , and from the results of electrophoresis of arachin after treatment with disulphide-splitting reagents. With only seven thiol groups (Tombs, 1965a) to form disulphides the simple scheme for dissociation in 8M-urea: $\alpha_8\gamma_2\delta_2 \rightarrow 4 \alpha\text{-S-S-}\alpha + 2\gamma + 2\delta$, which requires ν of 8 (as compared with 6.4 found) and the (S-S) value to be 0.5 (0.8 found), is not adequate. Nor can it account for the quite large aggregates which are clearly present by the electrophoresis criterion. Larger aggregates must be formed through disulphide-interchange reactions.

From their electrophoretic mobility, and their sedimentation coefficients (Tombs, 1965a), the $\alpha_2\beta_2$ and $\alpha\beta$ dimers are intermediate in size between the hexamer and the sub-unit. Given that the γ and δ chains were not involved, and that disulphides were present, the most probable compositions are $\alpha\text{-S-S-}\alpha$, $\alpha\text{-S-S-}\beta$, $\beta\text{-S-S-}\beta$, though many other forms could exist.

The aggregating effect of high concentrations of sulphite even in 8M-urea or 4M-guanidine was unexpected. A possible explanation is that at high concentration sulphite causes interchange reactions between intramolecular disulphide bonds and free thiol groups, and it is possible that even higher concentrations of sulphite would split all the disulphide bonds. Another possibility is that SO_4^{2-} ions, certainly present at the concentrations of sulphite used, had a specific effect. Sulphate is known to affect strongly the dissociation of arachin (Johnson & Shooter, 1950).

It seems to be rare for a globular protein to contain more than two types of chain, or for any stable form to contain an odd number of chains (Klotz, 1967). In arachin, the hexamer contains odd numbers of γ and δ chains though the dodecamer is symmetrical. It may be that the most stable form of a protein will contain not only an even number of sub-units, but an even number of each kind of sub-unit.

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