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INFLUENCE OF SINGLE AMINO ACID SUBSTITUTIONS ON ELECTROPHORETIC MOBILITY OF SODIUM DODECYL SULFATE-PROTEIN COMPLEXES

Wilfried W. de Jong, Anneke Zweers and Louis H. Cohen*

Department of Biochemistry, University of Nijmegen,
6500 HB Nijmegen, The Netherlands

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SUMMARY: The substitutions Thr → Ala, Gln → Leu and Pro → Thr or Ala in mammalian α-crystallin A chains (19,830 daltons) are found to increase the electrophoretic mobility in sodium dodecyl sulfate gel electrophoresis. Substitutions between residues of like hydrophobicity and small changes in intrinsic charge of the chain did not alter the mobility. Changes in hydrophobicity appear to influence the binding of sodium dodecyl sulfate, and therefore the mobility, whereas proline may affect the conformation of the sodium dodecyl sulfate-protein complex. These effects may depend on the position of the substitution in the chain. Sodium dodecyl sulfate gel electrophoresis is thus able to detect neutral substitutions not usually visible in regular electrophoresis.

In the course of studies on the biosynthesis of rat lens proteins (1) it was incidentally observed that the α-crystallin A chain of the rat migrates faster during SDS[+] polyacrylamide gel electrophoresis than the corresponding bovine chain. Both chains have a length of 173 residues, corresponding to a molecular weight of 19,830, and differ only by seven amino acid substitutions, not involving any charged residues (2). Such small differences are not expected to influence the mobility in SDS gel electrophoresis, although the theoretical basis of the method is still unclear (3). Electrostatic (4,5) and hydrophobic (6-8) interactions have both been stressed as major factors in the binding of SDS to proteins, and different models for the SDS-protein complex have been proposed (4, 9-11).

In order to elucidate the cause of the different mobilities in SDS gels of the αA chains of rat and calf we carefully compared the mobilities of several other mammalian αA chains, known to differ in a single or only a few

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*Present address: Department of Biological Chemistry, School of Medicine, UCLA, Los Angeles, California 90024, U.S.A.
[+]SDS: sodium dodecyl sulfate.

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residues. The results allow us to conclude that single amino acid substitutions involving changes in side chain hydrophobicity or involving prolyl residues may have a considerable effect on the electrophoretic mobility of α-crystallin A chains.

MATERIALS AND METHODS

Samples of α-crystallin from the following species with known αA chain sequences were used: calf (*Bos taurus*), pig (*Sus scrofa*), horse (*Equus caballus*), dog (*Canis familiaris*), rabbit (*Oryctolagus cuniculus*), rat (*Rattus norvegicus*), elephant (*Loxodonta africana*), hyrax (*Procavia capensis*), rhinoceros (*Ceratotherium simum*), whale (*Balaenoptera acutorostrata*), guinea pig (*Cavia porcellus*), lemur (*Lemur fulvus*) and pika (*Ochotona princeps*) (2, 12 and unpublished data of W.W. de J., A.Z., M. Goodman and M.C. McKenna). Aminoethylation, carboxymethylation and carboxamidomethylation of calf and elephant α-crystallin was carried out according to standard procedures (13).

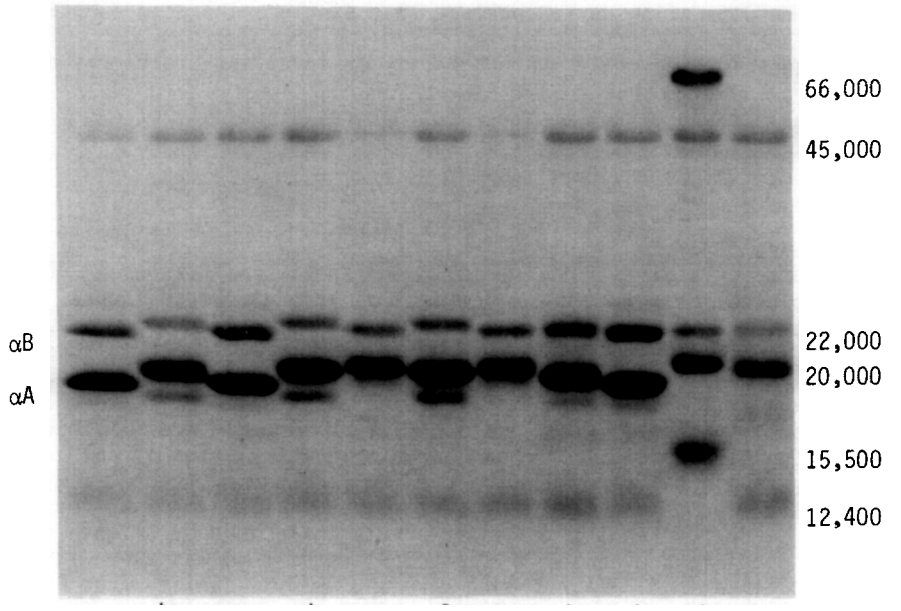
Chemicals and procedures for SDS gel electrophoresis are described in some detail because the results are considerably influenced by slight variations in conditions. The SDS-Tris-glycine system (14) was used with significant modifications. All reagents were zur Analyse quality from Merck, apart from glycine (Merck, für medizinische Zwecke) and SDS (Merck, für biochemische Zwecke). Acrylamide and methylenebisacrylamide were recrystallized and solutions were used within two months.

A Biorad model 220 dual vertical slab gel electrophoresis cell was used. The dimensions of the separation gels were 95 x 160 x 0.5 mm. For two 15% acrylamide slab gels 9.75 ml of acrylamide-methylenebisacrylamide solution (40% - 1.07%), 7.50 ml of 1.5 M Tris-HCl (pH 8.8), 0.30 ml of 10% SDS and 8.55 ml of water were mixed and deaerated. After adding 100 µl of 10% tetramethylethylenediamine and 75 µl of 10% ammonium persulfate (freshly dissolved) the mixture was poured between the glass-plates, overlayered with 0.1% SDS and left to polymerize overnight. The stacking gel was poured from 1.0 ml of acrylamide-methylenebisacrylamide solution (40% - 1.07%), 2.5 ml of 0.5 M Tris-HCl (pH 6.8), 0.1 ml of 10% SDS and 6.3 ml of water, with 100 µl of 10% tetramethylethylenediamine and 50 µl of 10% ammonium persulfate added after mixing and deaerating. The height of the stacking gel under the sample slots was 1 cm. Samples were loaded directly after polymerization of the stacking gel (which takes about 0.5 h).

The sample buffer consisted of 2 ml of 10% SDS, 100 mg of dithioerythritol, 1 ml of glycerol, 2 ml of water and a few drops of bromphenol blue. Samples were dissolved in sample buffer at a concentration of 2-4 µg per µl and heated for 2 h at 65°C or 3 min at 100°C (which made no difference for the final result). Sample solutions (2-4 µl per 4 mm slot) were loaded with a 10 µl Hamilton syringe. The reservoir buffers contained 3 g of Tris and 14.4 g of glycine per liter, and the upper reservoir buffer in addition 1 g of SDS per liter. During stacking the current was 15 mA for two slab gels, thereafter 30 mA. Total electrophoresis time was about 6 h. The electrophoresis apparatus was cooled with running tap water (12-14°C). Gels were stained with Coomassie brilliant blue (15).

RESULTS AND DISCUSSION

The SDS gel electrophoretic pattern shown in Fig. 1 most clearly reveals the effect of single amino acid substitutions on the mobility of α-crystallin A chains. The rat and guinea pig αA chains differ only by the substitution in



	a	b	c	d	e	f	g	h	i	j	k
13	Ala	Ala	Ala	Ala	Thr	Ala	Thr	Ala	Ala		
90	Leu	Gln	Leu	Gln	Gln	Gln	Gln	Gln	Leu		
101	Asn	Asn	Asn	Asn	Asn	Asn	Asn	Ser	Asn		

Figure 1: SDS gel electrophoretic pattern of α-crystallins from rat (a, c, i), guinea pig (b, d, f), rabbit (e, g), pika (h), horse (k) and marker proteins (j) of molecular weights 66,000 (bovine serum albumin), 45,000 (ovalbumin), 20,000 (calf α-crystallin A chain) and 15,500 (hemoglobin). The bovine α-crystallin B chain (20,070 daltons) has an apparent molecular weight of 22,000 (20). Ovalbumin and cytochrome c (0.5 μg of each) were added to all α-crystallin samples. Sequence differences between the α-crystallin A chains, in positions 13, 90 and 101, are indicated.

position 90 of leucine in rat for glutamine in guinea pig. This substitution results in a change of about 3% in the mobility of the αA chain. The guinea pig αA chain differs again in a single position from the rabbit αA chain by the substitution of residue 13-alanine for threonine. This substitution gives a smaller but clearly visible change of about 1.5% in the electrophoretic mobility. Also shown is the αA chain of the pika which moves approximately in the same position as guinea pig αA chain, although they differ by the substitution 101 Asn-Ser. The differences in mobility between pika αA and rabbit and rat αA chain correspond again to the substitutions 13 Thr-Ala and

90 Gln-Leu, respectively, which are present in addition to the substitution 101 Asn-Ser.

These observations were extended by comparing the electrophoretic mobilities of the αA chains of some other mammalian species differing in a limited number of residues. The differences in amino acid sequence of adjacent αA chains, shown in Fig. 2, are given in Table 1 and reveal that replacement of a hydrophobic for a hydrophilic residue (13 Thr-Ala; 90 Gln-Leu) results in an increased mobility. The hydrophobic residues leucine, isoleucine, valine and methionine can apparently be interchanged without influencing the electrophoretic mobility. The same is true for the hydrophilic residues threonine, serine, asparagine and glycine.

Proline, despite its hydrophobic character, visibly decreases the mobility of the αA chain when it replaces residue 13-Thr or 13-Ala, as can be seen from the comparison of lemur, rabbit and guinea pig αA chains.

Substitutions involving charge differences are rare among the αA chains (16), and their influence on mobility in SDS electrophoresis could not be assessed. However, the αA chain normally occurs in α-crystallin in two charge forms: αA_1 and αA_2, where αA_1 is thought to derive from αA_2 by deamidation of two amide residues (17). Both chains migrate as a single band in SDS electrophoresis, which implies that these substitutions of negatively charged for uncharged hydrophilic residues do not influence the mobility. Also the blocking of the single cysteine in bovine αA or the two cysteines in elephant αA by the cationic aminoethyl group, the anionic carboxymethyl group or the uncharged carboxamidomethyl group makes no difference for the mobility in SDS gel electrophoresis.

How can these findings be understood in the light of current knowledge about SDS-protein interaction? The constant binding ratio of approximately 1.4 g of SDS per g of protein (corresponding to about 1 molecule of SDS per two amino acid residues) is supposed to swamp out the intrinsic charge of proteins, resulting in a constant charge per unit mass for most SDS-protein

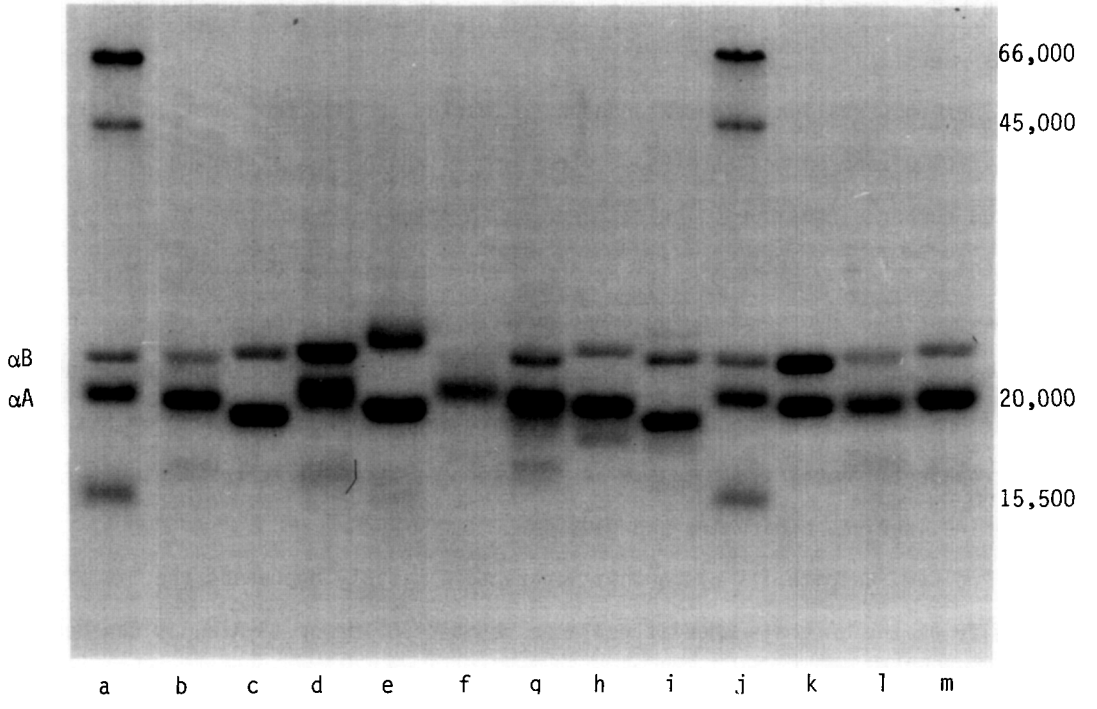


Figure 2: SDS gel electrophoretic patterns of α-crystallins from a. calf (plus marker proteins); b. whale; c. dog; d. elephant; e. hyrax; f. lemur; g. rabbit; h. guinea pig; i. rat; j. calf (plus markers); k. pig; l. horse; m. rhinoceros.

Table 1: Differences in the amino acid sequences of α-crystallin A chains of which the SDS gel electrophoretic patterns are shown in Fig. 2. Only the sequence differences within the four groups of adjacent αA chains are tabulated.

Position	13	72	90	146	150
Calf	Thr		Gln	Ile	Val
Whale	Ala		Gln	Val	Met
Dog	Ala		Leu	Val	Val
Elephant		Val	Gln	Ile	
Hyrax		Leu	Leu	Val	

	13	55	61	90	127	133	146	150	168	172
Lemur	Pro		Val	Gln						
Rabbit	Thr		Ile	Gln						
Guinea pig	Ala		Ile	Gln						
Rat	Ala		Ile	Leu						
Calf	Thr	Thr	Ile		Ser	Leu	Ile	Val	Ser	Ser
Pig	Ala	Thr	Val		Ser	Leu	Val	Val	Ser	Thr
Horse	Ala	Thr	Ile		Thr	Val	Ile	Met	Gly	Ser
Rhinoceros	Thr	Ser	Val		Thr	Leu	Ile	Met	Ser	Ser

complexes (18,19). Several examples are nonetheless known of homologous proteins of the same molecular weight but showing different mobilities in SDS gel electrophoresis: hemoglobin α and β chains (15), α-crystallin A and B

chains (20), parvalbumins (21), and cucumber virus coat proteins (22). In these cases the electrophoretic mobilities are thought to be influenced by differences in intrinsic charge or conformation, caused by the considerable differences in amino acid sequences.

In the case of the substitutions Thr → Ala and Gln → Leu in the αA chain there is neither a change in charge nor, most probably, in conformation. The faster electrophoretic mobility can therefore only be due to an increase in the negative charge of the complex by the binding of more SDS to the protein. Studies on the interaction of SDS with free amino acids have shown that acidic and neutral hydrophilic amino acids bind no SDS at all; some SDS is apparently bound to alanine and proline, but basic and hydrophobic amino acids bind most SDS (23). This agrees with the finding that a hydrophobic fragment of cytochrome b_5 binds 2.5 times the normal amount of SDS (8), which must correspond to 2-3 molecules of SDS per hydrophobic side chain.

The replacement of threonine by alanine means that at least one additional molecule of SDS can be bound to the αA chain, and in the case of the Gln → Leu replacement perhaps two or three molecules of SDS. The αA chain will bind approximately 85 molecules of SDS. The net intrinsic charge of an αA_2 chain at pH 8.8 is approximately six negative charge units. The addition of one to three molecules of SDS to the αA chain-SDS complex thus leads to an increase of about 1 to 3% in net negative charge, which may well account for the observed increase in mobility.

Paradoxically then is the finding that small changes in intrinsic charge of the αA chain do not influence the mobility. It is known that negative maleyl, succinyl or carboxymethyl groups lead, by electrostatic repulsion, to a lower binding of SDS and to a variable degree of reduction in electrophoretic mobility (18,22,24). The loss of a single amino group in a Qβ coat protein mutant gives a 1.5% faster mobility in SDS gels (25), whereas ε-acetylation of lysines in histones has no such influence (26). It is conceivable that the effects of small changes in charge or hydrophobicity on the mobility of

proteins in SDS gels are influenced by neighbouring residues, because SDS molecules seem to be unevenly distributed along the polypeptide chain (6,8,24). Likewise the effect of prolyl residues, which possibly influence the final conformation and thereby the frictional drag of the SDS-protein complex by interfering with the α-helix structure which predominates in the complex (4,6), may well depend on their position in the chain.

Our observations emphasize that differences in SDS gel mobility between closely related proteins should be interpreted with caution, and do not necessarily reflect changes in polypeptide length. On the other hand it opens the possibility to detect in proteins a number of neutral amino acid substitutions which can not be detected by regular electrophoresis. This is exemplified in Figs. 1 and 2 where also a considerable variation is shown in mobility of the α-crystallin B chains of different species, which are as yet of unknown sequence, apart from the bovine chain (20). These αB chains are all indistinguishable on alkaline urea gel electrophoresis (2,12), but apparently contain a considerable number of neutral substitutions.

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REFERENCES

1. Cohen, L.H., Smits, D.P.E.M., and Bloemendal, H. (1976) Eur. J. Biochem. 67, 563-572.
2. De Jong, W.W., Van der Ouderaa, F.J., Versteeg, M.L., Groenewoud, G., Van Amelsvoort, J.M., and Bloemendal, H. (1975) Eur. J. Biochem. 53, 237-242.
3. Maddy, A.H. (1976) J. Theor. Biol. 62, 315-326.
4. Mattice, W.L., Riser, J.M., and Clark, D.S. (1976) Biochemistry 15, 4264-4272.
5. Satake, I., and Yang, J.T. (1973) Biochem. Biophys. Res. Commun. 54, 930-936.
6. Nozaki, Y., Reynolds, J.A., and Tanford, C. (1974) J. Biol. Chem. 249, 4452-4459.
7. Satake, I., and Yang, J.T. (1976) Biopolymers 15, 2263-2275.
8. Robinson, N.C., and Tanford, C. (1975) Biochemistry 14, 369-378.
9. Reynolds, J.A., and Tanford, C. (1970) J. Biol. Chem. 245, 5161-5165.
10. Shirahama, K., Tsujii, K., and Takagi, T. (1974) J. Biochem. 77, 117-123.
11. Wright, A.K., Thompson, M.R., and Miller, R.L. (1975) Biochemistry 14, 3224-3228.
12. De Jong, W.W., Nuij-Terwindt, E.C., and Versteeg, M. (1977) Biochim. Biophys. Acta 491, 573-580.
13. Hirs, C.H.W. (ed.) (1967) Meth. Enzymol. Vol. XI, Academic Press, New York.

14. Laemmli, U.K. (1970) Nature 227, 680-685.
15. Weber, K., and Osborn, M. (1975) in The Proteins (Neurath, H., and Hill, R.L., eds.) Vol. I, pp. 179-223, Academic Press, New York.
16. De Jong, W.W., Gleaves, J.T., and Boulter, D. (1977) J. Mol. Evol. 10, 123-135.
17. Van Kleef, F.S.M., De Jong, W.W., and Hoenders, H. (1975) Nature 258, 264-266.
18. Pitt-Rivers, R., and Impiombato, F.S.A. (1968) Biochem. J. 109, 825-830.
19. Reynolds, J.A., and Tanford, C. (1970) Proc. Nat. Acad. Sci. U.S. 66, 1002-1007.
20. Van der Ouderaa, F.J., De Jong, W.W., Hilderink, A., and Bloemendal, H. (1974) Eur. J. Biochem. 49, 157-168.
21. Sullivan, B., Bonaventura, J., Bonaventura, C., Pennell, L., Elliott, J., Boyum, R., and Lambie, W. (1975) J. Mol. Evol. 5, 103-116.
22. Tung, J.-S., and Knight, C.A. (1972) Anal. Biochem. 48, 153-163.
23. Maley, F., and Guarino, D.U. (1977) Biochem. Biophys. Res. Commun. 77, 1425-1430.
24. Takagi, T., Tsujii, K., and Shirahama, K. (1975) J. Biochem. 77, 939-947.
25. Strauss, E.G., and Kaesberg, P. (1970) Virology 42, 437-452.
26. Panyim, S., and Chalkley, R. (1971) J. Biol. Chem. 246, 7557-7560.