Nearest-neighbour analysis of myoglobin antigenic sites

Nearest-neighbour residues whose replacement can alter the environment of binding-site residue(s) and thus change their characteristics and binding capability

A. Latif KAZIM and M. Zouhair ATASSI

Department of Immunology, Mayo Medical School, Rochester, MN 55901, U.S.A.

(Received 4 December 1979/Accepted 11 August 1980)

By using the known antigenic structure of sperm-whale myoglobin previously determined in this laboratory and the X-ray co-ordinates for the myoglobin molecule, we have calculated the nearest-atom distances between each of the residues of the antigenic sites and all the other amino acids of the myoglobin molecule. These calculations have enabled us to identify the nearest-neighbour residues to each of the residues in the five antigenic sites, and which thus describe the immediate molecular environment of the sites. The influences of chemical changes or replacements in these environmental residues on the binding capacity of an antigenic site, when considered together with replacements directly in the antigenic sites, are expected to account for the major effects and will be extremely useful in explaining the cross-reactions of myoglobins from various species. However, it is stressed that the analysis has limitations due to the qualitative estimates of the effects, the influences of substitutions at once-removed or even at more distant locations (especially when they are cumulative) and finally the influences of any conformational re-adjustments when these occur as a result of the replacement(s).

Recently we presented the results of calculations of the distances between each of the residues in the lysozyme antigenic sites and the amino acids of the entire lysozyme molecule (Atassi & Kazim, 1980). From those calculations the nearest-neighbour residues were identified and their minimum approach distances from the antigenic-site residues were provided. The nearest-neighbour residues then describe the environment that is responsible for the expressed chemical characteristics of an antigenic-site residue and that influences its contribution to the overall binding energy of the site. Substitutions in neighbouring residues resulting in any drastic re-adjustments or alterations in the environment of a site residue can be detrimental to the binding of the site or cause a decrease in its binding capacity, the extent of which will be dependent on the nature and magnitude of the environmental change. The latter is also a function of the distance of the neighbouring residue from the site residue, with the effect decaying rapidly as the distance increases. These are un-

doubtedly the major effects. Nevertheless, we have previously pointed out that influences resulting from replacements within a protein molecule in once-removed interactions and even at distant residues cannot be discounted (Atassi & Kazim, 1980). However, the latter effects were considered to be in general of smaller importance, even though undoubtedly occasional exceptions may arise. Also, conformation re-adjustments may occur as a result of substitutions and their effect should not be disregarded.

In the present work we have made distance calculations for the antigenic-site residues of myoglobin (Mb). The nearest-neighbour residues occurring within a 0.7 nm (7 Å) radius from each antigenic-site residue are presented here.

Methods

The complete antigenic structure of sperm-whale Mb was previously elucidated in this laboratory (Atassi, 1975). The five Mb antigenic sites correspond to continuous portions of its primary structure and are for: site 1, residues 15–22; site 2, residues 56–62; site 3, residues 94–99; site 4, residues

Abbreviation used: Mb, the major chromatographic component no. 10 obtained by chromatography of crystalline sperm-whale myoglobin on CM-cellulose.

113–119; site 5, residues 145–151. Calculations were performed on an Interdata computing system by using the atomic co-ordinates of Mb obtained at 0.2 nm (2 Å) resolution (Kendrew *et al.*, 1961), as reported by Watson (1969). The minimum distances occurring between each of the residues in the

five Mb antigenic sites and all other residues in the molecule as well as the atoms contributing to those distances were calculated as reported for hen lysozyme (Atassi & Kazim, 1980). The primary structure of Mb is from Edmundson (1965) and Romero-Herrera & Lehmann (1974).

Table 1. *Neighbouring residues to antigenic site 1*

The first atom notation refers to the neighbouring residue and the second atom refers to the site residue (for atom notations see Watson, 1969).

Nearest-neighbour residue	Site residue	Minimum distances [nm (Å)] and closest atoms to residues in antigenic site 1							
		Ala-15	Lys-16	Val-17	Glu-18	Ala-19	Asp-20	Val-21	Ala-22
Leu-11		0.64 (6.4)* CA-CA							
His-12		0.45 (4.5) CA-CB	0.23 (2.3) NE2-NZ						
Val-13		0.55 (5.5)* CA-CA	0.41 (4.1) CA-CB	0.45 (4.5) CG2-CG1					
Trp-14		0.37 (3.7)† CA-CA	0.51 (5.1)* CA-CA	0.39 (3.9) CA-CG1	0.33 (3.3) NE1-OE1			0.67 (6.7) CZ2-CB1	
Gly-23							0.32 (3.2) CA-OD1	0.54 (5.4)* CA-CA	0.38 (3.8)† CA-CA
His-24			0.64 (6.4) NE2-CA	0.37 (3.7) NE2-CA	0.65 (6.5)* ND1-CA	0.68 (6.8) CE1-CB	0.44 (4.4) ND1-CA	0.43 (4.3) CB-CA	0.55 (5.5)* CA-CA
Gly-25								0.61 (6.1) CA-CA	0.51 (5.1)* CA-CA
Gln-26									0.54 (5.4) CB-CA
Asp-27							0.62 (6.2)* OD2-OD1		
Ile-28				0.63 (6.3)* CD1-CG2					
Lys-62							0.62 (6.2) CD-OD2		0.37 (3.7) CD-CB
Lys-63									0.65 (6.5) CA-CB
Gly-65								0.66 (6.6) CA-CG2	0.58 (5.8) CA-CA
Val-66								0.43 (4.3) CA-CG2	0.36 (3.6) CG1-CB
Leu-69				0.39 (3.9) CD2-CG2				0.38 (3.8) CB-CG2	0.59 (5.9)* CD2-CA
Thr-70					0.62 (6.2) CA-OE1			0.47 (4.7) OG1-CG2	
Gly-73					0.52 (5.2)* CA-OE1				
Lys-77		0.67 (6.7) CE-CA			0.31 (3.1) NZ-OE2				
Leu-115				0.42 (4.2) CD1-CG1					
Arg-118				0.70 (7.0)* NE-CA		0.70 (7.0) NH1-CB	0.29 (2.9) NH1-OD1	0.70 (7.0)* NH1-CA	
His-119			0.40 (4.0) NE2-CB	0.47 (4.7) CE1-CA		0.62 (6.2)* NE2-CB			
Asp-122			0.35 (3.5) NOD-NZ						
Met-131				0.62 (6.2) CE-CG1					

* These residues are obstructed by third-party atoms.

† These residues are linked by peptide bonds.

Results

Tables 1–5 list the nearest-neighbour residues for antigenic sites 1–5 respectively. Since the field of influence of a neighbouring residue is expected to decrease rapidly with increasing distance from the site residues, we considered that the effects would, in general, become negligible at distances greater than

0.7 nm (7 Å). Accordingly, in these Tables we present only those residues situated within a minimum radius of 0.7 nm (7 Å) from the site residues. In addition, residues that are obstructed by intervening third-party atom(s) are also indicated. It should be noted that among these intervening atoms are atoms of the haem group, which obstruct the interaction of Thr-39, Lys-42 and Phe-43 with the residues of site

Table 2. *Neighbouring residues to antigenic site 2*

The first atom notation refers to the neighbouring residue and the second atom refers to the site residue (for atom notations see Watson, 1969).

Nearest-neighbour residue	Site residue ...	Minimum distances [nm (Å)] and closest atoms to residues in site 2						
		Lys-56	Ala-57	Ser-58	Glu-59	Asp-60	Leu-61	Lys-62
Asp-20								0.62 (6.2) OD2-CD
Ala-22								0.37 (3.7) CB-CD
Gly-23								0.55 (5.5) CA-CD
Gly-25								0.67 (6.7)* CA-CG
Gln-26		0.27 (2.7) NOE-CA	0.50 (5.0)* NOE-CA	0.58 (5.8) NOE-CA	0.60 (6.0)* NOE-OE2		0.41 (4.1) CG-CD1	0.36 (3.6) NOE-CG
Asp-27		0.67 (6.7) OD1-CG						
Leu-29							0.49 (4.9) CB-CD1	0.60 (6.0)* CD2-CA
Ile-30		0.38 (3.8) CD1-CA					0.37 (3.7) CG1-CD1	
Phe-33							0.49 (4.9)* CE2-CD2	
Leu-40							0.67 (6.7)* CD2-CD2	
Arg-45						0.27 (2.7) NE-OD1	0.63 (6.3)* NH1-CA	
Phe-46				0.51 (5.1) CE1-OG		0.36 (3.6) CE1-OD1	0.40 (4.0) CE1-CA	
His-48				0.58 (5.8) NE2-OG		0.66 (6.6) CE1-OD1		
Leu-49				0.46 (4.6) CD1-CB			0.27 (2.7) CD1-CD2	
Glu-52		0.36 (3.6) CG-CE						
Ala-53		0.46 (4.6) CA-CG	0.58 (5.8) CA-CB					
Glu-54		0.57 (5.7) CA-CA	0.42 (4.2) CA-CB	0.59 (5.9) CA-CB				
Met-55		0.38 (3.8)† CA-CA	0.51 (5.1)* CA-CA	0.46 (4.6) CA-CB			0.39 (3.9) CA-CD2	
Lys-63					0.52 (5.2) CG-OE1	0.40 (4.0) CB-CA	0.54 (5.4)* CA-CA	0.38 (3.8)† CA-CA
His-64						0.60 (6.0)* CA-CA	0.45 (4.5) CB-CA	0.52 (5.2)* CA-CA
Gly-65							0.64 (6.4) CA-CA	0.55 (5.5)* CA-CA
Val-66								0.55 (5.5) CG1-CG

* These residues are obstructed by third-party atoms.

† These residues are linked by peptide bonds.

Table 3. *Neighbouring residues to antigenic site 3*

The first atom notation refers to the neighbouring residue and the second atom refers to the site residue (for atom notations see Watson, 1969).

Nearest-neighbour residue	Site residue . . .	Minimum distances [nm (Å)] and closest atoms to residues in antigenic site 3					
		Ala-94	Thr-95	Lys-96	His-97	Lys-98	Ile-99
Thr-39							0.67 (6.7)* CA-CD1
Lys-42					0.55 (5.5)* CD-CA	0.27 (2.7)* NZ-CB	0.33 (3.3)* CD-CD1
Phe-43					0.70 (7.0)* CE2-CB		0.61 (6.1)* CE2-CG1
Leu-89					0.70 (7.0) CA-CD2		
Ala-90		0.55 (5.5)* CA-CB					
Gln-91		0.50 (5.0) CA-CB	0.44 (4.4) CA-CG2				
Ser-92		0.57 (5.7)* CA-CA	0.43 (4.3) CA-OG1	0.54 (5.4) CA-CB	0.37 (3.7) CB-NE2		
His-93		0.39 (3.9)† CA-CA	0.58 (5.8)* CA-CA	0.61 (6.1)* CA-CA	0.36 (3.6) CD2-CD2	0.56 (5.6)* CA-CA	0.40 (4.0) CD2-CG1
Pro-100						0.52 (5.2) CD-CA	0.28 (2.8)† CD-CA
Ile-101							0.59 (5.9) CA-CG2
Tyr-103							0.36 (3.6) CB-CG2
Leu-104							0.41 (4.1) CD1-CG2
Ile-142		0.68 (6.8) CG2-CB					
Tyr-146		0.31 (3.1) CE2-CB				0.60 (6.0) OH-CA	0.44 (4.4) OH-CB
Leu-149		0.49 (4.9) CD2-CB	0.58 (5.8) CD2-CG2				
Tyr-151		0.36 (3.6) CG-CB	0.38 (3.8) OH-CG2			0.62 (6.2) CE1-CG	

* These residues are obstructed by third-party atoms.

† These residues are linked by peptide bonds.

3. Neighbouring residues that influence the environment of more than one site are presented in Table 6. Substitutions at these locations therefore may be expected to have more of an effect on the immunochemical activity than substitutions that influence only one site.

Discussion

During our studies on the antigenic structures of Mb and lysozyme we have repeatedly demonstrated that the reactivity of an antigenic site (i.e. the ability to combine with its antibodies) is highly dependent on its chemical and conformational characteristics. Thus changes in the chemical composition of a site brought about, for example, either through chemical modification of its residue(s) (e.g., Atassi, 1967a, 1968; Atassi & Thomas, 1969) or their evolutionary

replacement in homologous proteins from different animal species (Atassi, 1970; Atassi *et al.*, 1970a,b) quite frequently decrease or abolish its reactivity (for reviews see Atassi, 1975, 1977a,b). The presence and extent of reduction will, of course, depend on the nature of the chemical change of a site residue (Atassi & Habeeb, 1969; Atassi *et al.*, 1971). Similarly, it is well documented that alterations in the conformation of an antigenic site will also influence its reactivity (Atassi, 1967b, 1970; Atassi *et al.*, 1970a,b; Andres & Atassi, 1970).

The chemical and conformational factors that contribute to and maintain the reactivity of an antigenic site are not, however, limited to only those that are localized within the site (Atassi, 1968; Atassi & Thomas, 1969). It must be remembered that protein antigenic sites exist as integral portions of the entire native molecule, and are therefore also

Table 4. *Neighbouring residues to antigenic site 4*

The first atom notation refers to the neighbouring residue and the second atom refers to the site residue (for atom notations see Watson, 1969).

Nearest-neighbour residue	Site residue ...	Minimum distances [nm (Å)] and closest atoms to residues in antigenic site 4						
		His-113	Val-114	Leu-115	His-116	Ser-117	Arg-118	His-119
Val-13				0.37 (3.7)* CG2-CD2				0.45 (4.5) CG2-ND1
Trp-14				0.58 (5.8) CE3-CD2				
Lys-16								0.40 (4.0) CB-NE2
Val-17				0.42 (4.2) CG1-CD1			0.70 (7.0)* CA-NE	0.47 (4.7) CA-CE1
Ala-19							0.70 (7.0) CB-NH1	0.62 (6.2)* CB-NE2
Asp-20							0.29 (2.9) OD1-NH1	
Val-21							0.70 (7.0)* CA-NH1	
Gly-23							0.39 (3.9) CA-NH2	
His-24			0.42 (4.2) CD2-CG2	0.41 (4.1) CD2-CD1			0.31 (3.1) CE1-NE	0.34 (3.4) NE2-CE1
Asp-27			0.39 (3.9) CB-CG1				0.28 (2.8) OD2-NH2	
Ile-28			0.39 (3.9) CA-CG1	0.52 (5.2) CD1-CD1				
Arg-31		0.36 (3.6) NH2-NE2	0.46 (4.6) CB-CG1			0.64 (6.4) NH2-OG		
Glu-109		0.60 (6.0) CA-CB						
Ala-110		0.42 (4.2)* CA-CB	0.53 (5.3) CA-CG1					
Ile-111		0.57 (5.7)* CA-CA	0.50 (5.0)* CA-CB	0.40 (4.0) CG2-CG				
Ile-112		0.38 (3.8)† CA-CA	0.51 (5.1)* CA-CA	0.40 (4.0)* CA-CB	0.55 (5.5) CG2-CB			
Pro-120					0.54 (5.4) CA-NE2	0.64 (6.4) CD-CA	0.48 (4.8) CD-CA	0.28 (2.8)† CD-CA
Gly-121								0.57 (5.7)* CA-CA
Asp-122							0.70 (7.0)* NOD-CA	0.35 (3.5)* NOD-CB
Phe-123		0.70 (7.0)* CE1-CA		0.37 (3.7) CE1-CB	0.40 (4.0) CD1-CA			0.42 (4.2) CB-CB
Gly-124					0.57 (5.7) CA-ND1			
Gln-128		0.62 (6.2) NOE-CA		0.69 (6.9)* NOE-CB	0.44 (4.4) NOE-CB			
Met-131				0.35 (3.5) CE-CD2				
Leu-135				0.70 (7.0) CD2-CD2				

* These residues are obstructed by third-party atoms.

† These residues are linked by peptide bonds.

subject to chemical and conformational influences exerted from regions outside the antigenic sites and that are transmitted to the site through mutual interactions. Indeed, we have described antigenic

sites as being in a certain electronic and steric equilibrium with the remainder of the protein molecule (Atassi & Kazim, 1980). An example of transmitted conformational effects may be taken

Table 5. *Neighbouring residues to antigenic site 5*

The first atom notation refers to the neighbouring residue and the second atom refers to the site residue (for atom notation see Watson, 1969).

Nearest-neighbour residue	Site residue ...	Minimum distance [nm (Å)] and closest atoms to residues in antigenic site 5						
		Lys-145	Tyr-146	Lys-147	Glu-148	Leu-149	Gly-150	Tyr-151
Lys-87		0.28 (2.8) NZ-NZ			0.39 (3.9) NZ-OE2	0.64 (6.4) CE-CD1		
Ala-90		0.36 (3.6) CB-CG	0.42 (4.2) CA-CE2			0.50 (5.0) CB-CD2		
Gln-91		0.39 (3.9) NOE-CE	0.58 (5.8)* CA-CE2		0.61 (6.1) NOE-OE1	0.46 (4.6) CG-CD2		
His-93			0.36 (3.6) CB-OH					
Ala-94			0.31 (3.1) CB-CE2			0.49 (4.9) CB-CD2		0.36 (3.6) CB-CG
Thr-95						0.58 (5.8) CG2-CD2		0.38 (3.8) CG2-OH
Lys-98			0.60 (6.0) CA-OH					0.62 (6.2) CG-CE1
Ile-99			0.44 (4.4) CB-OH					
Pro-100			0.51 (5.1) CA-CE1					
Ile-101			0.31 (3.1) CD1-CD1					0.66 (6.6)* CD1-CB
Leu-104			0.57 (5.7) CG-OH					
Asp-141		0.60 (6.0) CA-CB						
Ile-142		0.47 (4.7) CA-CB	0.38 (3.8) CG2-CE2					
Ala-143		0.55 (5.5)* CA-CA	0.43 (4.3)* CA-CB	0.61 (6.1) CA-CB				
Ala-144		0.38 (3.8)† CA-CA	0.53 (5.3)* CA-CA	0.44 (4.4) CA-CB	0.62 (6.2) CA-CB			
Gln-152			0.39 (3.9) CG-CB	0.39 (3.9) NOE-CB			0.68 (6.8)* CA-CA	0.38 (3.8)† CA-CA
Gly-153								0.65 (6.5) CA-CA

* These residues are obstructed by third-party atoms.

† These residues are linked by peptide bonds.

from our early studies on myoglobin, which showed that conformational changes intentionally imposed on this protein by chemical modification clearly outside its antigenic sites led to a decrease in its antigenic reactivity (Atassi, 1967*b*; Andres & Atassi, 1970). More recently, for lysozyme, we have demonstrated that a chemical change outside of an antigenic site but within the immediate interaction distance from an essential site residue (without detectable accompanying conformational changes) creates an electrostatic inductive effect that exerts a detrimental effect on the reactivity of that site (Lee & Atassi, 1977; Atassi, 1978).

Overall, these findings prompted us to undertake an analysis of the residues that comprise the environment of the antigenic sites of Mb and

lysozyme and that, when altered, may be expected to influence their reactivity. We have previously presented a description of the residues that constitute the environment of the antigenic sites of lysozyme, together with a somewhat detailed discussion of the general nature of protein binding sites and the forces affecting them (Atassi & Kazim, 1980). In the present paper we concentrate on the nearest-neighbour residues making up the immediate environment for each residue in the five antigenic sites of Mb (Atassi, 1975). Because of the expected rapid decay of the field of influence with increasing distance between a site residue and a nearest-neighbour residue, we have considered that the effects will become negligible in most cases when the distance is greater than 0.7 nm (7 Å). Therefore we

Table 6. *Residues neighbouring more than one antigenic site*
For distances between and identities of nearest atoms see Tables 1–5.

Nearest-neighbour residue	Closest residues in antigenic sites				
	Site 1	Site 2	Site 3	Site 4	Site 5
Val-13	Ala-15, Lys-16, Val-17			Leu-115, His-119	
Trp-14	Ala-15, Lys-16, Val-17, Glu-18, Val-21			Leu-115	
Asp-20		Lys-62		Arg-118	
Gly-23	Asp-20, Val-21, Ala-22	Lys-62		Arg-118	
His-24	Lys-16, Val-17, Glu-18, Ala-19, Asp-20, Val-21, Ala-22			Val-114, Leu-115, Arg-118, His-119	
Gly-25	Val-21, Ala-22	Lys-62			
Gln-26	Ala-22	Lys-56, Ala-57, Ser-58, Glu-59, Leu-61, Lys-62			
Asp-27	Asp-20	Lys-56		Val-114, Arg-118	
Ile-28	Val-17			Val-114, Leu-115	
Lys-63	Ala-22	Glu-59, Asp-60, Leu-61, Lys-62			
Gly-65	Val-21, Ala-22	Leu-61, Lys-62			
Val-66	Val-21, Ala-22	Lys-62			
Ala-90			Ala-94		Lys-145, Tyr-146, Leu-149
Gln-91			Ala-94, Thr-95		Lys-145, Tyr-146, Glu-148, Leu-149
His-93			Ala-94, Thr-95, Lys-96, His-97, Lys-98, Ile-99		Tyr-146
Pro-100			Lys-98, Ile-99		Tyr-146
Ile-101			Ile-99		Tyr-146, Tyr-151
Leu-104			Ile-99		Tyr-146
Asp-122	Lys-16			Arg-118, His-119	
Met-131	Val-17			Leu-115	
Ile-142			Ala-94		Lys-145, Tyr-146

have limited our analysis to those residues that fall within a 0.7 nm (7 Å) radius of the antigenic-site residues.

The information summarized in the present paper should be valuable for correlating the immunochemical cross-reactions of myoglobins from various species with amino acid replacements of site residues and of residues within interaction distances from the antigenic sites. It should be stressed that the use of this information should not ignore the effects of substitutions at once (or more)-removed interactions, or even of influences transmitted within the molecule to an antigenic site from distant replacements, or of conformational readjustments (even minor) that may occur. Even though the individual effects of such changes may each be smaller, their cumulative effect on an antigenic site in a protein may reach an appreciable degree.

We have analysed the cross-reactions of myoglobins from 14 species in the light of the above considerations and have succeeded in rationalizing their observed cross-reactions with antisera to sperm-whale Mb. These results are reported in the following paper (Twining *et al.*, 1980).

Note added in proof (Received 21 July 1980)

Recently, East *et al.* (1980) have suggested that evolutionary substitutions of residues in the vicinity of Mb site 1 (residues 15–22) may diminish the affinity of this site for its antibodies. Attenuation of the affinity of this site was ascribed by these authors to a more 'direct' role in the activity of site 1 for the residues at substituted locations, rather than to conformational effects of the substitutions. Residues 9, 13, 53, 113, 116, 117, 121, 122 and 124 were

considered as possible candidates for 'direct' participation in site 1. However, little or no attention was paid to indirect environmental effects on site 1 of these substitutions. By using calculation methods identical with those reported in the present paper we have determined the minimum distances of these residues from site 1 of sperm-whale Mb. These are 0.91, 0.41, 1.60, 1.15, 1.00, 1.13, 0.75, 0.35 and 1.02 nm (9.1, 4.1, 16.0, 11.5, 10.0, 11.3, 7.5, 3.5 and 10.2 Å) respectively. With the exception of residues 13 [0.41 nm (4.1 Å)] and 122 [0.35 nm (3.5 Å)], these residues occur at exceptionally large distances from site 1 for them to be directly involved in binding. Furthermore, it may be deduced from the present and the following paper (Twining *et al.*, 1980) that these residues are clearly not direct 'contact' residues for site 1. The effects of substitutions at positions 13, 122 and other nearest-neighbour residues (see Twining *et al.*, 1980) are cumulative environmental effects on the order of those described therein.

The work was supported by a grant (AM-18920) from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U.S. Public Health Service. A. L. K. is supported by a post-doctoral fellowship from the Muscular Dystrophy Association. We thank Mr. William Samayoa for his expert assistance in the computer programming.

References

- Andres, S. F. & Atassi, M. Z. (1970) *Biochemistry* **9**, 2268–2275
- Atassi, M. Z. (1967a) *Biochem. J.* **102**, 478–488
- Atassi, M. Z. (1967b) *Biochem. J.* **103**, 29–35
- Atassi, M. Z. (1968) *Biochemistry* **7**, 3078–3085
- Atassi, M. Z. (1970) *Biochim. Biophys. Acta* **221**, 612–622
- Atassi, M. Z. (1975) *Immunochemistry* **12**, 423–432
- Atassi, M. Z. (1977a) in *Immunochemistry of Proteins* (Atassi, M. Z., ed.), vol. 1, pp. 1–161, Plenum Press, New York
- Atassi, M. Z. (1977b) in *Immunochemistry of Proteins* (Atassi, M. Z., ed.), vol. 2, pp. 177–264, Plenum Press, New York
- Atassi, M. Z. (1978) *Immunochemistry* **15**, 909–936
- Atassi, M. Z. & Habeeb, A. F. S. A. (1969) *Biochemistry* **8**, 1385–1393
- Atassi, M. Z. & Kazim, A. F. S. A. (1980) *Biochem. J.* **187**, 163–172
- Atassi, M. Z. & Thomas, A. V. (1969) *Biochemistry* **8**, 3385–3394
- Atassi, M. Z., Habeeb, A. F. S. A. & Rydstedt, L. (1970a) *Biochim. Biophys. Acta* **200**, 184–187
- Atassi, M. Z., Tarlowski, D. P. & Paull, J. M. (1970b) *Biochim. Biophys. Acta* **221**, 623–635
- Atassi, M. Z., Perlstein, M. T. & Habeeb, A. F. S. A. (1971) *J. Biol. Chem.* **246**, 3291–3296
- East, I. J., Todd, P. E. & Leach, S. J. (1980) *Mol. Immunol.* **17**, 519–525
- Edmondson, A. B. (1965) *Nature (London)* **205**, 883–887
- Kendrew, J. C., Watson, H. C., Strandberg, B. E., Dickerson, R. E., Phillips, D. C. & Shore, V. C. (1961) *Nature (London)* **190**, 666–670
- Lee, C.-L. & Atassi, M. Z. (1977) *Biochem. J.* **167**, 571–581
- Romero-Herrera, A. E. R. & Lehmann, H. (1974) *Biochim. Biophys. Acta* **336**, 318–323
- Twining, S. S., Lehmann, H. & Atassi, M. Z. (1980) *Biochem. J.* **191**, 681–697
- Watson, H. C. (1969) in *Progress in Stereochemistry* (Aylett, B. J. & Harris, M., eds.), pp. 299–333, Butterworth, London