Biopharmaceutics

Proteins binding to APIs

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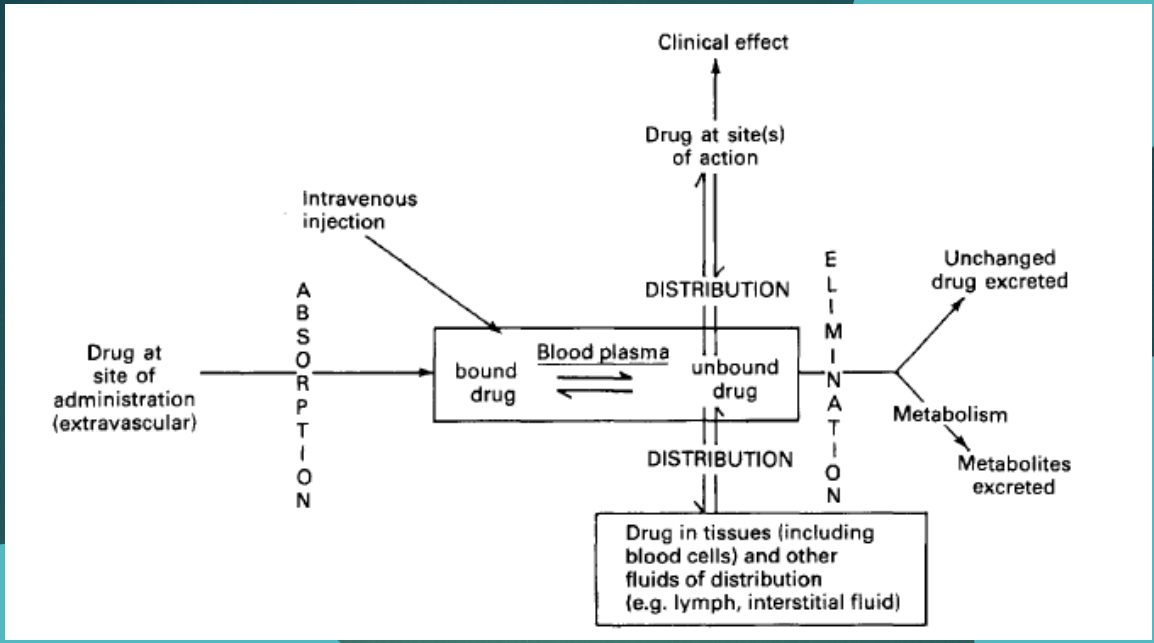
Outline of lectures

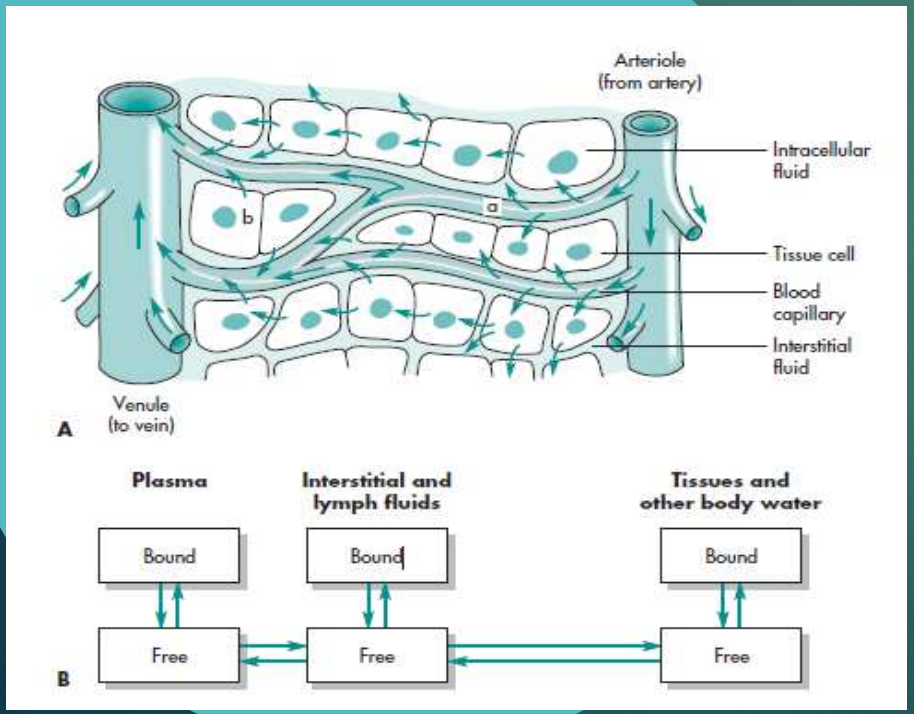
- Importance of protein binding in the field pharmaceutics
- How drug-protein binding/changes in drug-protein binding affect volume of distribution, metabolism, clearance, half-life etc
- Types of binding
- Extent of drug- protein binding
- Factors affecting drug-protein binding
- How to measure/estimate drug- protein binding
- Drug-protein binding as an advantageous approach in drug delivery
- Monitoring of free drug concentrations
- Case studies
- Summery
- Questions

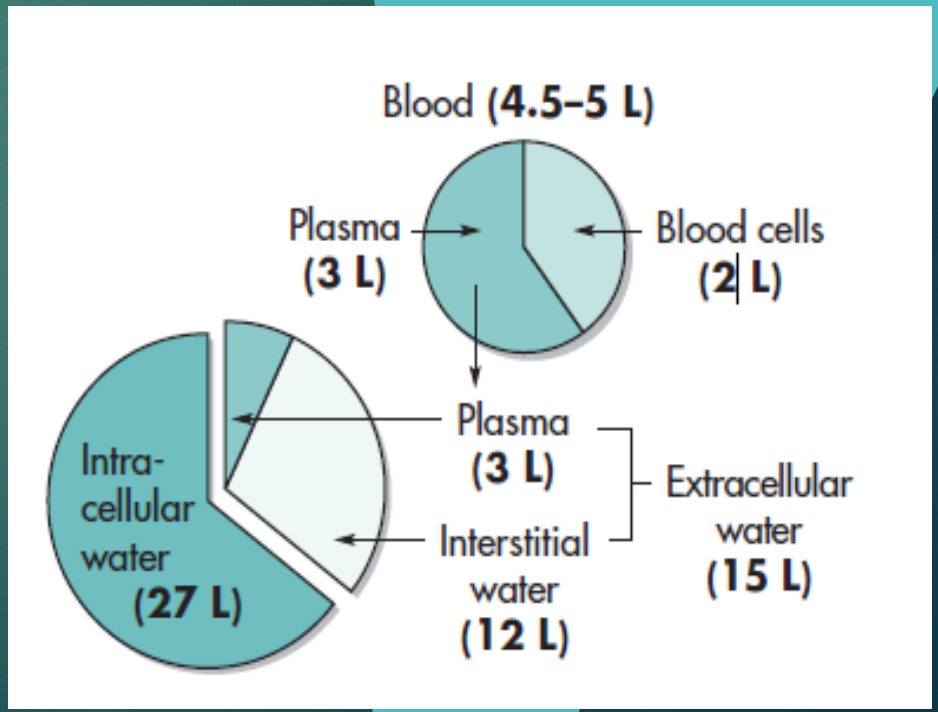
Drug-protein binding

Many drugs interact with plasma or tissue proteins or with other macromolecules, such as melanin and DNA, to form a *drug–macromolecule complex*.

Why drug-protein binding is important in drug delivery?


Clinical effect
Drug at site(s) of action
Intravenous injection
DISTRIBUTION
ELIMINATION
Unchanged drug excreted
Drug at site of administration (extravascular)
ABSORPTION
bound drug
Blood plasma
unbound drug
Metabolism
Metabolites excreted
DISTRIBUTION
Drug in tissues (including blood cells) and other fluids of distribution (e.g. lymph, interstitial fluid)


Arteriole (from artery)
Intracellular fluid
Tissue cell
Blood capillary
Interstitial fluid
Venule (to vein)
A
Plasma
Interstitial and lymph fluids
Tissues and other body water
Bound
Free
B


Blood (4.5–5 L)
Plasma (3 L)
Blood cells (2 L)
Intra-cellular water (27 L)
Plasma (3 L)
Interstitial water (12 L)
Extracellular water (15 L)

Types of drug-macromolecule interactions

- Reversible:

1. Involves weaker chemical bonds, such as hydrogen bonds or van der Waals forces.
2. The amino acids that compose the protein chain have hydroxyl, carboxyl, or other sites available for reversible drug interactions.
3. The protein-bound drug is a large complex that cannot easily transverse the capillary wall and therefore has a restricted distribution.
4. The drug-protein complex is usually pharmacologically inactive while the free form is the active form.

Table: passage 'permeability' of molecules through capillary walls

	Molecular Weight	Radius of Equivalent Sphere *A* (0.1 mm)	Diffusion Coefficient	
			In Water (cm^2/s) × 10^5	Across Capillary (cm^2/s × 100 g)
Water	18		3.20	3.7
Urea	60	1.6	1.95	1.83
Glucose	180	3.6	0.91	0.64
Sucrose	342	4.4	0.74	0.35
Raffinose	594	5.6	0.56	0.24
Inulin	5,500	15.2	0.21	0.036
Myoglobin	17,000	19	0.15	0.005
Hemoglobin	68,000	31	0.094	0.001
Serum albumin	69,000		0.085	<0.001

Types of drug-macromolecule interactions

- Irreversible:

1. usually a result of chemical activation of the drug, which then attaches strongly to the protein or macromolecule by covalent chemical bonding.
2. It might cause certain types of drug toxicity that may occur over a long time period, as in the case of chemical carcinogenesis.
3. It can cause short-term toxicity due to the formation of reactive intermediates such as hepatotoxicity of high doses of acetaminophen

What are the macromolecules involved in these complexes?

Macromolecule involved in these complexes

- Albumin
- alpha1-acid glycoprotein
- Lipoproteins
- Immunoglobulins (IgG)
- Erythrocytes (BC)

Protein	Molecular Weight (Da)	Normal Range of Concentrations (g/L)	Normal Range of Concentrations (mol/L)
Albumin	65,000	35–50	$5–7.5 \times 10^{-4}$
α_1-Acid glycoprotein	44,000	0.4–1.0	$0.9–2.2 \times 10^{-5}$
Lipoproteins	200,000–3,400,000	Variable	

The sequence of drug-protein binding is:
Albumin> Glycoprotein > Lipoproteins> Globulins

Human Serum Albumin (HSA)

- Albumin is a protein with a molecular weight of 65,000 to 69,000 Da that is synthesised in the liver.
- It comprises about 60% of total plasma protein content present in the blood
- In the body, albumin is distributed in the plasma and in the extracellular fluids of skin, muscle, and various tissues.
- The elimination half-life of albumin is 17 to 18 days.
- Normally, albumin concentration is maintained at a relatively constant level of 3.5% to 5.5% w/v.
- Albumin is responsible for maintaining the osmotic pressure of the blood and for the transport of endogenous and exogenous substances in the plasma.
- Albumin complexes with endogeneous substances such as free fatty acids (FFAs), bilirubin, various hormones (eg, cortisone, aldosterone, thyroxine, tryptophan), and other compounds.

Human Serum Albumin (HSA)

- Many weak acidic (anionic) drugs bind to albumin by electrostatic and hydrophobic bonds.
- Weak acidic drugs such as salicylates, phenylbutazone, and penicillins are highly bound to albumin.
- The strength of the drug binding is different for each drug.

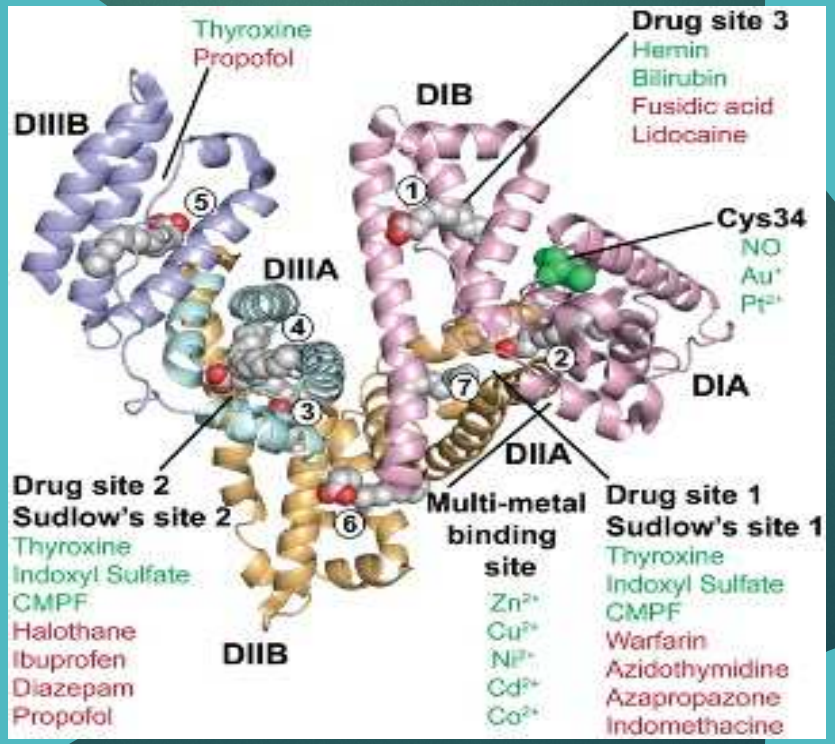


Fig. Structure of HSA

Alpha-1-acid glycoprotein (AAG)

- Also known as orosomucoid (ORM)
- It is synthesised and metabolised by the liver
- Its half-life is approximately 5.5 day
- Its molecular weight of about 44,000 Da
- The plasma concentration of AAG is low (0.4%–1%)
- Its high sialic acid content results in its acidic nature and low pKa.
- It is an acute phase reactant, and concentrations increase in stress situations including diseases
- At least four polymorphic patterns and four genetic variants in human plasma have been reported

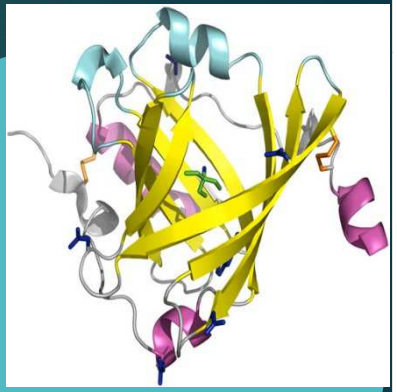


Fig. Structure of orosomucoid

Alpha-1-acid glycoprotein (AAG)

- Many basic drugs (cationic) such as propranolol, lidocaine, and neutral drugs bind significantly to AAG
- Generally, the affinity of a drug that binds to both albumin and AAG is higher for AAG
- AAG is referred to as a low-capacity, high-affinity protein
- AAG also binds to some acidic and neutral drugs such as carbamazepine and prednisolone

Some basic drugs which bind significantly to α_1-acid glycoprotein (AAG)

β-adrenoceptor blockers
Alprenolol (Piafsky & Borga, 1977)
Oxprenolol (Belpaire *et al.*, 1982)
Pindolol (Belpaire *et al.*, 1982)
Propranolol (Piafsky *et al.*, 1978)
Timolol (Belpaire *et al.*, 1982)

Miscellaneous
Chlorpromazine (Piafsky *et al.*, 1978)
Dipyridamole (Kopitar & Weisenberger, 1971)
Erythromycin (Prandota *et al.*, 1980)
Metoclopramide (Webb *et al.*, 1986)
Nicardipine (Urien *et al.*, 1985)
Phencyclidine (Giles *et al.*, 1982)
Prednisolone (Milsap & Jusko, 1983)
Progesterone (Ganguly & Westphal, 1968)
Triazolam (Kobroth *et al.*, 1984)

Antiarrhythmics
Aprindine (Teirlynck *et al.*, 1982)
Bupivacaine (Denson *et al.*, 1984)
Disopyramide (Lima *et al.*, 1981)
Lignocaine (Routledge *et al.*, 1980a)
Pirmenol (Hammill *et al.*, 1982)
Quinidine (Nilsen *et al.*, 1978)
Verapamil (McGowan *et al.*, 1983)

Opiates
Methadone (Romach *et al.*, 1981)
Pethidine (Nation, 1981)

Antidepressants
Amitriptyline (Pike & Skuterud, 1982)
Imipramine (Borga *et al.*, 1977)
Nortriptyline (Pike & Skuterud, 1982)

Lipoproteins

- They are heterogeneous group of proteins in the form of complexes of lipids and proteins (Molecular weight approx. 200,000 -3,400,000 Da)
- They are classified according to their density and separation in the ultracentrifuge into chylomicrons, VLDL, LDL, and HDL
- Lipoproteins are responsible for the transport of plasma lipids to the liver and may be responsible for the binding of drugs if the albumin sites become saturated.

Examples of drugs binding with different proteins

Table 1 Predominant binding proteins of drugs >70% bound to plasma proteins

Albumin (% bound)	Albumin and AAG (% bound)	Albumin and lipoproteins (% bound)	Albumin, AAG, and lipoproteins (% bound)
Ceftriaxone (A)	Alprenolol (B)	Cyclosporine (N)[a]	Amitriptylline (B)
Clindamycin (A)	Carbamazepine (N)	Probucol (N)[a]	Bupivacaine (B)
Clofibrate (A)	Disopyramide (B)[a]		Chlorpromazine (B)
Dexamethasone (N)	Erythromycin (B)		Diltiazem (B)
Diazepam (B)	Lidocaine (B)		Imipramine (B)
Diazoxide (A)	Meperidine (B)		Nortriptyline (B)
Dicloxacillin (N)	Methadone (B)		Perazine (B)
Digitoxin (N)	Verapamil (B)		Propranolol (B)
Etoposide (N)			Quinidine (B)
Ibuprofen (A)			
Indomethacin (A)			
Nafcillin (A)			
Naproxen (A)			
Oxacillin (A)			
Phenylbutazone (A)			
Phenytoin (A)			
Probenecid (A)			
Salicylic acid (A)			
Sulfisoxazole (A)			
Teniposide (N)			
Thiopental (A)			
Tolbutamide (A)			
Valproic acid (A)			
Warfarin (A)			

[a]Albumin is minor binding protein.
A, indicates acid; B, base; N, neutral.

Globulins (α,β,γ globulins)

- Globulins may be responsible for the plasma transport of certain endogenous substances such as corticosteroids.
- They have a low capacity but high affinity for the binding of these endogenous substances

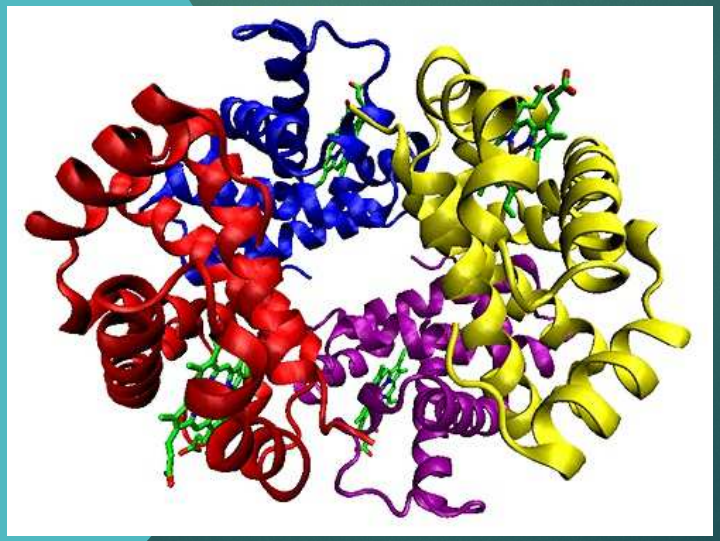


Fig. structure of β-globulin

Erythrocytes

- Erythrocytes, or red blood cells (RBCs), may bind both endogenous and exogenous compounds.
- RBCs consist of about 45% of the volume of the blood.
- Phenytoin, pentobarbital, and amobarbital are known to have an RBC/plasma water ratio of 4 to 2, indicating preferential binding of drug to the erythrocytes over plasma water.

Extent of drug-protein binding

DRUG	PERCENT BOUND
Naproxen (Naprosyn)	>99
Chlorambucil (Leukeran)	>99
Etodolac (Lodine)	>99
Warfarin sodium (Coumadin)	>97
Fluoxetine (Prozac)	>95
Ceftriaxone (Rocephin)	85–95
Cefoperazone (Cefobid)	82–93
Cefonicid (Monocid)	>90
Indomethacin (Indocin)	>90
Spironolactone (Aldactone)	>90
Digitoxin (Crystodigin)	>90
Cyclosporine (Sandimmune)	>90
Sulfisoxazole (Gantrisin)	>85
Diltiazem (Cardizem)	70–80
Penicillin V (Veetids)	>75
Nitroglycerin (Nitro-Bid)	>60
Penicillin G potassium	>60
Methotrexate	>50
Methicillin (Staphcillin)	>40
Ceftizoxime (Cefizox)	>30
Captopril (Capoten)	25–30
Ciprofloxacin (Cipro)	20–40
Digoxin (Lanoxin)	20–25
Ampicillin (Omnipen)	>20
Amoxicillin (Amoxil)	>20
Metronidazole (Flagyl)	>20
Mercaptopurine (Purinethol)	>19
Cephradine (Velosef)	8–17
Ranitidine (Zantac)	>15
Ceftazidime (Tazicef)	>10
Nicotine (ProStep)	>5
Minoxidil (Loniten)	>0

Average literature values based on conditions usually associated with drug therapy.

Factors affecting drug-protein binding

- API related factors

1. Physiochemical characteristics of the drug
2. Concentration of drug in the body
3. Drug's affinity towards protein/tissue

- Protein related factors

1. Number of binding sites on the protein
2. Concentration of protein/binding component

- Drug interactions
- Patient related factors

Factors affecting drug-protein binding
Patient related factors

- Physiologic conditions

1. Age
2. Pregnancy
3. Ethnicity
4. Gender
5. Smoking
6. Obesity
7. Nutritional status
8. Surgery

- Disease states

1. Renal disease
2. Liver disease
3. Inflammatory conditions
4. Cancer and burn injury
5. Diabetes mellitus
6. Thyroid disease
7. Cystic fibrosis

Further readings

- Chapter 11: Applied Biopharmaceutics and Pharmacokinetics, 7th Edition; 2012
- Encyclopaedia of Pharmaceutical Technology; 3rd Edition; 2007; pages 3027-3041